ELSEVIER
ACADEMIC PRESS

SYSTEMIC LUPUS ERYTHEMATOSUS

Fourth Edition

Edited by

Robert G. Lahita

LIST OF CONTRIBUTORS

Numbers in parentheses indicate the chapter numbers of the authors' contribution.

GYÖRGY ÁBEL (6), Clinical Immunology Laboratory, Lahey Clinic Medical Center, Burlington, Massachusetts 01805-0001.

VINCENT AGNELLO (6), Department of Immunology Research, Lahey Clinic Medical Center, Edith Norse Rogers Memorial Veterans Affairs Hospital, Burlington, Massachusetts 01805-0001.

DONATO ALARCÓN-SEGOVIA (3), National Autonomous University of Mexico, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, 14000 Mexico, D.F.

MARTA E. ALARCÓN-RIQUELME (3), Department of Genetics and Pathology, Rudbeck Laboratories, Uppsala University, Uppsala, Sweden.

HOWARD AMITAL (1), Center for Autoimmune Diseases and Department of Medicine "B," Affliated with Sackler Faculty of Medicine, Sheba Medical Center, Tel-Aviv University, Tel-Hashomer 52621, Israel.

RONALD A. ASHERSON (41), Rheumatic Diseases Unit, Department of Medicine, University of Cape town School of Medicine, Cape Town 8001, South Africa.

JOHN P. ATKINSON (5), School of Medicine, Washington University, St. Louis, Missouri 63110.

TATSUYA ATSUMI (39), Department of Medicine II, School of Medicine, Hokkaido University, Sapporo 060-8638, Japan.

HOWARD A. AUSTIN (30), National Institutes of Health, Bethesda, Maryland 20892-1818.

ROBERTO BACCALA (8), Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

JAMES E. BALOW (30), National Institutes of Health, Bethesda, Maryland 20892-1818.

DIMITRI BOUMPAS (30), National Institutes of Health, Bethesda, Maryland 20892-1818.

ROBIN L. BREY (26), Department of Medicine, Division of Neurology, University of Texas Health Science Center, San Antonio, Texas 78229.

JILL BUYON (17), Department of Rheumatology, Hospital for Joint Diseases, New York, New York 10003

EDWARD K. L. CHAN (13), Department of Oral Biology, University of Florida College of Dentistry, Gainesville, Florida 32610–0424.

RICARD CERVERA (41), Department of Autoimmune Diseases, Hospital Clinic, 08036 Barcelona, Catalonia, Spain.

WENDY S. COHEN (27), School of Medicine, New York University, New York, New York 10016.

JOHN C. DAVIS (47), Clinical Trials Center, Lupus Clinic, San Francisco, California 94143.

CRAIG J. DELLA VALLE (37), Department of Orthopaedic Surgery, Rush-Presbyterian-St. Lu8ke's Medical Center, Chicago, Illinois 60612.

PAUL J. DEMARCO (18), Department of Psychological and Brain Sciences, University of Louisville, Lousville, Kentucky 40292.

JUDAH A. DENBURG (28), Department of Psychiatry and Behavioural neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

SUSAN D. DENBURG (28), Department of Psychiatry and Behavioural neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

BETTY DIAMOND (10), Albert Einstein College of Medicine, Bronx, New York 10461.

PAUL E. DICESARE (37), Department of Orthopaedic Surgery, Hospital for Joint Diseases, New York University, New York, New York 10003.

Iris Dotan (34), Division of Clinical Immunology, Mount Sinai Medical Center, New York, New York 10029-6574.

Barri Fessler (32), Division of Clinical Immunology and Rheumatology, University of Alabama, Birmingham, Alabama 35294.

Martin J. Fritzler (13), University of Calgary, Calgary ALB T2N 4N1, Canada.

Dafna D. Gladman (23), Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital, Toronto, Ontario M5T 2S8, Canada.

Oscar S. Gluck (38), Department of Medicine, College of Medicine and Arizona Rheumatology Center, Phoenix, Arizona.

Richard E. W. Halliwell (9), Veterinary Field Station, University of Edinburgh, Easter Bush, Rosling, Midlothian EH25 9RG, Scotland.

Evelyn V. Hess (45), Division of Immunology, University of Cincinnati Medical Center, Cincinnati, Ohio 45221.

Gary S. Hoffman (32), Department of Rheumatic and Immunologic Diseases, Cleveland Clinic Foundation, Cleveland, Ohio 44195.

Robert W. Hoffman (24), Division of Rheumatology and Immunology, University of Miami, Miami, Florida 33101.

Graham R. V. Hughes (40), Lupus Research Unit, Department of Rheumatology, Rayne Institute, St. Thomas Hospital, London SE1 7EH, England.

Munther A. Khamashta (40), Lupus Research Unit, Department of Rheumatology, Rayne Institute, St. Thomas Hospital, London SE1 7EH, England.

John Klippel (47), NIAMS, Arthritis Foundation, Atlanta, Georgia 30309.

Robert P. Kimberly (46), Division of Clinical Immunology and Rheumatology, University of Alabama, Birmingham, Alabama 35294.

Takao Koike (39), Department of Medicine II, School of Medicine, Hokkaido University, Sapporo 060-8638, Japan.

Dwight H. Kono (8), Department of Immunology, Scripps Research Institute, La Jolla, California 92037.

Robert G. Lahita (7, 16), Department of Medicine and Rheumatology, LibertyHealth, Jersey City, New Jersey 07304.

Lynne Larocque (28), department of Psychiatry and Behavioural Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada.

James L. Levenson (27), Liaison Psychiatry, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

Jeffrey Laurence (36), Weill Medical College, Cornell University, New York, New York 10021.

E. Clinton Lawrence (33), McKelvey Lung Transplantation Center, School of Medicine, Emory University, Atlanta, Georgia 30322.

Matthew Liang (20), Department of Medicine/Rheumatology/Immunology/PBB-B2, Brigham and Women's Hospital, Boston, Massachusetts 02115.

Michael D. Lockshin (22), Barbara Volker Center, Hospital for Special Surgery, Weill-Cornell Medical College, New York, New York 10021.

Lisa A. Mandl (20), Department of Medicine, Weill Cornell Medical College, Hospital for Special Surgery, New York, New York 10021.

Mart Mannik (14), Department of Medicine, University of Washington, Seattle, Washington 98195.

Michael J. Maricic (38), University of Arizona, Southern V. A. Health Care System, Tucson, Arizona 85723.

Eiji Matsuura (39), Department of Cell Chemistry, Graduate School of Medicine and Dentistry, Okayama University, Okayama, Japan.

Lloyd Mayer (34), Division of Clinical Immunology, Mount Sinai Medical Center, New York, New York 10029-6574.

Ian R. MacKay (35), Department of Biochemistry and Molecular Biology, Monash University, Clayton 3800, Victoria, Australia.

Joan T. Merrill (43), Clinical Pharmacology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104.

Daniel Mimouni (29), Division of Dermatoimmunology, Johns Hopkins Hospital, Baltimore, Maryland 21205.

Anne-Barbara Mongey (45), Division of Immunology, University of Cincinnati Medical Center, Cincinnati, Ohio 45267.

Carlos H. Nousari (29), Department of Dermatology, Cleveland Clinic Florida, Weston, Florida 33326.

Elena Peeva (10), Albert Einstein College of Medicine, Bronx, New York 10461.

Michelle Petri (31), School of Medicine, Johns Hopkins University, Baltimore, Maryland 21205.

Wesley H. Reeves (15), Division of Rheumatology and Clinical Immunology, University of Florida, Gainesville, Florida 32610.

Morris Reichlin (11), Arthritis/Immunology Program, Oklahoma Medical Research Foundation, Department of Medicine, Oklahoma University Health Sciences Center, Oklahma City, Oklahoma 73104.

Karen E. Rendt (32), Department of Rheumatic and Immunologic Diseases, Cleveland Clinic Foundation, Cleveland, Ohio 44195.

John D. Reveille (4), Department of Rheumatology, University of Texas at Houston, Health Science Center, Houston, Texas 77225.

Hanno B. Richards (15), Division of Rheumatology and Clinical Immunology, University of Florida, Gainesville, Florida 32610.

W. Neal Roberts (27), School of Medicine, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

Garcia-Cavazos Rogelio (26), Department of Medicine, University of Texas Health Science Center, San Antonio, Texas 78229.

Robert L. Rubin (12), Department of Molecular Genetics and Microbiology, School of Medicine, University of New Mexico, Albuquerque, New Mexico 87131.

Lisa R. Sammaritano (22), Barbara Volker Center, Hospital for Special Surgery, Weill-Cornell Medical College, New York, New York 10021.

Minoru Satoh (15), Division of Rheumatology and Clincal Immunolgoy, University of Florida, Gainesville, Florida 32610.

Peter M. Schneider (5), Institute of Legal Medicine, University of Mainz, Mainz, Germany D55131.

Peter H. Schur (21), Harvard Medical School, Department of Rheumatology, Brigham and Women's Hospital, Boston, Massachusetts 02115.

Sergio Schwartzman (22), Barbara Volker Center, Hospital for Special Surgery, Weill-Cornell Medical College, New York, New York 10021.

Joseph C. Shanahan (46), Division of Clinical Immunology and Rheumatology, University of Alabama, Birmingham, Alabama 35294-0007.

Lorraine G. Shapeero (19), Department of Musculoskeletal Radiology, Bone and Soft Tissue Program, U.S. Military Cancer Institute, Uniformed Services University, Bethesda, Maryland 20814.

Sandor S. Shapiro (42), Cardeza Foundation for Hematologic Research, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107.

Yehuda Shoenfeld (1), Department of Medicine, Concepts of Autoimmunity, Sheba Medical Center, Tel-Aviv University, Tel-Hashomer 52621, Israel.

Ronit Simantov (36), Weill Medical College, Cornell University, New York, New York 10021.

Stephen E. F. Spurgeon (42), Cardeza Foundation for Hematologic Research, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107.

Ilona Szer (18), Division of Rheumatology, Children's Hospital of San Diego, San Diego, California 92123.

Argyrios N. Theofilopoulos (8), Department of Immunology, Scripps Research Institute, La Jolla, California 92037.

George C. Tsokos (2), Department of Medicine Uniformed Services, University of the Health Sciences, Bethesda, Maryland 20814.

Murray Urowitz (Foreword), Department of Medicine, University of Toronto Hospital, Toronto, Ontario M5T2S8, Canada.

Ronald F. van Vollenhoven (48), Rheumatology Unit, Karolinska Hospital and Institute, Stockholm, Sweden.

Mark H. Wener (14), Department of Laboratory Medicine, Division of Rheumatology, University of Washington, Seattle, Washington 98195.

John B. Winfield (25), Department of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599.

Raymond L. Yung (44), Department of Medicine, University of Michigan, Ann Arbor, Michigan 48109.

Joseph D. Zuckerman (37), Department of Orthopedic Surgery, Hospital for Joint Disease, New York, New York 10003.

FOREWORD

"One small step for man . . ." Neal Armstrong's intended first utterance when he stepped onto the surface of the moon on July 20, 1969 can indeed be the motto of every researcher and clinician involved in the investigation of systemic lupus erythematosus, which also blossomed in the late 1960s. Each can point to their contribution as one small piece of the puzzle in the understanding of the etiology, pathogenesis, clinical presentation and treatment of lupus. In the last three decades the puzzle has indeed begun to take shape! The immune system abnormalities and their pathogeneic consequences have been extensively elaborated and are now serving as guides for the development of new therapies. Prognosis has improved dramatically from less than 50% at 5 years in the 1950s to 70% at 20 years today.

Quality of life has also improved as we learn to modulate our therapies more appropriately. At the same time, new clinical presentations have come to the forefront, either as unrecognized features of the disease itself or as a consequence of our therapies, including accelerated atherosclerosis, cognitive dysfunction, osteoporosis/osteonecrosis, and antiphospholipid antibody syndrome. The intensive investigations of these new features in lupus will give insights into their underlying mechanisms in people in the general population with atherosclerosis, dementia, osteoporosis, and clotting disorders. However, the etiology section of the puzzle still continues to elude us. Although investigations in many laboratories and clinics around the world continue to probe the genome, no clear understanding of susceptibility genes or disease expression genes has yet emerged. Similarly, the putative viral or hormonal etiologic factors remain elusive. Systemic Lupus Erythematosus, Fourth Edition, brings together the many pieces of the lupus puzzle to allow the researcher, clinician, teacher, and student to begin to see the puzzle coming together. Researchers, physicians, and patients look forward to the day when the puzzle will come together as a clear picture . . . *"one giant leap for mankind."*

Murray Urowitz, MD
University of Toronto

PREFACE

A book about the disease systemic lupus erythematosus (SLE) has to be remarkable. As we have heard many times, patients who acquire this illness are young, in the prime of life, surprised, and often diagnosed on average of five years after their first symptoms. Moreover, this is a deadly disease, even though patients look well. More and more people develop related autoimmune diseases like lupus, and the disease shares many clinical characteristics with these other maladies. In fact, I will reiterate the notion held by some that SLE is more than one illness. This notion is likely based on the varied presentation, organ selectivity, and waxing severity even after therapy is complete. Understanding this complexity, this book is designed to explore both the clinical and basic aspects of immunology as it applies to lupus. The book is divided into sections, which deal with the basic immunology, the overall clinical presentations, system analysis, and then treatment options. However, it is not that easy, since conditions like fibromyalgia and the antiphospholipid syndrome have to be included. I have attempted to make the division of sections and the grouping of chapters logical and easy to follow.

It is a book directed to both the student of immunology and the experienced clinician. In addition, it is a book that includes work by some of the most experienced clinicians and researchers among us. Each chapter is a compelling treatment of a specific area in exhaustive detail, and the authors of these detailed chapters have taken great time to write for this book. For them we are grateful.

This fourth edition of *Systemic Lupus Erythematosus*, a book born at the Rockefeller University in the laboratory of the late Professor Henry G. Kunkel, serves as a testimony to his dedication and work in immunology. It is an inspiration for all who take the findings at the patient's bedside and bring it to the laboratory in order to understand. Wherever possible, I have taken the progeny of the many investigators who passed through the Kunkel laboratory and included their work between these covers. Dr. Kunkel wrote the first Foreword for this book and encouraged me to include as much science as possible in the first edition. The inclusion of science continues. Eng Tan, Graham Hughes, and now Murray Urowitz followed Dr. Kunkel's tradition and wrote forewords for the next three editions. Dr. Urowitz, author of the current Foreword, is the director of the successful Toronto Center for Lupus Research and is a guiding force in the current thinking of the science of SLE.

Many authors from the last edition have revised their chapters, and much new material has been added. By necessity, overlap has occurred in some of the chapters. Purposefully, Some material is presented in different ways in different chapters. Some newer additions to the book include George Tsokos, who covers the molecular aspects of cellular immunology as it applies to lupus in great detail. James Levenson and colleagues chapter on the psychiatry of SLE, which places some perspective on the variant forms of behavior that can occur either because of the effects lupus has on a patient due to the severity of the illness or the actual production of psychosis by the effects of the immune dysfunction on the brain. Robin Brey and colleagues present the latest information on all aspects of the nervous system and lupus, a particularly challenging area. Carlos H. Nousari, Department of Dermatology, Cleveland Clinic Florida, details the dermatologic aspects of lupus, a system that was studied at Johns Hopkins by William Osler more than 100 years ago. John Winfield, in a new chapter, carefully dissects

fibromyalgia in association with SLE. Finally, Oscar Gluck presents osteoporosis and lupus in a chapter that covers both the steroid-treated patient and the effects of natural loss of bone in a patient with a chronic disease.

Lupus is a complex illness that often requires a physician's considerable time and effort. It is not an easy disease to diagnose or manage. This book is written as an aid to help the physician understand the subtle nuances of this enigma and use knowledge herein to manage the patient. It is written with the idea that new knowledge is urgently needed to diagnose and treat those patients for whom this illness is nothing short of a major catastrophe—a life emergency that deserves all our efforts and attention.

Numerous worldwide groups of patients dedicate their time and effort to social programs and funding of basic and clinical research, in the hope of an eventual cure. I salute the patients and their families for their efforts on behalf of our knowledge. I hope that the accuracy of the information given here in this book will educate and inform until the fifth edition comes forward. It is always the hope of this editor that subsequent editions might include both the cause and cure of this devastating illness.

Finally, I thank my publisher Elsevier for its patience and high standards of excellence and dedication to helping the sick. I specifically note my developmental editors Tari Paschall and Judy Meyer for sticking with the project and enduring my many letters, telephone calls, and emails regarding the authors and their materials included in the book.

Robert G. Lahita
Jersey City, N. J., 2003

INTRODUCTION

OVERVIEW

As I have noted in previous editions of this work, systemic lupus erythematosus (SLE), known to the public as *lupus*, is not a rare disease, but it is not common either. It is a chronic disease of unknown etiology that is very difficult to diagnose, not easy to treat, and yet sometimes over diagnosed. Since the last edition of this book, major advances have been noted in the treatment of illnesses like rheumatoid arthritis using biological therapy but still nothing that is able to completely reverse the ravages of a autoimmune diseases like lupus. This absence of therapeutic agents is the subject of discussion by a host of groups established to look at criteria for diagnosis, biomarkers of severity, and instruments of clinical activity. The establishment of clear indices that would allow regulatory agencies to see some consistency of response is lacking because lupus is a disease that often does not respond to either therapy or conventional wisdom.

I am unsure that lupus is really only one illness, and that may be one of the difficulties. I am more convinced that lupus is a series of illnesses that has, as its basis, an autoimmune response that devastates the whole body or destroys a single organ. Its classification and drugs for treatment might be better designed if it were a simple organ-specific entity that had consistency of presentation.

This protean illness presents in a variety of ways. It really depends on the organ chosen by the immune system for attack. The diagnostic difficulty revolves around the standard issues surrounding all illnesses, namely, the factors associated with the disease. This disease favors women, has a genetic association, and has an elusive trigger. Although we get closer to a cause by looking at many different possibilities, the problem is not easily solved because the immune system seems to become more complex by the year. In this fourth edition, the issues remain the same for clinicians and investigators. Reading the chapters in this book will not solve the mystery, but it will give you the impression that we are closer to understanding the illness. Most importantly, you will understand the disease better. The newer association of fibromyalgia and phospholipid antibody with this illness and the presence of cognitive disability apart from any gross neurological findings would seem to indicate the lupus is also evolving as time goes on. The limited numbers of drugs available to treat lupus despite years of research speak for the difficulty the scientific community has with understanding the biology of lupus.

In this fourth edition, we have revised every chapter. It includes new chapters on fibromyalgia and certain aspects of treatment. New authors write older topics like the dermatology of lupus. Older chapters such as the imaging of lupus are considerably enhanced with a thorough discussion of the current and future roles of the different modalities for imaging each organ system of patients with SLE. The section on the overview of the cellular and molecular aspects of lupus is probably the most comprehensive treatment of this area in print. The central nervous system and psychiatric aspects of lupus are written by new authors and are new and exciting. Apart from books dealing solely with the antiphospholipid syndrome, there is no better discussion of this topic and all of the aspects of the syndrome from clinical presentation to theories about pathogenesis. All in all, the book is enhanced in all areas to provide the reader with the latest information about SLE, its science, presentation, and treatment.

We have also produced a CD-ROM of the illustrations in this book in hopes that you might use it

to teach students and young physicians who may delight in a new frontier in medicine. Lupus gives us a handle on molecular biology, insight into common ailments like atherosclerosis, and a better understanding of the way the brain works. I have said before that it is a disease of the twenty-first century, and this book is testimony to that statement.

HISTORY OF LUPUS

The history of the disease SLE is colorful. The term *lupus* is attributed to the thirteenth century physician Rogerius, who described the facial lesions that were reminiscent of a "wolf's bite"(1). In 1851, Cazenave applied the term *lupus erythematosus* for the first time to a disease described by his teacher Laurent Biett (2). In 1845, von Hebra, a Viennese physician, used *butterfly rash* to describe the familiar malar rash of the disease. For most of the nineteenth century, lupus was thought to be a dermatologic disease. Von Hebra, in his 1856 book, published the first illustrations of the disease in the *Atlas of Skin Diseases*. However, when Moretz Kaposi described the visceral forms of the disease in 1872, physicians began to suspect that this disease was a more generalized form of the illness and the term *acute disseminated* was included in the description (3). Kaposi proposed two types of lupus: disseminated and discoid. In his early writings, he supposed that the disseminated form consisted of (1) subcutaneous nodules, (2) arthritis, (3) lymphadenopathy, (4) fever, (5) weight loss, (6) anemia, and (7) central nervous system involvement. In 1904, William Osler described two women who developed renal failure within 10 months of the appearance of a facial erythema, which in retrospect was the facial rash of von Hebra (4). Osler described a number of other illnesses at the time, among them, Henoch Schoenlein purpura and disseminated gonococcemia, which could be confused with the lesions on the two women. In Vienna at this time, Jedasson described similar syndromes in a few patients. Both he and Osler therefore established SLE as a distinct entity by the turn of the century, even though many practitioners still thought of SLE as a form of skin tuberculosis (5). Very typical cases of SLE were reported under a variety of names (6), and it was not until the 1920s and 30s that the disease was well defined. The disease, at that time, was carefully described by pathologists who studied the morbid anatomic changes that so characterized someone with lupus. The atypical bacterial endocarditis of Emmanuel Libman and Benjamin Sacks, described in 1924, was a classic example of the pathology found in some patients with SLE and were likely lesions associated with antiphospholipid antibody (7). Following that description, George Baehr, published a series of 23 autopsied cases of the renal *wire loop* lesions of lupus nephritis and described the solar sensitivity we know so well. Thus, pathologists further elucidated the disease known as lupus. In 1936 Frieberg, Gross and Wallach autopsied a young woman with lupus and no skin lesions, indicating that the disease was not primarily a skin condition and was even less associated with tuberculosis. Klempner, Pollack, and Baehr in 1941 suggested that collagen was a part of the disease because of the many instances of fibrinoid necrosis that they found with the disease. This gave rise to the name *collagen disease* as a grouping for all of the diseases that affected connective tissue, a term that is not used widely today.

Hack and Reinhart were the first to describe the false-positive syphilis test in SLE, and in 1940 Keil similarly reported ten cases of SLE with false-positive syphilis tests. Haserick and Lang wrote about an additional series of cases where the presence of the false-positive syphilis serology predated the clinical lupus by up to eight years. In all of these cases, the false-positive syphilis tests probably resulted from the presence of antiphospholipid antibodies, the discovery of which was to take an additional 30 years. In 1955, Moore (8) studied another 148 patients who were positive for syphilis and found that some 7% developed lupus with time, whereas 30% had symptoms relegated to *collagen vascular disease.* In 1949, Phillip Hench (9) discovered cortisone, and the future of the connective tissue diseases changed. Rheumatoid arthritis patients and those with lupus erythematosus (LE) were manageable, and "cures" were reported.

In 1948 Hargraves, Richmond, and Morton described the LE cell in the bone marrow of SLE patients (10). The test was later adapted to peripheral blood. This discovery laid the foundation for our confirmation of the disease lupus as an autoim-

mune disease. These were phagocytes eating cells coated with autoantibody. Until it was described in other illnesses like rheumatoid arthritis (RA), it was thought to be pathognomonic of lupus. Dubois in 1953 and Harvey in 1954 (11, 12) sought to set the record straight by stressing the chronicity of SLE and stressed the diagnostic importance of the LE test. However, its presence in only 50% to 70% of lupus cases made it a useful adjunct to the diagnosis of SLE, but the discovery later of more sensitive and more specific tests made its use limited. Meanwhile, other serum abnormalities such as hypergammaglobulinemia, detected in newly discovered techniques like immunoelectrophoresis, suggested that gamma globulins were present in great numbers that behaved like antibodies that reacted with normal tissues. These were later called *autoantibodies.* George Friou in 1957 (13) applied the indirect immunofluorescent test of Coons to the study of these autoantibodies. The fluorescent antinuclear antibody test (FANA) is positive in some 95% to 98% of SLE cases. At about the time of the Friou discovery, Deicher, Holman, and Kunkel (14), along with three other groups, described antibodies to DNA. In 1956, Eng Tan and Henry Kunkel described an antibody to a glycoprotein called SM after the first two initials of the patient's name, Smith (15). This was a practice that would follow over many years after for several new antibodies. Although present in only 30% of patients with lupus, this antibody would be one of the few that is found with relative specificity to SLE.

Many more antibodies were described after the findings of the antiDNA and the antiSM. Among these were the discovery of Gordon Sharp of an overlap syndrome characterized by high titers of anti ribonucleoprotein antibody, a syndrome that we now call *mixed connective tissue disease (MCTD).* Moreover the discovery of countless autoantibodies to cellular components such as the signal proteins of the nucleus, high molecular group proteins, and others have in large part helped the foment revolution in molecular biology by providing reagents with which to study cell function.

Dixon and colleagues (16) assisted with the development of many murine strains that develop diseases like lupus and afford models for genetic study. Leonhardt in 1954 described the familial nature of lupus, something studied later by Shulman and Arnett at Hopkins (17) and more deeply by Hahn, Harley, and others in recent years (18). Such studies allowed for the description of large multiplex families, identical twins, and again the application of these studies to molecular biology. From this came our knowledge of the role of the MHC class II and III in lupus and other related illnesses, work which goes forward today.

Finally, the work of Hughes, Harris, Gharavi, Asherson, and Alarcón-Segovia (19) to name a few opened the door to our understanding of the antiphospholipid antibody as an important aspect of lupus. The frequency of this unusual syndrome, which can be secondary to SLE, is one of great curiosity and the subject of intensive research much as the early studies of autoantibody were. The role of these antibodies in lipid metabolism and biological processes such as atherosclerosis again places lupus in the knowledge "revolution" and makes this global scourge known as SLE relevant to the most common causes of morbidity and mortality, something that the early researchers found implicit in the urgency of their work.

This brief history of lupus does not give justice to the countless investigators who make history every day with small steps. In this book, you will find that many of the historical discoveries that provided data and added terms to our immunological lexicon are discussed within the cover of this book in great detail. Moreover, the academic progeny of those who I mention previously are those who write about lupus and this disease as it applies to modern medicine. I am among those progeny. We write with humility and eloquence, since our knowledge of lupus and all of medical science is very young and evolving for the good of all humanity.

Robert G. Lahita
Jersey City, N. J., 2003

REFERENCES

1. Blotzer, J. W. 1983. Systemic Lupus Erythematosus 1: Historical Aspects. *Marlyand State Med J* 32:439.
2. Talbot, J. H. 1974. Historical Background of discoid and systemic lupus erythematosus. In Lupus Erythematosus.

E.L. Dubois, editor. University of California Press, Los Angeles. 1.
3. Kaposi, M. K. 1872. Neue Beitrage zur Keantiss des lupus erythematosus. *Arch Dermatol Syphilol* 4:36.
4. Osler, W. 1904. On the visceral manifestations of the erythema group of skin diseases. *Am J Med Sci* 127:1.
5. Benedek, T. G. and Rodnan, G. P. 1983. Brief history of the rheumatic diseases. *Bull Rheum Dis* 32:59.
6. Reifenstein, E. C., Refifenstein, E. C. Jr., and Reifenstein, G. H. 1939. Variable symptom complex of undetermined etiology with fatal termination. *Arch Int Med* 63:553.
7. Libmann, E. and Sacks, B. 1924. A hitherto undescribed form of valvular and murla endocarditis. *Arch Int Med* 33:701.
8. Moore, J. E. and Lutz, W. B. 1955. The natural history of systemic lupus erythematosus: an approach to the study through chronic biological false positive reactions. *J Chron Dis* 2:297.
9. Hench, P. S. 1952. The reversibility of certain rheumatic and non-rheumatic conditions by the use of cortisone or of the pituitary adrenocorticotrophic hormone. *Ann Int Med* 36:1.
10. Hargraves, M. M., Richmond, H., and Morton, R. 1948. Presentation of two bone marrow elements: The tart cell and the LE cell. *Proc Staff Meet Mayo Clinic* 23:25.
11. Dubois, E. L. 1953. The effect of the LE cell test on the clinical picture of systemic lupus erythematosus. *Ann Int Med* 38:6.
12. Harvey, A. M., Shulman, L. E., and Tumulty, P. A. 1954. Systemic lupus erythematosus: review of the literature and clinical analysis of 138 cases. *Medicine* 33:291.
13. Friou, G. J. 1957. Clinical applicatoin of lupus serum nucleoprotein reaction using fluorescent antibody technique. *J. Clin Invest* 36:390.
14. Deicher, H. R., Holman, H. R., and Kunkel, H. G. 1959. The precipitin reaction between DNA and a serum factorin SLE. *J Exp. Med* 109:97.
15. Tan, E. M. and Kunkel, H. G. 1966. Characteristics of a soluble nuclear antigen precipitating with sera from patients with systemic lupus erythematosus. *J Immunol* 96:464.
16. Theofilopoulos, A. N. and Dixon, F. J. 1985. Murine models of systemic lupus erythematosus. *Adv. Immunol* 37:269–390.
17. Arnett, F. C. and Shulman, L. E. 1976. Studies in familial systemic lupus erythematosus. *Medicine* 55:313.
18. Stein, C. M., Olson, J. M., Gray-McGuire, C., Bruner, G. R., Harley, J. B., and Moser, K. L. 2002. Increased prevalence of renal disease in systemic lupus erythematosus families with affected male relatives. *Arthritis Rheum* 46:428–435.
19. Harris, E. N., Gharavi, A. E., Wasley, G. D., and Hughes, G. R. V. 1988. Use of an enzyme linked immunosorbent assay and of inhibition studies to distinguish between antibodies to cardiolipin from patients with syphilis or autoimmune disorders. *J Inf Dis* 157:23–31.

1

AUTOIMMUNITY AND AUTOIMMUNE DISEASES SUCH AS SYSTEMIC LUPUS ERYTHEMATOSUS

Howard Amital
Yehuda Shoenfeld

THE NATURE OF AUTOIMMUNITY

Autoimmune disorders affect approximately 5% of the population in the Western world and there are about 80 different autoimmune diseases [1]. Autoimmunity develops when an individual mounts an anti-self response. This reaction is signaled by the presence of self-reactive antibodies (e.g., humoral-mediated autoimmune diseases) or self-reactive T cells (e.g., cell-mediated autoimmune diseases). An autoimmune disease is a condition in which injury to the organs or tissues is caused by autoreactive antibodies or cells. According to these definitions, it is clear that these two entities usually coexist; however, a milieu of autoimmunity can exist without an overt autoimmune disease. Various clinical conditions of diverse etiologies may express autoimmune phenomena, e.g., malignant tumors, infections, and immune deficiencies (IgA or complement component deficiencies). Many are not accompanied with a substantial loss of organ function or body impairment, which can be classified as a sign or symptom. In all of these conditions one can find an increased incidence of autoantibody production with a great diversity of ligand-binding characteristics. Nevertheless, in most of these conditions it is not possible to link the affinity of the autoantibodies to the clinical expression of the patients [1–12]. This phenomenon is demonstrated most clearly in patients with monoclonal gammopathies, a group of diseases in which an uncontrolled proliferation of plasma cells takes place [9–13]. The outcome of the monoclonal proliferation of these cells is the production of an extremely high amount of immunoglobulins with a concomitant decline in the concentration of the other polyclonal immunoglobulins, usually only with minimal autoimmune phenomena, if at all. In a series of studies, approximately 8–10% of serum monoclonal immunoglobulins were found to be autoreactive with antibodies that bound to DNA, histones, Sm/RNP, Ro SSA, and La SSB [9–11]. By and large, only a minority of these patients develop signs that are related to the binding characteristics of the monoclonal immunoglobulins as a bleeding diathesis or as a peripheral neuropathy [12, 13]. Autoimmune features are associated with monoclonal gammopathies clarify several important principles.

1. The existence of an autoantibody is not sufficient in order to evoke an autoimmune disease. Even huge amounts of such an autoantibody may fail to induce an autoimmune disorder. For an autoimmune disease to be clinically apparent, additional genetic, hormonal, and environmental parameters are essential [14, 15]. Interestingly, Marmont [16] demonstrated four decades ago that the transfer of lupus LE cells to human recipients stimulated the generation of similar cells but no clinical signs or symptoms.
2. For decades it was accepted that the clonal deletion theory elucidated the maintenance of healthy

immune equilibrium, leaving a repertoire of T and B cells that recognize foreign antigens. Today we hold the view that a certain degree of autoreactivity is essential to the normal function of the immune system. Yoles *et al*. [17] inserted the term "protective autoimmunity" to the scientific vocabulary. They have shown that active or passive immunization with central nervous system (CNS) myelin-associated self-antigens reduced secondary neuronal loss. Autoantigens help form the repertoire of mature lymphocytes that secrete natural autoantibodies. These autoantibodies are generated in minute amounts, usually of the IgM isotype being polyspecfic in nature, with low avidity to autoantigens. Several physiological and regulatory roles have been attributed to these natural autoantibodies. Grabar [18] was the first to postulate that natural autoantibodies fulfill the role of biological scavengers binding to catabolic end products, cleansing the circulation from debris and other degraded tissue components. Concomitantly, these complexes facilitate opsonization and phagocytosis. Others stressed their role in achieving self-tolerance and balance of the idiotypic network [19–21]. Because autoreactivity is physiological, the challenge is to understand the mechanisms that divert normal function to pathological processes that lead to tissue injury.

What Leads from Autoimmunity to an Autoimmune State?

Many individuals remain in an "autoimmune state" throughout their entire lives without a subsequent "autoimmune disease." Others are destined to develop a full-blown disease. We believe that alteration of the delicate equilibrium that distinguishes physiological from harmful effects of autoantibodies may be caused by infections, sunshine, drugs, and smoking [14, 15, 22–25]. The concept of the "kaleidoscope of autoimmunity" describes this concept in a virtual manner. Any change in the immune system may induce remission or cure from one autoimmune disease but may set the foundation to the emergence of another [26–29].

Which Diseases Are Really Autoimmune Ones?

The list of diseases in which autoimmune pathogenesis is implicated has grown to embrace diverse disorders such as cataracts, peptic diathesis, and alopecia [30, 31]. Several indications even pointed that schizophrenia has an autoimmune "flavor" [32–34].

In most cases, there is no explicit evidence of autoimmunity except than the presence of an autoantibody or an autoreactive cell. By strict application of the criteria set out in Table 1, very few diseases would be regarded as truly autoimmune (e.g., myasthenia gravis, autoimmune thyroiditis, autoimmune hemolytic anemia, Lambert–Eaton's syndrome, idiopathic thrombocytopenic purpura, pemphigus vulgaris). In addition, autoimmunity can be transferred by T cells as in the case of experimental antiphospholipid syndrome. In this syndrome, the passive transfer of autoreactive T cells to naive mice induced the disease [35].

TABLE 1 Criteria for Autoimmune Diseases

A defined circulating antibody or cell-mediated immunity to autoantigens
The ability to generate the autoantibody or self-reacting cells following immunization with the self-antigen (with complete Freund's adjuvant)
The ability to produce the disease in an experimental animal by passive transfer of the antibody or the self-reacting cells
The ability to produce the disease in an experimental animal by immunization with the self-antigen (with complete Freund's adjuvant)
Definition of a specific autoantigen

Paradoxically, even systemic lupus erythematosus (SLE), the subject of this book, does not fulfill the strict criteria required for a disease to be classified as autoimmune (Table 1): passive transfer of various autoantibodies (e.g., anti-DNA, anti-Sm antibodies) was not followed by disease development [36]. Indeed, some antinucleic acid autoantibody specificities could be achieved by immunization of experimental animals with protein–nucleic acid complexes (nucleosomes). However, it is almost impossible to stimulate an immune response to intact native DNA, ribosomal RNA, or tRNA unless certain biochemical modulations are carried out [37–39]. However, despite these interesting attempts to generate immunogenic DNA, no one has been able to induce SLE following immunization of naive animals with either DNA or another autoantigen emulsified with adjuvant [40]. Furthermore, most passive transfer experiments employing either monoclonal or polycolnal anti-DNA antibodies failed to induce manifestations of SLE [41, 42]. According to the most recent data, it is inconceivable that anti-DNA antibodies are ascribed to all the actual organ damage of SLE. Anti-DNA antibodies probably cross-react with antiheparan sulfate, antiproteoglycans, or antilaminin antibodies, hence evoking renal and placental injury [43–45]. Nevertheless, SLE remains one of the classical autoimmune diseases due to several reasons.

- It is associated with the appearance of more than a hundred different autoantibodies (Table 2) [46, 47].
- It is associated with other parameters characteristic of the classical autoimmune conditions, e.g., human lymphocyte antigen (HLA), sex hormone influence, Gm allotypes, T-cell defects in apoptosis and

TABLE 2 Autoantibodies in SLE[a]

No.	Target of autoantibody
1	Antinuclear antibodies (ANA)
2	Nucleosomes
3	Deoxyribonucleic acid (DNA) Double-stranded (native) DNA (dsDNA) Single-stranded (denatured) DNA (ssDNA) Z-DNA
4	Telomeres
5	Nucleosides, nucleotides
6	Histones (H) (H2A-H2B)–DNA complex H1, H2A, H2B, H3, H4
7	DNA-dependent ATPase
8	DNA polymerase α
9	Replication protein A (RPA) RPA-70 RPA-32
10	Ku
11	Poly(ADP-ribose) polymerase (PARP)
12	Poly(ADP-ribose)
13	Topoisomerase-I (Scl-70)
14	High-mobility group protein HMG17
15	DEK oncoprotein
16	Centromere proteins CENP-A CENP-B
17	Antisense to the ERCC-1 DNA excision repair enzyme gene (ASE-1)
18	Proliferating cell nuclear antigen (PCNA)
19	RNA polymerase (RNAP) I, II, III
20	Transcription factor TFIIF (Rap74 subunit)
21	Transcription factor TFIIB
22	Protein kinase NII
23	Ro (SS-A)
24	La (SS-B)
25	Nuclear poly(A) polymerase (polynucleotide adenyltransferase)
26	Nucleolar RNA helicase (Gu) protein
27	SR proteins SRp20, SRp30a, SRp30b, SRp40, SRp55, SRp75
28	Alu RNA–protein complex
29	Small nuclear ribonucleo-protein particles (snRNP) spliceosomal Sm-B/B′, D1, D2, D3 proteins and E-F-G protein complex U1 snRNP-70K, A, C polypeptides U2 snRNP-A′, B″ polypeptides nonspliceosomal P ribonuclease (RNase P)
30	Heterogeneous nuclear ribonucleoprotein particles (hnRNP) Protein A1 Protein A2 (RA33)
31	Fibrillarin
32	Nucleolin
33	B23/nucleophosmin
34	90-kDa protein of the nucleolus organizer region/human upstream-binding factor (NOR-90/hUBF)
35	Interferon-inducible protein IFI 16
36	Mitotic spindle apparatus Type 1 nuclear mitotic apparatus protein (NuMA-1, MA-I) Spindle kinesin-like protein HsEg5 (NuMA-2, MA-II)
37	Ki-67
38	p53
39	c-myc oncogene product
40	Proteins phosphorylated during apoptosis
41	Ki [probably identical to sicca lupus (SL) system]
42	Nuclear lamins B-type lamins B1 and B2
43	Su
44	Prothymosin α
45	DA1, DA2
46	MA
47	Me
48	Outer ring subunit HC9 (α3) of 20S proteasome
49	Ribosomal P proteins (P0, P1, P2)
50	Ribosomal protein S10
51	Ribosomal protein L12
52	28S ribosomal RNA (rRNA)
53	JA
54	Eukaryotic protein L7
55	Golgi apparatus proteins Golgin-95/gm 130, golgin-160, golgin-67
56	Transfer RNA (tRNA)
57	Cytoskeletal antibodies Microfilaments Actin Fimbrin T- and L-isoforms (T-f, L-f) α-Fodrin (α-f) 135-KDa antigen microtubuli Tubulin Intermediate filaments Vimentin Neurofilaments (NF) Cytokeratins (CK)
58	Calpastatin
59	Follistatin-related protein

(*continues*)

TABLE 2 (*continued*)

No.	Target of autoantibody
60	Heat shock protein 90 (HSP 90)
61	FK506-binding protein 12
62	Carbonic anhydrase (CA) I,II
63	Pyruvate dehydrogenase (mitochondrial antibodies of M2 type)
64	Profilaggrin (perinuclear factor)
65	CD45
66	CD4
67	FcγR FcγRI (CD 64) FcγRII (CD 32) FcγRIII (CD16)
68	Insulin receptor
69	Gangliosides (Gang) GM_1, GD_{1a}, GD_{1b}, GT_{1b} Galactocerebrosides (Gal)
70	Cell membrane-associated DNA (cmDNA)
71	Cell membrane DNA-binding protein
72	Lipocortin-1
73	β_2-Microglobulin
74	Lupus anticoagulant (LAC)
75	Anionic phospholipids Cardiolipin (CL)
76	Zwitterionic phospholipids Phosphatidylethanol-amine (PE) Phosphatidylcholine (PC) (including bromelain-treated erythrocytes) Platelet-activating factor (PAF)
77	β2-Glycoprotein I (β2GPI)
78	Prothrombin (PT, factor II)
79	Annexins (Anx) Anx IV Anx V (placental anticoagulant protein I) Anx XI [calcyclin-associated protein (CAP-50)]
80	Protein S
81	Protein C
82	Thrombomodulin (TM)
83	Factor XII
84	Tissue type plasminogen activator (t-PA), bound to fibrin
85	High and low molecular weight kininogens (HMWK, LMWK) Kininogen-binding proteins: factor XI and prekallikrein
86	Thromboplastin (tissue factor)
87	Vascular heparan sulfate proteoglycan
88	Oxidized low-density lipoprotein (ox-LDL)
89	Malonaldehyde (MDA)-modified lipoprotein (a) (MDA-Lp[a])
90	Antimitochondrial antibodies of M5 type (AMA5)
91	Lysophosphatidylcholine (LPC)
92	Platelets Glycoprotein (GP) IIb-IIIa GPIb-IX CD36 (GPIV)
93	Antineutrophil cytoplasmic antibodies (ANCA) with cytoplasmic staining pattern Proteinase 3 (PR3) ANCA with perinuclear staining pattern (pANCA) Myeloperoxidase (MPO) ANCA with specificity other than MPO and PR3 Lactoferrin (LF) Cathepsin G (CTG) High mobility group proteins HMG1, HMG2 Lysozyme (LZ) Elastase (EL) α-Enolase (EN)
94	Endothelial cells (EC) candidate antigens: Ribosomal P protein P0 Endothelial cell-specific plasminogen activator inhibitor (EPAI) Ribosomal protein L6 (L6) Elongation factor 1α (E1α) Adenyl cyclase-associated protein (CAP 1) DNA replication licensing factor (LF) Profilin II Tubulin Vimentin Human endothelial-associated putative lupus antigens HEAPLA 1, HEAPLA 2
95	Nervous system (neuronal antibodies) Gangliosides Galactocerebrosides 50-kDa antigen 97-kDa antigen Ribosomal P protein Neurofilaments
96	Lymphocytes T cells, B cells CD45 CD4 T-cell receptor (TCR) Major histocompatibility complex (MHC) class I and II antigens Ribosomal antigens including P protein
97	Red blood cells (RBC) "Warm" autoantibodies (WA) Rh family antigens p34 and gp37-55 Glycophorin A Band 3 anion transporter Other blood group system antigens Wr^b, LW, U, K, Kp^b, K13, Fy "Cold" autoantibodies (CA)
98	Collagen-like region of C1q

(*continues*)

TABLE 2 (*continued*)

No.	Target of autoantibody
99	C1 inhibitor
100	Erythropoietin (EPO)
101	Idiotypes (Id) Anti-DNA (16/6 Id)
102	Anti-idiotypic antibodies
103	Calreticulin
104	Prolactin
105	Rheumatoid factor (RF)
106	Von Willebrand factor
107	Factor VIII
108	Apolipoprotein A1
109	Acute-phase proteins C-reactive protein (CRP) Ceruloplasmin (CP) α_1-Antitrypsin (α_1AT)
110	Collagen (C) types I, II, III, IV, V, and VI
111	Fibronectin
112	PL 4
113	Corpus luteum
114	Retroviruses (RV) Endogeneous (ERV) Human T lymphotropic virus type 1 (HTLV-1)-related endogenous sequence (HRES-1) ERV-9 envelope C-terminal surface glycoprotein (ECTSU) Human ERV (HERV)-H- derived human transmembrane sequence clones (HUTM) 1-1 peptide Human immunodeficiency virus (HIV)-1 Glycoprotein gp24 gp41 gp120
115	Citrullinated peptide
116	Poly(amino acids) Polyhistidine Polyproline
117	Nerve growth factor
118	Entactin (nidofen)

[a] SLE, systemic lupus erythematosus; SS, Sjogren's syndrome; SSc, systemic sclerosis; RA, rheumatoid arthritis; MCTD, mixed connective tissue disease; UCTD, undetermined connective tissue disease; PMR, polymyalgia rheumatica; APS, antiphospholipid syndrome; PM, polymyositis; ANA, antinuclear antibodies; aCL, anticardiolipin; LAC, lupus anticoagulant; CNS, central nervous system; CSF, cerebrospinal fluid.

cytokine production, and association with IgA and complement component deficiency [14, 15]. Therefore, we have to accept the definition of several clinical conditions as autoimmune because they are associated with those typical characteristics of SLE, although they do not fulfill the criteria listed in Table 1.

Classification of Autoimmune Diseases

For practical reasons, autoimmune diseases can be classified according to how the damage is induced: humoral-mediated autoimmune diseases (e.g., myasthenia gravis and immune thrombocytopenic purpura) and cell-mediated autoimmune diseases (e.g., insulin-dependent diabetes mellitus, thyroiditis, and rheumatoid arthritis). The division between the two is not distinct, and evidence for the involvement of T cells in induction of the damage in antibody-mediated disease and *vice versa* has been documented. Another classification is based on the number of organs afflicted. Thus, autoimmune diseases may be categorized according to organ-specific diseases and multisystemic diseases (Table 3).

Autoimmune diseases have also been sorted according to the type of the immunodysregulation that evokes the disorder. Diseases that originate from a reactive immune system, mainly composed of an excess of T-cell

TABLE 3 Classification of Autoimmune Diseases

Disease	Target organ/tissue
Multisystemic	
SLE	Skin, joints, kidneys, brain, heart, lungs
Rheumatoid arthritis	Joints, lungs, skin, pericard
Sjogren's syndrome	Exocrine glands (particularly lacrimal and parotid)
Diffuse systemic sclerosis	Skin, joints, kidneys, gastrointestinal tract, lungs
Goodpasture's syndrome	Kidneys, lung
Organ specific	
Graves' disease	Thyroid
Hashimoto's disease	Thyroid
Myasthenia gravis	Muscles
Polymyositis	Muscles
Pernicious anemia	Stomach
Addison's disease	Adrenal
Insulin-dependent diabetes	Pancreas
Primary biliary cirrhosis	Liver
Autoimmune hemolytic anemia	Red blood cells
Idiopathic thrombocytopenic purpura	Platelets
Pemphigus	Skin, mucosal membranes

TABLE 4 Primary versus Secondary Autoimmune Diseases (AID)

Primary AID	Secondary AID
Cause unknown	Cause known or suspected
Autoantigens normal	Autoantigens altered or mimicry with exogenous antigens or leakage of sequestered autoantigens
Excess of T-cell help	Immune system normal
Permanent disease	Transient autoimmune phenomena
Can be treated	Curable autoimmune phenomena
Mainly women	Both sexes
Rarely in children	All ages
Clinical overlap with other primary AID	No clinical overlap with other AID
Serological overlap with other primary AID	No serological overlap with other AID
Familial predisposition	Rarely familial predisposition

activity, are often termed "primary autoimmune diseases." They should be distinguished from autoimmune diseases that develop in a completely normal immune system, often called "secondary autoimmune diseases." These latter diseases are probably triggered by certain autoantigens that were altered, by certain exogenous antigens that resemble autologous antigens leading to cross-reactions, or by certain previously well-sequestered autoantigens that broke out of the tolerance. Table 4 gives differences between primary and secondary autoimmune diseases [48].

The Mosaic of Autoimmunity or Why Are Autoimmune Diseases so Diversified?

The etiology of autoimmune diseases is multifactorial [49, 50] (Table 5). It is interesting to note that in almost all autoimmune conditions there are familial tendencies and an increased incidence of autoantibodies among healthy first-degree relatives of affected patients [49]. This trend is very familiar with diabetes type 1 patients [51]. Most autoimmune diseases are multigenic, with multiple susceptibility genes working in concert ending with an abnormal manifestation or disorder [52]. In many of the different disorders an increased incidence of B8 or DR2 and DR3 or DR4 is found [49]. Some HLA alleles protect against the development of autoimmunity even when a susceptibility allele is present. For example, the HLA-DQB1*0602 allele protects against type 1 diabetes even if the HLA-DQB1*0301 or DQB1*0302 susceptibility gene is present [53]. There is hardly an autoimmune disease in

TABLE 5 Major Pieces of the Mosaic of Autoimmunity[a]

Genetic
- Increased incidence of the disease in families
- Increased incidence of autoantibodies in first-degree relatives of the patients
- Increased incidence of the disease in monozygotic twins
- HLA studies: increased incidence of HLA-B8, -DR2, -DR3, and -DR4 in some diseases. A protective effect (e.g., HLA-DR2 in type 1 diabetes noted in others or aspartic acid in position 57 in HLA-DQ3β)
- Increased incidence of common idiotype of autoantibodies in patients and first-degree relatives
- Gm allotypes
- Complement component deficiencies

Defects in immune system
- IgA deficiency
- Complement component deficiencies (e.g., C1q, C2, and C4)
- Qualitative and quantitative defects in suppressors
- Defects in natural killer cells
- Defects in secretion, response to, and receptors for IL-2 and other lymphokines (including interferons and tumor necrosis factor)
- Defects in phagocytosis
- AIDS

Hormonal
- Increased prevalence of disease among females
- Increased prevalence of disease in Klinefelter's syndrome
- Increased prevalence of autoantibodies among females
- Exacerbation of diseases during puberty, pregnancy, and postpartum periods and while taking contraceptives
- Association between hyperprolactinemia and autoimmunity
- The protective effects of testosterone-like drugs

Environmental
- Infecting agents: viruses, bacteria, parasites
- Drugs (e.g., in idiopathic thrombocytopenic purpura, myasthenia gravis, SLE)
- Toxins (e.g., scleroderma)
- Ultraviolet light (e.g., SLE)
- Smoking (Goodpasture's syndrome)
- Stress (e.g., SLE)
- Nutritional influence (e.g., rheumatoid arthritis)

[a] Based on Shoenfeld and Isenberg [49].

which a defect in T suppressor cells has not been reported with an associated natural killer cell function impairment [54]. Many of these diseases have been reported in conjunction with an interleukin-2 deficiency [55] or IgA deficiency [56]. Complete deficiencies of the early components C1q, C2, and C4 in the classical pathway of complement are associated with the most striking risk to the development of SLE. Deficiency of C4A is probably the most common inherited complement deficiency, occurring at varying frequency in different population groups [57]. Hereditary homozygous deficiencies of C1q, C1r and C1s, and C4 are each strongly associated with susceptibility to SLE, with respective prevalences of 93%, 57% (because deficiencies of C1r and C1s are usually inherited together), and

75%. The hereditary C1q deficiency therefore represents the most powerful susceptibility gene identified for the development of SLE in humans. In some populations, 50–80% of SLE patients have the C4A deficiency. C2 deficiency is associated with SLE at a lower frequency and with a mild clinical presentation than other complement deficiencies [58, 59] (discusses further in Chapters 5 and 6).

The high concentration of immune complexes detected in the blood of SLE has raised the suspicion that Fcγ receptor polymorphism might be associated with SLE. Fcγ receptors are active natural immune complex scavengers; therefore, low-affinity-binding characteristics of these molecules to circulating immune complexes might result in excessive deposition of such complexes in various organs. Low-affinity variants of both Fcγ IIA and IIIA have been shown to be associated with lupus nephritis [57, 60]. The mannose-binding protein is a serum acute-phase reactant that activates the classical and alternative pathways of the complement independently of antibody. An allele of this protein shown to be incapable of activating the classical pathway of the complement was found to have a moderate association to SLE [60].

External modulations of the immune system by drugs or by allogenic bone marrow transplantation (causing a graft-versus-host response) evoke autoimmune phenomena [58, 61, 62]. D-Penicillamine is eminent among drugs known to induce autoimmune disease: this compound was used to treat patients with severe rheumatoid arthritis, diffuse systemic sclerosis, and primary biliary cirrhosis but was reported to induce autoimmune conditions as SLE, rheumatoid arthritis, pemphigus, myasthenia gravis, polymyositis, thrombocytopenia, systemic sclerosis, and Goodpasture's syndrome. Similarly, the induction of graft-versus-host disease in animal models and even in humans is associated with various autoimmune conditions, such as SLE, systemic sclerosis, and polymyositis. In graft-versus-host disease, T cells of the donor collaborate with recipient B cells to secrete autoantibodies [49, 62].

Thus, it seems that the different clinical presentations of autoimmune conditions stem from various combinations of factors. In the propositus, a particular setup will evoke a certain manifestation, whereas in another member of even the same family, a different constellation will be associated with an involvement of completely different organs. This phenomenon has been referred to as the *mosaic of autoimmunity* [25, 49, 61] using the definition of mosaic in the shorter "*Oxford English Dictionary:*" "the process of producing pictures or patterns by cementing together small pieces of stone, glass, etc." This definition implies that by reassembling the same pieces in a different order, another pattern or picture will emerge. We share this concept in a similar manner concerning diseases of the immune system; hence by rearranging much of the same parameters, diverse patterns of autoimmune diseases become evident. The integration of many factors into the etiology of one autoimmune disease and the association of the same defects with different diseases may explain some clinical phenomena.

TABLE 6 Association between Autoimmune Diseases[a]

First disease	Associated disease
Multisystemic	
Systemic lupus erythematosus (SLE)	SSc, mixed connective tissue disease (MCTD), SS, HT, MG, PM
Rheumatoid arthritis (RA)	SS, HT
Systemic sclerosis (SSc)	SLE, RA, SS, PM, PBC
Sjogren's syndrome (SS)	SLE, RA, SSc, MCTD
Organ specific	
Immune thrombocytopenic purpura	SLE, CAH
Myasthenia gravis	SLE, SS, HT
Hashimoto's thyroiditis	SLE, SS
Pernicious anemia	Graves' disease, HT, Addison's disease
Chronic autoimmune hepatitis	SS
Insulin-dependent diabetes mellitus	Thyroiditis and pernicious anemia
Primary biliary cirrhosis	SLE, RA, Ssc, SS, HT

[a] The first disease preceded or occurred concomitantly with the associated disease.

- The most prevalent autoimmune disorders are multisystemic.
- There are overlapping syndromes.
- Many organ-specific autoimmune diseases are associated in the same patient with other autoimmune diseases or with other conditions that affect the immune system as malignancies or primary and secondary immunedeficiency syndromes [6, 7, 28, 29, 58–64].

These associations are summarized in Tables 6 and 7. Remarkably, these phenomena go beyond the individual patient. Family members of patients suffering from autoimmune diseases have increased levels of autoantibodies [65, 66] and of similar or other autoimmune conditions [48, 51–53].

The Kaleidoscope of the Autoimmune Mosaic

The origin of autoimmunity is complex and stands probably above all the assumptions and speculations that are prevailing today. Several reports have raised the

TABLE 7 Association between Autoimmune and Malignant Diseases

Autoimmune disease	Malignant disease
Systemic lupus erythematosus	Few cases of lymphomas, thymoma, multiple myeloma
Rheumatoid arthritis	Hodgkin and non-Hodgkin lymphoma, multiple myeloma, leukemia
Systemic sclerosis	Lung cancer (especially the bronchoalvaeolar type), breast cancer
Sjogren's syndrome	Lymphoma, Waldenström's macroglubulinemia, pseudolymphoma
Dermatomyositis, polymyositis	Malignancies of breast, lung, ovary, stomach, uterine, colon, Hodgkin lymphoma
Chronic lymphocytic thyroiditis	Thyroid lymphomas, extrathyroid lymphoma, myeloproliferative diseases
Vasculitis	Hairy cell leukemia, lung cancer (mainly nonsmall cell)
Autoimmune hemolytic anemia and thrombocytopenia	Chronic lympocytic leukemaia, B-cell lymphoma
POEMS syndrome (polyneuropathy, hepatosplenomegaly, endocrinopathy, M protein and skin changes)	IgA plasmacytoma
Hypertrophic osteoarthropathy	Nonsmall cell lung cancer, metastasis to lungs
Sweet's syndrome (fever, neutrophilia, tender erythematosus cutaneous plaques of the arms, neck, and head. Pulmonary, hepatic and musculoskeletal involvement may be seen)	Acute myeloblastic leukemia
Lambert–Eaton myasthenic syndrome	Small cell carcinoma of lung
Stiff man syndrome	Small cell carcinoma of lung, breast cancer, thymoma
Peripheral neuropathy	Waldenström's macroglobulinemia

attention to another aspect, namely that amelioration of one disease "opens the door" for another autoimmune disorder. Levine *et al*. [64] described two patients with immune thrombocytopenic purpura who, following a successful splenectomy, developed chronic active hepatitis, probably of an autoimmune origin. From an etiological viewpoint, one can interpret these events as follows: factors associated with autoimmunity were deranged and in a combination that "favored" the development of immune thrombocytopenic purpura. Subsequently, one or more of the surrounding conditions were altered (in these patients the spleen was removed), bringing about a rearrangement to the antecedent immune equilibrium, leading to the emergence of a different autoimmune condition this time, autoimmune hepatitis. There is an additional report of a patient with immune thrombocytopenic purpura who developed chronic active hepatitis after a splenectomy [67]. Other occurrences reported are of patients with myasthenia gravis that developed aggressive SLE and antiphospholipid syndrome (APS) after thymectomy [28, 68–70]. Gerli *et al*. [68] were uncertain what had more impact on the development of SLE in these patients: the history of myasthenia gravis or the immunomodulation that was caused by the thymectomy. Rodrigue *et al*. [71] described six patients in whom SLE and thyrotoxicosis coexisted. In three of them, hyperthyroidism preceded the SLE, but the cause for the change remained obscure. Interestingly, Krause *et al*. [72] were able to block the development of diabetes in nonobese diabetes (NOD) mice (a model that develops insulin-dependent diabetes mellitus spontaneously) by inducing a lupus-like syndrome by their immunization with an anti-DNA idiotype. The mice developed clinical features of SLE instead of the classical clinical and pathological presentation of diabetes. It seems that the clinical shift in this animal model resulted from a change in the immune milieu after this idiotypic manipulation.

We refer to such a switch from one abnormal immune balance to another as the ***kaleidoscope of the autoimmune mosaic*** [73]. We prefer to use the term kaleidoscope because it gives emphasis to the notion that new emerging autoimmune conditions can not be attributed to a novel exposure to a presumed autoantigen, whereas shuffling of the perturbed self and environment results in a new autoimmune disease.

Why does the kaleidoscope phenomena occur? As suggested earlier, it is probably due to an additional change in one or a few of the factors that compose the autoimmune mosaic. For instance, the spleen is a major regulator of immunocytes, and its removal in predisposed patients undoubtedly modifies the immune function. Similarly, following thymectomy, the loss of thymic T-cell regulation contributes to the subsequent occurrence of SLE. In a similar manner, in patients in whom a certain disease was treated by certain drugs or by immunomodulation, an autoimmune disorder might develop consequently (Table 8). Many other environmental factors known to affect the immune system may play a role in the kaleidoscope phenomenon, such as

TABLE 8 Association between Autoimmune Diseases and Drugs

Disease	Drugs
Drug-induced lupus	Hydralazine, isoniazide, procainamide, captopril, acebutalol, chlorpromazine, labetolol, methyldopa, carbamezapine, phenytoin, lithium, D-penicillamine, propylthiouracyl
Drug-induced myasthenia gravis	D-Penicillamine, trimethadione, long-term therapy with diphenylhydantoin, "unmasking" by therpay with quinidine, propanolol, and lithium
Drug-induced myositis	Amiodorone, clofibrate, enalapril, colchicine, cyclosporine, chloroquine, ε-aminocaproic acid, glucocoticosreoids, vincristine, amphotericin B, salbutamol, D-penicillamine, penicillin, hydralazine, phenytoin, procainamide, propylthiouracyl, lovastatin
Drug-induced hemolytic anemia	Methyldopa, quinidine, quinine, sulfonylurea, phenacetin
Drug-induced thrombocytopenia	Quinidine, quinine, gold salts, D-penicillamine, heparin, digitoxin, methyldopa, carbamezapine
Drug-induced pemphigus	D-Penicillamine, captopril
Drug-induced systemic sclerosis	D-Penicillamine, bleomycin, carbodopa, pentazocine, ergotamines, mazindol, amphetamines

infections and nutrition. For instance, James *et al*. [74] showed that exposure to the Epstein–Barr virus (EBV) is highly associated with the development of SLE. Of the 196 lupus patients they tested, all but 1 had been exposed to EBV, while 22 of the 392 controls did not have antibodies consistent with previous EBV exposure, implying that this viral infection might tilt the kaleidoscope to be more autoimmune.

A certain degree of heresy is needed to accept the inevitable fact that autoantigens are not essential for the induction of the autoimmune process. It is difficult to depart from the role that anti-DNA antibodies have as a tragic hero playing in this scene called SLE and to refer to them as spectators accompanying the performance. These data led us to the comprehension that the classical anti-DNA reaction in SLE is due merely to the fortuitous selection of DNA from a *"shelf"* rather than plainly indicating DNA as a true *self-target antigen*.

Is it possible that there is no true autoantigen in SLE? We probably have to acknowledge the distinction between classical, organ-specific autoimmune disorders (Graves' disease, Hashimoto's disease, myasthenia gravis, pernicious anemia, insulin dependent diabetes, etc.) and multisystemic autoimmune diseases. However, these distinctions do not shed light on the autoimmune kaleidoscope puzzle.

Why Do Patients Differ from Each Other?

It remains ambiguous why two patients with SLE with the same antidouble-stranded DNA (anti-dsDNA) may tremendously differ from each other. In one patient, convulsions may be the first expression of CNS affliction, which may develop into chronic renal failure, whereas another patient may experience a mild butterfly rash with an increased erythrocyte sedimentation rate with slight arthralgia. We would like to offer an additional mechanism to resolve this enigma: the pathogenic potential of different idiotypes. Thus, although two patients may both have anti-dsDNA autoantibodies, one idiotype may induce CNS damage and renal failure and the other may be deposited in the skin or joints [75, 76].

One of the possible solutions to this puzzle may rise from various autoimmune models. It has been shown that immunization of animals with a given antigen can lead to spreading or the antibody response to include not only the targeted antigen but also related epitopes. This "epitope spread" was noticed in various animal autoimmune models, such as experimental allergic encephalomyclitis in which the immunization of mice with the myelin basic protein peptide 1-11 led to the breakdown of T-cell tolerance to three other endogenous peptides [77]. Similarly, immunization of healthy mice with individual protein components of the La/Ro ribonucleoprotein (RNP) targeted in primary Sjogren's syndrome and in SLE induced autoantibodies recognizing Ro60 (SSA), Ro52 (SS-A), and La (SS-B) and, in some cases, the molecular chaperones calreticulin and Grp78 [78]. The endogenous antigen driving determinant spreading might be derived from physiological apoptosis, which could explain the involvement of some chaperone proteins in the autoimmune response. In has been shown that active immunization of mice with a given autoantibody may lead to the production of murine self-autoantibodies and certain autoimmune disease expression. The concept, initially proposed by Jerne [79], suggests that the immune response may be governed by unique antigenic determinants of the immunoglobulin variable regions (idiotypes). These idiotypes are regulated by other antibodies, comprising a dynamic and balanced idiotypic network with intrinsic regulatory properties. Interestingly, when immunizing mice with a given antibody, the generation of murine self-antibodies is often accompanied by the production of apparently unrelated autoantibodies. For instance,

immunization with the anti-DNA antibody 16/6 idiotype leads to the production of antibodies to Ro (SSA) and histones. Additionally, immunization with the anti-Sm idiotype led to the production of anti-dsDNA and ssDNA, as well as anti-RNP antibodies [75, 80, 81]. Thus, immunization with a given antibody can drive spreading of the autoantibody response toward associated molecules as well as to apparently unrelated antigens. Therefore, *autoantibody spreading* may be one of the mechanisms by which heterogeneous autoantibodies emerge in autoimmune conditions. Fluctuating levels of autoantibodies may be one of the mechanisms that determine the clinical presentation of a patient at a certain time.

Physiologic and Pathologic Autoimmunity

Autoimmune diseases are conditions in which the immune system damages normal components of the individual. Initially, it was thought that autoimmune diseases were an inevitable outcome of the presence of clones of lymphocytes with receptors that recognize self-antigens. Thus, tolerance to self, or nonautoimmunity, was due to the absence of autoreactivity, the "forbidden" culprit of autoimmune diseases. As a bacteriologist, Burnet knew that lymphocytes proliferate in response to antigenic stimulation as microbial infections, thus somatic mutations occur in lymphocytes just as they do in bacterial populations. According to his hypothesis, antibodies and lymphocytes may have a myriad of affinities to innumerable antigens. However, because of the random element in the somatic mutation, sometimes clones with autoreactive traits will arise. Burnet referred to such clones as "forbidden clones." In order to be born free of autoimmune diseases, the fetus has to destroy or persistently suppress any expression of clones reactive with host antigens [82]. In "a normal" individual, self-reactive clones are automatically eliminated following their appearance. Furthermore, any case of autoimmunity could be attributed to the failure of suppression or exclusion of a particular autoreactive lymphocytic clone. Proliferation of such a clone with time entails danger due to the emergence of an autoimmune condition.

In contrast to the "Burnetian" theory, it is now known that lymphocytes are produced throughout life, namely their clonal segregation is not only confined to developmental stages of a fetus surrounded by self-antigens. This fact introduces an entirely new concept that tolerance is acquired along with the development of the organism. We believe that it is evident that self-recognition is vital for the maintenance of normal physiology. Grabar [18] argued that the presence of a large variety of autoantibodies in the blood may be required for scavenging effete molecules and cells. Furthermore, autoreactivity is essential for the sustenance of immune tolerance. Thus, cells that "protect" autologous tissues from immune attack must be per definition. This last statement does not negate the concept of clonal deletion, but it does raise the necessity of certain clones to escape it [69].

It has been suggested that a certain degree of autoreactivity is essential to the maintenance of normal immune behavior. Self-recognition has a seminal role in the detection of major histocompatibility complex antigens on most of the cell membranes and, as mentioned earlier, self-recognition is the cornerstone of the binding properties within the idiotypic network. It has been suggested that autoimmune conditions result from many foci of prematurely aging cells. Those inherently damaged cells adapt to *in vivo* challenges by beginning to transform into cancer cells. However, as long as those stressed cells have not fully transformed, they will continue to signal "danger" to the innate immune system. The clinical outcome of that struggle between incipient neoplasia and immunity will vary depending on the degree of tumor proneness and resistance of the individual. This hypothesis is in concordance with the frequent association between neoplasia and autoimmunity [83, 84].

MECHANISMS OF AUTOIMMUNITY

Breaking Peripheral Tolerance

As a consequence of constant reshuffling of the genes that encode T- and B-cell receptors, theoretically more than 10^9 different antigen-binding sites can be generated, some of them with affinities to autoantigens. Tolerance is the process that eliminates or neutralizes autoreactive cells. Whenever a breakdown in the process occurs, autoimmunity might evolve. Distinguishing self from nonself is fundamental to the normal function of the immune system. For T cells, this discrimination is a dangerous balancing act, swaying between extremes of nonresponsiveness and autoimmunity. Several mechanisms are involved in order to preserve this equilibrium. Clonal deletion of immature B cells takes place in the bone marrow, whereas the deletion of autoreactive B cells occurs in the T-cell zones of the spleen and lymph nodes [85, 86]. Tolerance of B cells can be obtained *via* the lack of T-cell assistance and by "receptor editing," a mechanism that changes the specificity of the B-cell receptor when an autoantigen is encountered [87]. However, the relative importance of these mechanisms to the prevention of autoimmunity remains to be determined [88].

The main mechanism of T-cell tolerance is the deletion of self-reactive T cells in the thymus. Immature T

cells migrate from the bone marrow to the thymus, where they bind to peptides derived from autoantigens bound to MHC molecules. Only T-cell clones with an intermediate affinity for such complexes avoid apoptosis. The induction of central tolerance requires the presence of autoantigens in the thymus [89]. Because not all self-antigens exist in the thymus, peripheral mechanisms complete other aspects of peripheral T-cell tolerance by inhibitory mechanisms or deletion of autoreactive clones (*via* the Fas receptor) [90]. The importance of the thymus to the acquisition of a stable degree of tolerance was demonstrated by Saoudi *et al*. [91], who showed that thymectomy or split-dose irradiation to the thymus of adult mice induced latent insulin-dependent diabetes mellitus (IDDM) in a normally resistant strain of mice. Moreover, administration of $CD4^+CD8^-$ thymocytes prevented the development of IDDM in thymectomized and irradiated recipients, indicating that the thymus generates a T-cell repertoire that is highly effective in preventing tissue-specific autoimmune diseases. This process occurs in humans with multisystemic diseases as we learned from patients with myasthenia gravis who underwent thymectomy and developed aggressive SLE afterward [69–71]. Nevertheless, a normal thymus does not render us "autoimmune proof." In the thymus, complexes of self-peptides and self-MHC molecules delete one subset of autoreactive clones and select another subset of T cells. The second group leaves the thymus, enters the circulation, and can encounter complexes of MHC molecules with peptides derived from self-proteins not found in the thymus. T cells that react with such complexes are not destroyed but are tolerated by inactivation (anergy) or suppression by other cells [92].

One idea is that this state of peripheral tolerance is broken because of changes in immune response to environmental antigens and microorganisms. Structural similarity between microbial and self-antigens ("molecular mimicry") probably plays a key role in activating autoreactive T cells [90]. Considerable evidence ties infections with autoimmunity as in multiple sclerosis, catastrophic antiphospholipid syndrome, type 1 diabetes mellitus, and many other disorders [2, 23, 93, 94]. Some T cells recognize both microbial peptides and self-peptides with similar amino acid sequences. Additionally, the release of sequestered autoantigens following tissue damage and the activation of a large fraction of the T-cell population by superantigens consequently induce the secretion of inflammatory cytokines and costimulatory molecules, enhancing the autoimmune reaction [95–98]. Interestingly, infections are crucial for the development of protective T-cell clones, and depriving the immune system from combating infectious agents has also been associated with a higher frequency of autoimmune phenomena. Sasazuki *et al*. [99] reported that diabetes in NOD mice was increased by keeping the animals pathogen free and was decreased by a variety of treatments that activate T-cell responses. A similar finding was also reported in humans, multiple infections during the first year of life are associated with a significant reduction in the risk of autoimmune diabetes [100].

While class I molecules are expressed on all nucleated cells, the expression of MHC class II molecules is normally restricted to cells of the immune system. Not only the genetic composition of the MHC class II, but also changes in the amount of surface molecules determine the type and magnitude of antigen presentation, and hence the consequences of the immune response [101]. Therefore, their expression is tightly regulated. The appearance of such antigens can be induced *in vitro* mainly by interferon-γ (IFN-γ), and synergistically with tumor necrosis factor (TNF)-α, on a wide variety of nonimmunological cells, such as astrocytes, keratinocytes, melanocyte, and many other cell types. Expression of MHC class II molecules can be induced on tissue cells, including thyrocytes, during viral infection, independent of cytokine effects [102]. *In vivo* aberrant expression of MHC class II molecules has been observed on autoimmune and malignant tissue cells, including autoimmune and neoplastic thyrocytes [101].

Reovirus and cytomegalovirus have been demonstrated to induce MHC class II expression on cultured thyrocytes in the absence of T cells [103, 104]. A direct correlation between the high expression of MHC class II and the degree of the inflammatory response has been observed in autoimmune thyroiditis [105].

Population studies have demonstrated that Graves' and Hashimoto thyroiditis, as well as type 1 diabetes mellitus and many other autoimmune disorders, are associated with specific MHC class II alleles [101, 106]. The efficiency with which certain MHC classes II allotypes present autoantigens may affect either failure or success to mount a productive immune response, thereby determining the fate of the patient whether normal immune homeostasis will prevail or an autoimmune reaction will emerge.

Autoimmunity Induction by the Idiotype–Anti-idiotype Interaction

In the past decade a growing body of evidence has accumulated suggesting that the idiotype network may play an important role in the development of autoimmunity. The roots of this concept lie in the initial hypothesis postulated by Jerne (79) in 1974, stating that the immune response may be modulated via unique antigenic determinants of the immunoglobulin variable

region, e.g., its idiotype. We referred to it earlier in an attempt to explain the diversity of manifestations in SLE as dependent on the appearance of different idiotypes of autoantibodies.

One mechanism by which anti-idiotypes could become pathogenic and induce autoimmune diseases involves antireceptor antibodies as experimental myasthenia gravis, IDDM, and Graves' disease [15, 107]. An anti-idiotype that combines with the ligand-binding site at the variable region of the autoantibidy can mimic the ligand by specifically combining with their receptors. Such anti-idiotypic/antireceptor antibodies have been produced that bind to receptors for insulin [108], acetylcholine [107], thyroid-stimulating hormone [109], adrenergic catecholamines [110], and other ligands.

Consequently, the development of receptor-binding anti-idiotypic autoantibodies can be harmful to the host and initiate autoimmunity. Data support the idea that anti-idiotypes bearing an internal image of viral antigens participate in the induction of several autoimmune disorders. Indeed, it has been shown that myasthenia gravis developed in five individuals a few weeks after rabies virus vaccination [111]. Because the rabies virus has been shown to bind to the acetylcholine receptor [112], the antiviral response can induce anti-idiotype antibodies and bind to the acetylcholine receptor, thus indicating an autoimmune process.

The idiotypic network may be involved in the induction of autoimmunity in yet another mechanism, namely bypass of the suppressor arm of the immune system. The bypass mechanism is based on the demonstration that antigenic stimulation can induce the production of antibodies not directed against the challenging antigen, but sharing a cross-reactive idiotype (CRI). If the antibody sharing the CRI with the antigen-specific antibody is an autoantibody, an autoimmune response may result. Autoimmune reactions may be initiated by anti-idiotype antibodies triggered by environmental agents that share a CRI with autoantibodies. Anti-DNA antibodies and antibodies to *Klebsiella* have been shown to share a CRI, namely the 16/6 idiotype. Patients with *Klebsiella* infections have an increased incidence of high titers of 16/6 idiotype, and both the 16/6 idiotype and anti-*Klebsiella* antibodies can be absorbed by the *Klebsiella* K30 antigen [113].

It has been shown that immunization of different mice strains with either murine or human anti-DNA carrying the pathogenic 16/6 idiotype or with their anti-idiotypes resulted in the production of a wide array of lupus-associated antibodies (anti-ssDNA, anti-Sm, and anti-Ro/SSA antibodies). In parallel, the mice developed clinical findings consistent with human SLE, including an increased erythrocyte sedimentation rate, leukopenia, thrombocytponia, proteinuria, alopecia, and paralysis [75, 114, 115]. Another interesting animal model we developed was the experimental anti-phospholipid syndrome (APS) [116]. We demonstrated that immunization of naive mice with either human or mouse monoclonal or polyclonal anticardiolipin antibodies led to the production of anticardiolipin antibodies 3–4 months following administration of the antibody. Concomitant with the serological parameters, the immunized mice developed a prolonged activated thromboplastin time, thrombocytopenia, and a low fecundity rate with increased fetal resorptions upon mating. The hypercoagulable state was also manifested by the detection of multiple thrombi in some of the fetuses [116]. Idiotypic manipulation enabled us to produce an animal model of Wegener's granulomatosis-like disease as well [117]. This disease is a granulomatous disease that affects the upper respiratory tract, lungs, and kidneys. The disease is considered autoimmune due to its association with the antineutrophil cytoplasmic antibodies (ANCA), which is primarily detected against proteinase-3, a serine protease in neutrophil azurophil granules. Immunization of naive mice with ANCA-enriched IgG led to the production of mouse ANCA. Starting from 6 months following immunization, the mice developed signs of vasculitis, focal mononuclear infiltrates within the lungs, and granular deposition of immunoglobulins with glomeruli detected by immunofluorescence studies [117].

By immunizing mice with idiotypes of antithyroglobulin and antiglomerular basement membrane antibodies, we were able to produce clinical characteristics of a compatible autoimmune diseases as decreased thyroid hormone secretion and the appearance of erythrocyturia and proteinuria, respectively [118, 119]. These studies emphasize the important role that idiotypes play in the induction and pathogenesis of autoimmune diseases; however, this finding may also be used in order to divert the progression of one autoimmune condition to another.

As mentioned previously, we succeeded in preventing the development of diabetes in NOD mice by immunizing them with an anti-DNA antibody carrying the 16/6 id. Only 25% of the immunized mice developed diabetes in comparison to 90% of the control animals. Interestingly, the mice acquired a lupus-like syndrome regardless of their "usual" proneness to develop diabetes, pointing out that the "kaleidoscope phenomenon" can also be recruited by idiotypic manipulation and harnessed in order to avert the development autoimmune conditions [72].

Role of Cytokines in the Induction of Autoimmunity

Although both $CD4^+$ and $CD8^+$ T cells secrete cytokines, $CD4^+$ cells secrete them in significantly larger

quantities. $CD4^+$ T cells can be further subdivided by the patterns of cytokine released: Th1 cells produce interleukin-2 and IFN-γ, which are critical for cell-mediated immunity and acute allograft rejection, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-9, and IL-10, which promote antibody production and humoral immunity [120]. IFN-γ and IL-12 are thought to be the most potent cytokines that enhance Th1 cell differentiation but they do not exert any effect on Th2 cells [122, 123]. However, IL-4 is a potent inducer of Th2 cells and is essential for their maturation, whereas the major role of IL-10 is to suppress the synthesis of Th1 cytolkines [124].

When naive T cells first exit the thymus they secrete both Th1 and Th2 cytokines (at that maturational phase they are termed Th0). After an antigen activates Th0 cells they turn into memory cells and become committed to a certain T-cell type. Both low and high antigen concentrations tend to induce Th2 responses preferentially, whereas intermediate doses induce Th1 responses. These mechanisms are likely part of the tolerance mechanisms mentioned in detail earlier; these pathways probably favor specific T-cell type responses.

The discovery of this polarized immune response is of great significance in the understanding of autoimmunity, and many efforts have been carried out in order to immunomodulate autoimmune disorders by tilting one T-cell type response to the other. Autoimmune conditions can be classified according to their dependence on Th1 or Th2 responses (Table 9). Th1 responses are involved primarily in the pathogenesis of organ-specific autoimmune disorders (experimental autoimmune encephalomyelitis, autoimmune thyroid disease, and type 1 diabetes mellitus) and acute allograft rejection. In contrast, allergic reactions involving IgE production and mast cell activation are typical Th2 responses. However, "classical" autoimmune diseases such as SLE and rheumatoid arthritis (RA) share Th1 and Th2 response characteristics [125].

Many abnormal cytokine concentrations were measured in SLE patients. Elevated concentrations of IL-2, IL-4, IL-6, IFN-γ, and TNF-α serum levels were detected [126]. A heterogeneous cytokine profile (high levels of IL-1β, TNF-α, and IL-10 and low levels of IL-2, IL-4, IL-6, and IFN-γ) was also reported in murine SLE models [125, 127]. We have described that repeated administration of IFN-γ to an experimental lupus model that was induced by the immunization of BALB/c mice with the 16/6 anti-DNA intensified the degree of clinical, histological, and serological parameters. All the laboratory parameters reflected acceleration of the disease in the IFN-γ-treated animals. An elevated sedimentation rate, a decreased white blood cell court, and the development of massive proteinurea meant worsened disease. IFN-γ unregulated the levels of typical Th2 cytokines and increased the number of IL-4 and IL-6 secreting splenocytes [128].

TABLE 9 Association between Autoimmune Diseases and T Helper Subtype

T helper subtype	Associated disease
Th1 cells	
	Autoimmune thyroid disease (Hashimoto's thyroiditis, Graves' disease)
	Multiple sclerosis (and experimental allergic encephalomyelitis)
	Insulin-dependent diabetes mellitus
	Crohns' disease
	Primary biliary cirrhosis
	Lyme's arthritis
	Recurrent abortions
	Yersinia reactive arthritis
	Contact dermatitis
	Acute allograft rejection
Th2 cells	
	Hypereosinophilic syndromes
	Allergic reactions
Heterogeneous Th1 and Th2 cytokines	Systemic lupus erythematosus
	Rheumatoid arthritis
	Primary systemic vasculitis
	Myasthenia gravis
	Sjogren's syndrome

Llorente *et al*. [129] assessed the clinical efficacy of administering an anti-IL-10 monoclonal antibody to six SLE patients with active steroid-dependent disease. In all the patients, cutaneous lesions and joint symptoms improved with the beginning of anti-IL-10 administration and a decrease of the steroid dosage continued during the 6 months of follow-up. The SLE disease activity index decreased markedly from a mean ± SEM of 8.83 ± 0.91 on day 1 to 1.33 ± 0.80 with only one of the six patients remaining clinically active.

Abnormal IFN production has been found in SLE patients. This cytokine has a role in the upregulation of major histocompatibility complex class II molecules and in this way contributes to the pathogenesis of autoimmunity [130]. The B-cell hyperactivity found in SLE and in other autoimmune diseases may be related to a generic increase in Th2 cytokines. However, it has been shown that IL-6 production in SLE can take place in cells other than T cells and that excessive IL-10 levels are actually produced in monocytes and B cells rather than in T lymphocytes [131].

Success in diverting the course of autoimmune models was achieved with recombinant IL-4 in rat experimental autoimmune uveoretinitis [132] and with anti-IFN-γ in experimental autoimmune thyroiditis and collagen-induced arthritis [133, 134]. Nowadays, many efforts are made in attempts to develop medications

that target cytokines that are seminal to the formation of the autoimmune process.

Inobe *et al*. [135] unraveled some of the complexity of this cytokine equilibrium using experimental allergic encephalomyelitis (EAE) as a model. They showed that T cells generated from gut-associated lymphoid tissue can suppress the eruption of EAE following oral administration of the myelin basic protein (MBP). These regulatory T cells (also termed Th3 cells) are known to produce TGF-β with various amounts of IL-4 and IL-10. Interestingly, treatment of (PLJ × SJL)F1 mice with intraperitoneal or oral IL-4 with low-dose oral MBP reduced the severity of EAE, whereas injection of IL-4 alone or oral MBP alone given in these suboptimal doses showed no protection. Spleen cells from protected mice produced increased amounts of TGF-β and reduced IFN-γ upon stimulation with MBP *in vitro*. Moreover, mucosal MBP-specific IgA production was increased significantly in IL-4 plus MBP-fed animals. Their findings demonstrated that IL-4 is a differentiation factor for TGF-β-secreting Th3 cells.

Similar results have been obtained employing oral tolerance in our mouse model of APS [induced by immunizing with β2-glycoprotein I (β2GPI)] [136]. Promising outcomes have been reported on the administration of TNF-α inhibitors to patients with long-standing refractory RA [137, 138]. In the near future, similar agents will be part of the therapeutic arsenal of many autoimmune conditions.

Autoimmunity and MHC: Dissecting the Molecular Basis

The susceptibility to many autoimmune conditions was mapped to genes of the human and murine MHC [14, 15, 139].

Some autoimmune diseases are tightly related to a genetic defect such as an autoimmune lymphoproliferative syndrome, the syndrome of autoimmune polyglandular endocrinopathy with candidiasis and ectodermal dysplasia (APECED) [140, 141].

However, most autoimmune disorders are polygenic by nature with multiple susceptibility genes working in concert to produce the autoimmune condition. Mostly the diseases manifest with the presence of other predisposing factors. Some genes confer a higher risk than others, whereas others are protective by nature; e.g., the major histocompatibility complex makes an important contribution to disease susceptibility or protection [14, 15]. Tissue typing was first confined to class 1 HLA molecules, and in type 1 diabetes mellitus, it was initially correlated with HLA-B8 and B-15 antigens. A subsequent discovery of class II MHC antigens revealed stronger associations with HLA-DR3 and -DR4 class II antigens with the disease. It is now known that the HLA-DQB1*0602 allele protects against type 1 diabetes even in individuals with coexisting HLA-DQB1*0301 or DQB1*0302 genes that act as predisposing genes [142, 143]. There is a variance between HLA alleles and a particular disease in relation to different ethnic populations and that may vary among different populations. SLE in Europeans is associated with allele B8 at the HLA-B locus, allele *0501 at the DQA1 locus, and with alleles DR2 and DR3 at the HLA-DR locus. Risk ratios associated with DR3 loci are generally similar in African-Americans (2.7) to the risk ratios in Europeans (2.4) [143].

Studies in transgenic mice have led to the discovery of numerous genes that contribute to the pathogenesis of autoimmune conditions. These genes encode cytokines, antigen coreceptors, cytokine ligands, molecules involved in pathways that promote apoptosis, and other important molecules. Because they have either stimulatory or inhibitory effects, their deletion or overexpression results with either amplification or amelioration of the autoimmune cascade. These research outcomes will join other biological therapies that find their place in the treatment of autoimmune diseases.

Apoptosis and Its Role in Autoimmunity

Apoptosis is an active cell death process essential for a large spectrum of physiological functions, including T-cell—mediated cytotoxicity and immunosurveillance. Whenever normal apoptosis function is impeded, deviation from normal immune homeostasis may emerge, manifesting as neoplasm or autoimmunity. Concentrations of self-antigens depend, among other factors, on the clearance rate of apoptotic cells. During apoptosis, the cell membrane forms cytoplasmic blebs, some of which are shed as apoptotic bodies. Casciola-Rosen *et al*. [144] and Rosen and Casciola-Rosen [145] have shown that UV-induced apoptosis of keratinocytes leads to a redistribution of several autoantigens to apoptotic blebs. Phosphatidylserine, an acidic phospholipid autoantigen that normally resides on the inside of the cell, flips outward while the cell undergoes apoptosis [146]. Interestingly, immunizing mice with apoptotic cells induced autoantibody formation [147]. The hypothesis that apoptosis is one of the mechanisms that lead to the development of SLE provides a possible solution to some long-standing enigmas. The traditional intracellular boundaries in apoptosis lose their significance as nuclear, cytosolic, and membranous materials undergo structural modification blebs, which are partially presented to the outer surface [148]. The acceleration of apoptotic processes following viral infections

and sunlight exposure may also elucidate their linkage to SLE [149]. Association between the lupus-like syndromes of patients or mice and inherited deficiencies of complement components (particularly Clq and C4) may be explained by their impaired capability to clear apoptotic cells [150].

The presumed linkage between apoptosis and SLE has led many investigators to unravel this association via genes that take part in this apoptosis. Analysis of the genetic background of Mexican-American lupus patients disclosed a synergistic effect between alleles of the IL-10 and bcl-2 genes in determining the susceptibility to SLE [151]. However, it seems that this finding has ethnic correlates, as similar results were not obtained in Italian patients [152].

Apoptotic cells were initially regarded as anti-inflammatory because they inhibit the liberation of proinflammatory molecules by macrophages; it is now well known that some forms of apoptosis can be inflammatory and can induce dendritic cell maturation, particularly when apoptotic cells are coated with antibodies [153].

One of the most important antigens involved in apoptosis is Fas. Fas is a transmembranous receptor belonging to the TNF/nerve growth factor receptor superfamily [154]. The Fas ligand is also a transmembranous protein that induces apoptosis by binding to the Fas receptor. Suzuki *et al*. [155] reported that 33% of SLE patients have anti-Fas ligand antibodies in their sera. This interaction has an inhibitory effect on Fas–Fas ligand-mediated apoptosis. This finding might be one of the mechanisms that promote autoreactivity by Fas-positive lymphocytes from apoptosis observed so commonly in SLE [155].

USING THE PRINCIPLES OF AUTOIMMUNITY TO DESIGN THERAPEUTICS

Modulation of the Idiotypic Network as a Possible Treatment Approach to Autoimmune Diseases

The realization that the expression of idiotype-bearing antibodies is regulated by anti-idiotypes [79] has led to a new approach to the treatment of autoimmune diseases via manipulation of idiotype–anti-idiotypes interactions. Essentially, the idea is very simple; to suppress the production of autoantibodies bearing pathogenic idiotypes by immunizing with specific anti-idiotypes. This in turn can prevent the damage produced in target organs by the pathogenic idiotypes. Kim *et al*. [156] demonstrated that anti-DNA production by anti-DNA-secreting hybridomas can be inhibited by the addition of anti-idiotypes to anti-DNA. Anti-idiotypic therapy has been shown to suppress autoantibody synthesis in various experimental models of autoimmune diseases, including autoimmune thyroiditis and autoimmune tubulointerstitial nephritis [157, 158]. In lupus mice, models of unequivocal findings were reported. Immunization of MRL mice with an anti-idiotype directed against H-130 (a dominant idiotype of MRL anti-DNA antibodies) augmented the production of the H-130 idiotype and anti-DNA antibodies, whereas others abrogated anti-DNA production, immunizing them with another anti-idiotype carried by an anti-DNA natural autoantibody [159, 160]. This conflicting evidence can be elucidated if we accept that diverse anti-idiotypes regulate idiotype-bearing autoantibodies in various ways: some acting to suppress idiotype expression, whereas others amplify its production.

Another method used to manipulate the idiotypic network is to produce anti-idiotypes by active immunization with the idiotype instead of passive administration of the anti-idiotype. As mentioned previously, we have generated a lupus-like mouse model by immunization with an anti-DNA antibody carrying the 16/6 idiotype. In order to potentiate the effects of anti-idiotypes, we have conjugated a widely used toxic compound termed saporin to an anti-idiotype to the 16/6 id. The suppressive impact of this treatment was specific: *in vitro* the idiotype synthesis was halted and *in vivo* therapy diminished the clinical manifestations and decreased the level of the autoantibodies in the mice sera [161].

Although anti-idiotypic therapy has not been accepted as a therapeutic modality for human disease, it is believed that an abundance of idiotypes within preparations of intravenous γ-globulin infusion (IVIG) is one of the mechanisms that confer an inhibitory effect on autoimmunity.

Intravenous Gamma Globulin Infusion

Intravenous immune globulin has been used to treat primary and secondary antibody deficiencies since the mid-1970s. It is composed of IgG pooled from plasma originating from a large number of blood donors. This pooling of intact antibodies entails the basic characteristics of the IVIG preparation [162].

1. *Fc receptors.* An intact immunoglobulin ensures appropriate interactions of all the components of the molecule, e.g., the Fc portion can bind to complement components or with the Fcγ receptor and it may block Fc receptors on macrophages and

effector cells and induce antibody-dependent cellular toxicity.
2. *Inflammation.* IVIG attenuates and decreases the damaged caused by complement-mediated processes, it has the capacity to induce anti-inflammatory cytokines and abrogates the activation of endothelial cells, and it neutralizes microbial toxins.
3. *B-cell regulation.* IVIG can control the B-cell repertoire via the idiotypic network. Selectively, it can down- or upregulate antibody production by specific B-cell clones. In mice, IVIG administration controlled the migration of B-cell populations from the bone marrow to secondary lymphoid organs.
4. *T-cell regulation.* IVIG plays a role in the modulation of T-cell cytokine synthesis. IVIG was shown to selectively decrease the production of IL-2 and IFN-γ by antigen-reactive T cells. IVIG also has the ability to restore the balance between Th1 and Th2 responses various autoimmune conditions.
5. *Cell growth.* As mentioned earlier, IVIG via antibodies to antigens that have key roles in apoptosis can modulate cell proliferation. This mechanism implies that IVIG therapy may have be promising in cancer therapy [163].

Since this primal report of Shulman *et al*. [164], who reported on a positive response to fresh frozen plasma infusions in patients with chronic thrombocytopenia, IVIG therapy has been studied extensively and found to confer significant benefits to patients with autoimmune conditions such as idiopathic thrombocytopenic purpura (ITP), Guillian–Barre syndrome, myasthenia gravis, Kawasaki's syndrome, corticosteroid-resistant polymyositis, autoimmune uveitis, multiple sclerosis, and vasculitis [165–172].

Rheumatoid Arthritis

RA remains the most controversial indication for IVIG. Despite a few encouraging open study reports, results from controlled trials have not shown a significant effect. However, none of these studies were carried out with high-dose IVIG (2 g/kg). More impressive results were achieved in adult Still's disease and in juvenile rheumatoid arthritis [173, 174].

SLE

Corvetta *et al*. [175] described successful treatment with IVIG in three patients with life-threatening manifestations of SLE (encephalitis, nephritis) unresponsive to conventional high-dose corticosteroids or immunosuppressive therapy. We reported on various manifestations of SLE that responded favorably to treatment with IVIG: 7 patients with lupus nephritis that failed on therapy with cyclophosphamide and prednisone had good responses to six courses of IVIG [176]. We measured the effect of IVIG therapy on 20 patients with SLE. A beneficial clinical response following IVIG treatment was noted in 17 out of 20 patients (85%). Few clinical manifestations responded more favorably to treatment: arthritis, fever, thrombocytopenia, and neuropsychiatric lupus. In 9 of the patients that were evaluated before and after IVIG, a significant decrease in the mean systemic lupus activity measure (SLAM) score was recorded [177].

Antiphospholipid Syndrome

The employment of IVIG in the treatment of antiphospholipid syndrome was considered after demonstration of the potential of IVIG to neutralize lupus anticoagulant LA activity *in vitro* [178]. As IVIG appeared less toxic in pregnant women than prednisolone, most of the reports focus on the use of IVIG in the obstetric complications of antiphospholipid syndrome. The use of IVIG either alone or in combination with aspirin or heparin resulted in a successful pregnancy outcome in the vast majority of patients with antiphospholipid syndrome who had previously had recurrent abortions. Also, IVIG was beneficial in antiphospholipid antibody-positive patients undergoing *in vitro* fertilization. We have reported on the favorable outcomes of treatment with IVIG in our experimental models of APS and lupus [179].

Other Diseases

IVIG therapy was reported to favorably affect patients with insulin-dependent diabetes mellitus; however, the results were not based on large population studies and the reported follow-up was of short duration [180]. Successful reports indicate that IVIG therapy may have a pertinent role in the treatment of thyroid ophtalmopathy even in cases in which immunosuppressive therapy failed [181]. Similar tendencies have been documented after IVIG treatment of patients with chronic inflammatory demyelinating polyneuropathies and multiple sclerosis [182]. IVIG is a safe therapeautical agent; we reported that among 56 patients with various autoimmune diseases treated with IVIG, 20 (36%) had at least one adverse reaction (headache, low-grade fever, chills, anemia, low-back pain, transient hypotension, nausea, diaphoresis, and superficial vain thrombosis). These reactions were mostly mild and transient and were not correlated with the patients' response to the therapy [183].

Oral Tolerance

Oral tolerance refers to the observation that oral administration of an antigen followed by immunization might evoke a state of hyporesponsiveness to the specific antigen. Low doses of antigen administration favor the induction of active cellular regulation, whereas higher doses favor the induction of clonal anergy or deletion [184–186]. Active suppression is mediated by the induction of regulatory T cells in the gut-associated lymphoid tissue (GALT). These cells migrate to the systemic immune system and secrete Th2-type cytokines as TGF-β, IL-4, and IL-10 that suppress the immune reaction [187]. Benson *et al.* [188] demonstrated the downregulation of T-cell receptors as an immediate but reversible effect following high-dose feeding of mice with myelin basic protein (MBP). Carrying a detailed analysis of cytokine responses following a single high-dose feeding, they found that on day 1 there was an increase in the number of cells expressing IL-2, IFN-γ, IL-5, and IL-4, followed by their subsequent decrease and apparent deletion by day 14, a consequence of apoptosis. Cells secreting TGF-β, referred to as Th3 cells, have been implicated in the maintenance of oral tolerance [189]. These cells were distinctive in that they were not deleted following oral exposure to MBP. This effect was associated with protection from experimental autoimmune encephalomyelitis [188].

The promising prospects of oral tolerance in the treatment of autoimmune conditions were shown in our mouse model of APS (induced by immunizing with β2GPI [136]. Oral tolerance was induced in BALB/c mice by feeding low-dose β2GPI. β2GPI-fed mice did not develop serologic or clinical markers of experimental APS upon immunization with the autoantigen. β2GPI given orally before priming with β2GPI resulted in complete prevention of experimental APS development; β2GPI given at an early stage of the disease reduced clinical manifestations, whereas administration of β2GPI 70 days postimmunization conferred a less significant effect. Tolerized mice exhibited a diminished T lymphocyte proliferation response to β2GPI in comparison with β2GPI-immunized mice fed ovalbumin. When nontolerant β2GPI-primed T lymphocytes were mixed with T lymphocytes derived from tolerized mice, a significant inhibition of proliferation upon exposure to β2GPI was observed. Furthermore, $CD8^+$ T cells adoptively transferred the tolerance from the tolerized mice into naive mice.

Several teams worldwide have reported on successful trials suppressing animal models of EAE, collagen-induced arthritis, experimental autoimmune uveoretinitis, myasthenia, and diabetes in NOD mice by the oral administration of the inducer autoantigen [188, 190, 191].

Human trials have shown some success in patients with juvenile rheumatoid arthritis, rheumatoid arthritis, and uveoretinitis [192–194]. However, in multiple sclerosis and type 1 diabetes, patients' oral administration of a myelin preparation and insulin using different doses, respectively, failed to block progression of the disease [195, 196].

Despite the preliminary phase in which clinical trials seem to appear very challenging due to the simplicity of administration and lack of adverse effects, further progress in this field is expected in the near future.

HEMOPOIETIC STEM CELL TRANSPLANTATION IN AUTOIMMUNE DISEASES

Almost a decade ago Yin and Jowitt [197] described two patients with a long history of psoriasis and ulcerative colitis who received allogenic bone marrow transplantation (BMT) for leukemia. Four years posttransplantation they remained in full remission of psoriasis and ulcerative colitis and their leukemia.

The ability to transfer autoimmune disease to normal animals by the infusion of hemopoietic stem cells opened new avenues for investigating the pathophysiology of autoimmunity, as well as for altering this modality into an optional therapeutic intervention in established autoimmune processes. The substantial risk of morbidity and mortality associated with allogeneic BMT has so far prevented its application in the treatment of patients with severe autoimmune disease. In contrast, the lower complication rate (3–5% mortality risk) of autologous transplants justified trying this approach in patients with severe autoimmune disease who were refractory to current treatments, particularly among those with a severe and refractory course [198].

It is believed that BMT confers its effect by the following mechanisms.

1. Eradicating autoreactive mature and progenitor cells that contribute to the autoimmune condition, replacing them with a normal immune system.
2. Reducing the number of autoreactive cells to low levels that allow self-tolerance to overcome.
3. Altering immunoregulatory circuits by the use of cytotoxic agents to reestablish more efficient immune control mechanisms.

Several individual cases and a phase I study including patients with severe SLE treated with autologous stem cell transplantation have been published. SLE remained clinically inactive in all cases, sometimes after

more than 2 years [199, 200]. Tyndall and Gratwohl [201] reviewed the outcome of BMT in around 350 patients with multiple sclerosis, systemic sclerosis, rheumatoid arthritis, juvenile idiopathic arthritis, idiopathic thrombocytopenic purpura, and SLE. The procedure-related mortality was around 9% (being higher in systemic sclerosis and juvenile idiopathic arthritis). A benefit has been recorded in around two-thirds of cases.

Peptide Immunotherapy

$CD4^+$ helper T lymphocytes and $CD8^+$ cytotoxic T lymphocytes play major roles in the normal activation of the immune system to foreign antigens. These cells are triggered when their specific T-cell receptors (TCRs) recognize foreign antigenic peptide fragments hound to MHC molecules. $CD8^+$ T cells usually recognize peptides complexed to class I MHC molecules, whereas $CD4^+$ T cells usually recognize peptides complexed to class II MHC molecules. The activated T helper cell consequently secretes diverse cytokines that conduct the type of the immune reaction. The current understanding of the role of T cells in the immune response has led extensive research in attempts to modulate these steps and to alter disease outcome.

One of the major breakthroughs that was accomplished with peptide immunotherapy was obtained with copolymer I (cop 1, glatiramer acetate, *Copaxone*). Cop I, a synthetic amino acid copolymer, synthesized randomly from a pool of four amino acids—L-glutamic acid, L-alanine, L-lysine, and L-tyrosine—immunologically simulates myelin basic and was shown to suppress experimental autoimmune encephalomyelitis in mice when administered parenterally [203]. The beneficial effect of oral cop I was associated with specific inhibition of the proliferative and cytokine secretion responses of Th1 cells to MBP. The tolerance induced by cop I was adoptively transferred with spleen cells from cop I-fed animals and cop I-specific T-cell lines that inhibited EAE induction. These T-cell lines secreted IL-10 and TGF-β in response to both cop I and MBP. Therefore, oral cop I has a beneficial effect on the development of EAE that is associated with the down-regulation of T-cell immune responses to MBP [204]. In MS patients, a 29% reduction in attack frequency was observed over the 2 years of treatment with cop I, which became a 32% reduction when a 6-month extension of the trial was taken into account [205].

Singh *et al*. [205] examined the possibility that in mice with SLE, autoantibody molecules may be upregulating their own production by autoactivation of self-reactive T cells to their own processed peptides. They demonstrated that lupus-prone (NZBINZW)Fl (BWFl) mice developed spontaneous T-cell autoimmunity to peptides from variable regions of heavy chains (VH) of syngeneic anti-DNA MAbs. Tolerizing young BWFl mice with intravenous injections of autoantibody-derived determinants substantially delayed the development of anti-DNA antibodies and nephritis and prolonged survival. Thus, in such an autoantibody-mediated disease, the presence of autoreactive T cells against VII region determinants of autoantibodies may represent an important mechanism involved in the regulation of autoimmunity, hence tolerizing such autoreactive T cells might postpone the development of SLE.

In the (SWR x NZB)F1 mouse model of lupus, treatment with peptides corresponding to the critical autoepitopes (localized in the core histones of nucleosomes) markedly delayed the onset of severe lupus nephritis. Chronic therapy with the peptides injected into older mice with established glomerulonephritis prolonged survival and even halted the progression of renal injury [206].

Waisman *et al*. [207] employed a model of murine SLE induced by active immunization with a pathogenic anti-DNA MAb bearing the 16/6 idiotype. Strain-dependent differences were observed in the proliferative responses of lymph node cells of mice immunized with two peptides based on the sequences of the complementarity-determining region (CDR) I and 3 of a pathogenic anti-DNA monoclonal antibody. Immunization of high responder strains with the CDR-based peptides led to the production of autoantibodies and clinical manifestations characteristic of experimental SLE. The CDR-based peptides, however, could prevent autoantibody production in neonatal mice that were immunized later with either the peptide or the pathogenic autoantibody. Furthermore, the peptides inhibited specific proliferation of lymph node cells of mice immunized with the same peptide or with the pathogenic anti-DNA monoclonal antibody.

We have three hexapeptides that react specifically with anti-β2GPl MAbs that elicited endothelial cell activation and induced experimental APS. The peptides specifically inhibited both *in vitro* and *in vivo* the biological functions of the corresponding anti-β2GPl MAbs. Exposure of endothelial cells to anti-β2GPl MAbs and their corresponding peptides led to the inhibition of endothelial cell activation, as shown by a decreased expression of adhesion molecules and monocyte adhesion. *In vivo* infusion of each of the anti-β2GPl MAbs into BALBIc mice, followed by administration of the corresponding specific peptides, prevented the peptide-treated mice from developing experimental APS [208].

Five prospective clinical studies in lupus patients have shown that the LJP 394 peptide can reduce circulating anti-dsDNA antibody levels without causing gen-

eralized immunosuppression. The compound is currently being evaluated in a phase III clinical trial for the prevention of renal flares in patients with high-affinity antibodies to LJP 394 and a history of lupus nephritis [209]. These studies reflect that the use of synthetic peptides that focus on neutralization of the immune response may entail new avenues to the therapy of autoimmune diseases [210].

What Are We Expecting in the Future?

Despite the complexity of the mechanisms that are engaged in the induction of autoimmunity, this decade will be remembered as the period in which modern therapeutic modalities, mainly those impeding seminal steps of the immune response, have emerged. IVIG and the exploitation or the principles of oral tolerance seem promising. However, other novel therapeutic avenues such as bone marrow transplantation, immunomodulation by drugs and peptides, and utilization of MAbs directed against essential components of effector cells are undergoing great change and are encountered more commonly as medications seen at the patient bedside. We believe that all these advances will lead to more progress in the comprehension and combat against autoimmune diseases, turning the therapeutical measures we employ today more specific, targeting precise steps of the autoimmune reaction [211].

References

1. Jacobson, D. L., Gange, S. J., Rose, N. R., and Graham, N. M. (1997). Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin. Immunol. Immunopathol.* **84**, 223.
2. Shoenfeld, Y., and Cohen, I. R. (1987). Infection and autoimmunity. *In* "The Antigens" (M. Sela, ed.), pp. 317–325, Vol. VII. Academic Press, San Diego.
3. Isenberg, D. A., Shoenfeld, Y., Walport, M., *et al*. (1985). Detection of cross-reactive anti-DNA antibody idiotype in the serum of lupus patients and their relatives. *Arthritis Rheum.* **28**, 999.
4. Shoenfeld, Y., Segol, G., Segol, O., *et al*. (1987). Detection of antibodies to total histones and their subfractions in systemic lupus erythematosus patients and their symptomatic relatives. *Arthritis Rheum*. **45**, 169.
5. Bjoro, T., Holmen, J., Kruger, O., *et al*. (2000). Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The Health Study of Nord-Trondelag (HUNT). *Eur. J. Endocrinol*. **143**, 639.
6. Swissa, M., Amital-Teplizki, H., Haim, N., Cohen, Y., and Shoenfeld, Y. (1990). Autoantibodies in neoplasia: An unsolved enigma. *Cancer* **65**, 2554.
7. Abu-Shakra, M., Buskila, D., Ehrenfeld, M., Conrad, K., and Shoenfeld, Y. (2001). Cancer and autoimmunity: Autoimmune and rheumatic features in patients with malignancies. *Ann. Rheum. Dis.* **60**, 433.
8. Jonsson, V., Kierkegaard, A., Salling, S., *et al*. (1999). Autoimmunity in Waldenstrom's macroglobulinaemia. *Leukocyte Lymphoma* **34**, 373.
9. Shoenfeld, Y., Ben-Yehuda, O., and Naparstek, Y. (1986). The detection of a common idiotype of anti-DNA antibodies in the sera of patients with monoclonal gammopathies. *Clin. Immunol.* **42**, 194.
10. Shoenfeld, Y., El-Roeiy, A., Ben-Yehuda, O., and Pick, A. I. (1987). Detection of antihistone activity in sera of patients with monoclonal gammopathies. *Clin. Immunol. Immunopathol.* **42**, 250.
11. Buskila, D., Abu Shakra, M., Amital Teplizki, H., Coates, A. R., and Krupp, M. (1989). Serum monoclonal antibodies derived from patients with multiple. *Clin. Exp. Immunol.* **76**, 378.
12. Noerager, B. D., Inuzuka, T., Kira, J., Blalock, J. E., Whitaker, J. N., and Galin, F. S. (2001). An IgM anti-MBP Ab in a case of Waldenstrom's macroglobulinemia with polyneuropathy expressing an idiotype reactive with an MBP epitope immunodominant in MS and EAE. *J. Neuroimmunol.* **113**, 163.
13. Choufani, E. B., Sanchorawala, V., Ernst, T., *et al*. (2001). Acquired factor X deficiency in patients with amyloid light-chain amyloidosis: Incidence, bleeding manifestations, and response to high-dose chemotherapy. *Blood* **97**, 1885.
14. Davidson, A., and Diamond, B. (2001). Advances in immunology: Autoimmune diseases. *N. Engl. J. Med.* **345**, 340.
15. Shoenfeld, Y., and Schwartz, R. S. (1984). Immunologic studies and genetic factors in autoimmune diseases. *N. Engl. J. Med.* **311**, 1019.
16. Marmont, A. M. (1965). The transfusion of active L.E. plasma into nonlupus recipients, with a note on the L.E. like cell. *Ann. N.Y. Acd. Sci.* **124**, 838.
17. Yoles, E., Hauben, E., Palgi, O., *et al*. (2001). Protective autoimmunity is a physiological response to CNS trauma. *J. Neurosci.* **21**, 3740.
18. Grabar, P. (1983). Autoantibodies and the physiological role of immunoglobulins. *Immunol. Today* **4**, 337.
19. Cohen, I. R. (1986). Regulation of autoimmune disease physiological and therapeutic. *Immunol. Rev.* **94**, 5.
20. Kearney, J. F., Bartels, J., Hamilton, A. M., Lehuen, A., Solvason, N., and Vakil, M. (1992). Development and function of the early B cell repertoire. *Int. Rev. Immunol.* **8**, 247.
21. Shoenfeld, Y., and Isenberg, D. A. (1993). "Natural Autoantibodies: Their Physiological Role and Regulatory Significance." CRC Press, Boca Raton, FL.
22. Cohen, A. D., and Shoenfeld, Y. (1995). The viral-autoimmunity relationship. *Viral. Immunol.* **8**, 1.
23. Asherson, R. A., and Shoenfeld, Y. (2000). The role of infection in the pathogenesis of catastrophic antiphospholipid syndrome: Molecular mimicry? *J. Rheumatol.* **27**, 12.
24. Bar-Meir, E., Amital, H., Levy, Y., Kneller, A., Bar-Dayan, Y., and Shoenfeld, Y. (2000). Mycoplasma-

pneumoniae-induced thrombotic thrombocytopenic purpura. *Acta Haematol.* **103**, 112.
25. George, J., Levy, Y., and Shoenfeld, Y. (1997). Smoking and immunity: An additional player in the mosaic of autoimmunity. *Scand. J. Immunol.* **45**, 1.
26. Lorber, M., Gershwin, M. E., and Shoenfeld, Y. (1994). The coexistence of systemic lupus erythematosus with other autoimmune diseases: The kaleidoscope of autoimmunity. *Semin. Arthritis Rheum.* **24**, 105.
27. Mevorach, D., Perrot, S., Buchanana, N. M., *et al*. (1995). Appearance of systemic lupus erythematosus after thymectomy, four case reports and review of the literature. *Lupus* **4**, 33.
28. Alarcon-Sgovia, D., Galbaith, R. F., and Maldonado, J. E. (1963). Systemic lupus erythematosus following thymectomy for myasthenia gravis, report of two cases. *Lancet* **2**, 662.
29. Dalal, I., Levine, A., Somekh, E., Mizrahi, A., and Hanukoglu, A. (2000). Chronic urticaria in children: Expanding the "autoimmune kaleidoscope." *Pediatrics* **106**, 1139.
30. Rose, N., and Mackay, I. R. (1985). "Autoimmune Diseases." Academic Press, New York.
31. Ernst, P. B., Crowe, S. E., and Reyes, V. E. (1997). How does *Helicobacter pylori* cause mucosal damage? The inflammatory response. *Gastroenterology* **113**, S35.
32. Ganguli, R., Brar, J. S., and Rabin, B. S. (1994). Immune abnormalities in schizophrenia: Evidence for the autoimmune hypothesis. *Harv. Rev. Psychiat.* **2**, 70.
33. Amital-Teplizki, H., Sela, B., and Shoenfeld, Y. (1992). Autoantibodies to brain lipids among patients with schizophrenia a puzzle. *Immunol. Res.* **11**, 66.
34. Shinitzky, M., Deckmann, M., Kessler, A., Sirota, P., Rabbs, A., and Elizur, A. (1991). Platelet autoantibodies in dementia and schizophrenia. *Ann. N.Y. Acad. Sci.* **621**, 205.
35. Blank, M., Cohen, J., Toder, V., and Shoenfeld, Y. (1991). Induction of anti-phospholipid syndrome in naive mice with mouse. *Proc. Natl. Acad. Sci. USA* **88**, 3069.
36. Shoenfeld, Y., and Krause, I. (1996). Immunosuppression and immunomodulation of experimental models of systemic lupus erythematosus and antiphospholipid syndrome. *Transplant. Proc.* **28**, 3096.
37. Bell, D. A., Morrison, B., and VandenBygaart, P. (1990). Immunogenic DNA-related factors: Nucleosomes spontaneously released from normal murine lymphoid cells stimulate proliferation and immunoglobulin synthesis of normal mouse lymphocytes. *J. Clin. Invest.* **85**, 1487.
38. Gilkeson, G. S., Grudier, J. P., Karounos, D. G., and Pisetsky, D. S. (1989). Induction of anti-double stranded DNA antibodies in normal mice. *J. Immunol.* **142**, 1482.
39. Satake, F., Watanabe, N., Miyasaka, N., Kanai, Y., and Kubota, T. (2000). Induction of anti-DNA antibodies by immunization with anti-DNA antibodies: Mechanism and characterization. *Lupus* **9**, 489.
40. Madaio, M. P., Hodder, S., Schwartz, R. S., and Stollar, B. D. (1984). Responsiveness of autoimmune and normal mice to nucleic acid. *J. Immunol.* **132**, 872.
41. Tsao, B. P., Ebling, F. M., and Roman, C. (1990). Structural characteristic of the variable regions of immunoglobulin genes encoding a pathogenic autoantibody in murine lupus. *J. Clin. Invest.* **85**, 530.
42. Ohnishi, K., Ebling, F. M., Mitchell, B., Singh, R. R., Hahn, B. H., and Tsao, B. P. (1994). Comparison of pathogenic and non-pathogenic murine antibodies to DNA: Antigen binding and structural characteristics. *Int. Immunol.* **6**, 817.
43. van Bruggen, M. C., Kramers, C., Walgreen, B., *et al*. (1997). Nucleosomes and histones are present in glomerular deposits in human lupus nephritis. *Nephrol. Dial. Transplant.* **12**, 57.
44. Qureshi, F., Yang, Y., Jaques, S. M., *et al*. (2000). Anti-DNA antibodies cross-reacting with laminin inhibit trophoblast attachment and migration: Implications for recurrent pregnancy loss in SLE patients. *Am. J. Reprod. Immunol.* **44**, 136.
45. Mostoslavsky, G., Fischel, R., Yachimovich, N., *et al*. (2001). Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: A case for tissue injury by molecular mimicry. *Eur. J. Immunol.* **31**, 1221.
46. Peters, J. B., and Shoenfeld, Y. (1996). "Autoantibodies." Elsevier Science, Amsterdam.
47. Gorstein, A., Sherer, Y., and Shoenfeld, Y. (2003). Autoantibodies explosion in systemic lupus erythematosus. *Semin. Arthritis Rheum.* (in press).
48. Feltkamp, T. E. W. (1999). The mystery of autoimmune diseases. "The Decade of Autoimmunity" (Y. Shoenfeld, ed.), pp. 1–5. Elsevier, Amsterdam.
49. Shoenfeld, Y., and Isenberg, D. A. (1989). The mosaic of autoimmunity. *Immunol. Today* **10**, 123.
50. Rahamim-Cohen, D., and Shoenfeld, Y. (2001). The mosaic of autoimmunity: A classical case of inhalation of a polyclonal activating factor in a genetically and hormonally susceptible patient leading to multiple autoimmune diseases. *Isr. Med. Assoc. J.* **3**, 381.
51. Ito, T., Nakamura, K., Umeda, E., *et al*. (2001). Familial predisposition of type 1 diabetes mellitus in Japan, a country with low incidence: Japan Diabetes Society Data Committee for Childhood Diabetes. *J. Pediatr. Endocrinol. Metab.* **14**(Suppl. 1), 589.
52. Encinas, J. A., and Kuchroo, V. K. (2000). Mapping and identification of autoimmunity genes. *Curr. Opin. Immunol.* **12**, 691.
53. Tomer, Y., and Shoenfeld, Y. (1989). The significance of T suppressor cells in the development of. *J. Autoimmun.* **2**, 739.
54. Becker, K. G. (1999). Comparative genetics of type 1 diabetes and autoimmune disease: Common loci, common pathways? *Diabetes* **48**, 1353.
55. Crispin, J. C., and Alcocer-Varela, J. (1998). Interleukin-2 and systemic lupus erythematosus: Fifteen years later. *Lupus* **7**, 214.
56. Barka, N., Shen, G. Q., Shoenfeld, Y., *et al*. (1995). Multireactive pattern of serum autoantibodies in asymptomatic individuals with immunoglobulin A (IgA) deficiency. *Clin. Diag. Lab. Immunol.* **2**, 469.

57. Johanneson, B., and Alarcon-Riquelme, M. E. (2001). An update of the genetics of systemic lupus erythematosus. *Isr. Med. Assoc. J.* **3**, 88.
58. Wu, J., Edberg, J. C., Redecha, P. B., *et al*. (1997). A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest.* **100**, 1059.
59. Dragon-Durey, M. A., Quartier, P., Fremeaux-Bacchi, V., *et al*. (2001). Molecular basis of a selective C1s deficiency associated with early onset multiple autoimmune diseases. *J. Immunol.* **166**, 7612.
60. Davies, E. J., Snowden, N., Hillarby, M. C., *et al*. (1995). Mannose-binding protein gene polymorphism in systemic lupus erythematosus. *Arthritis Rheum.* **38**, 110.
61. Rahamim Cohen, D., and Shoenfeld, Y. (2001). The mosaic of autoimmunity: A classical case of inhalation of a polyclonal activating factor in a genetically and hormonally susceptible patient leading to multiple autoimmune diseases. *Isr. Med. Assoc. J.* **3**, 381.
62. Hess, A., Thoburn, C., Chen, W., and Horwitz, L. (2001). Autoreactive T-cell subsets in acute and chronic syngeneic graft-versus-host disease. *Transplant. Proc.* **33**, 1754.
63. Rossi, F., Guilbert, B., Tonnelle, C., Ternynck, T., Fumoux, F., and Avrameas, S. (1990). Idiotypic interactions between normal human polyspecific IgG and. *Eur. J. Immunol.* **20**, 2089.
64. Levene, N. A., Varon, D., Shtalrid, M., and Berrebi, A. (1991). Chronic active hepatitis following splenectomy for autoimmune thrombocytopenia. *Isr. J. Med. Sci.* **27**, 199.
65. Goshen, E., Livne, A., Krupp, M., *et al*. (1989). Antinuclear and related autoantibodies in sera of healthy subjects with IgA deficiency. *J. Autoimmun.* **2**, 51.
66. Isenberg, D. A., Shoenfeld, Y., Walport, M., *et al*. (1995). Detection of cross-reactive anti-DNA antibody idiotype in the serum of lupus patients and their relatives. *Arthritis Rheum.* **28**, 999.
67. Kleiner Baumgarten, A., Schlaeffer, F., and Keynan, A. (1983). Multiple autoimmune manifestations in a splenectomized subject with HLA-B8. *Arch. Intern. Med.* **143**, 1987.
68. Gerli, R., Paganelli, R., Cossarizza, A., *et al*. (1999). Long-term immunologic effects of thymectomy in patients with myasthenia gravis. *J. Allergy. Clin. Immunol.* **103**, 865.
69. Sherer, Y., Bar-Dayan, Y., and Shoenfeld, Y. (1997). Thymoma, thymic hyperplasia, thymectomy and autoimmune disease. *Int. J. Oncol.* **10**, 939.
70. Shoenfeld, Y., Lorber, M., Yucel, T., and Yazici, H. (1997). Primary antiphospholipid syndrome emerging following thymectomy for myasthenia gravis: Additional evidence for the kaleidoscope of autoimmunity. *Lupus* **6**, 474.
71. Rodrigue, S., Laborde, H., and Catoggio, P. M. (1989). Systemic lupus erythematosus and thyrotoxicosis: A hitherto little recognized association. *Ann. Rheum. Dis.* **48**, 424.
72. Krause, I., Tomer Y., Elias D., *et al*. (1999). Inhibition of diabetes in NOD mice by idiotypic induction of SLE. *J. Autoimmun.* **13**, 49.
73. Shoenfeld, Y. (1997). Anti-DNA Antibodies: Is DNA the Self Antigen or a Shelf Antigen?" pp. 139–148. Academic Press, San Diego.
74. James, J. A., Neas, B. R., *et al*. (2001). Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure. *Arthritis Rheum.* **44**, 1122.
75. Ziporen, L., Shoenfeld, Y., Levy, Y., and Korczyn, A. D. (1997). Neurological dysfunction and hyperactive behavior associated with antiphospholipid antibodies: A mouse model. *J. Clin. Invest.* **100**, 613.
76. Mendlovic, S., Brocke, S., Shoenfeld, Y., *et al*. (1988). Induction of a SLE-like disease in mice by a common anti-DNA idiotype. *Proc. Natl. Acad. Sci. USA* **85**, 2260.
77. Lehmann, P. V., Forsthuber, T., Miller, A., and Sercarz, E. E. (1992). Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. *Nature* **358**, 155.
78. McCluskey, J., Farris, A. D., Keech, C. L., *et al*. (1998). Determinant spreading: Lessons from animal models and human disease. *Immunol. Rev.* **164**, 209.
79. Jerne, N. K. (1984). Idiotypic networks and other preconceived ideas. *Immunol. Rev.* **79**, 5.
80. Dang, H., Ogawa, N., Takei, M., Lazaridis, K., and Talal, N. (1993). Induction of lupus-associated autoantibodies by immunization with native and recombinant Ig polypeptides expressing a cross-reactive idiotype 4B4. *J. Immunol.* **151**, 7260.
81. George, J., Gilburd, B., and Shoenfeld, Y. (1999). Autoantibody spread may explain multiple antibodies. *Immunologist* **716**, 189.
82. Burnet, M. (1959). "The Clonal Selection Theory of Acquired Immunity." Vanderbilt Univ. Press, Nashville, TN.
83. Reines, P. B. (2001). Bystanders or bad seeds? Many autoimmune-target cells may be transforming to cancer and signaling "danger" to the immune system. *Autoimmunity* **33**, 121.
84. Shoenfeld, Y., Gershwin, M. E. (eds.) (2000). "Cancer and Autoimmunity." Elsevier, Amsterdam.
85. Nemazee, D. A., and Burki, K. (1989). Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. *Nature* **337**, 562.
86. Rathmell, J. C., Townsend, S. E., Xu, J. C., Flavell, R. A., and Goodnow, C. C. (1996). Expansion or elimination of B cells in vivo: Dual roles for CD40- and Fas (CD95)-ligands modulated by the B cell antigen receptor. *Cell* **87**, 319.
87. Goodnow, C. C., Crosbie, J., Adelstein, S., *et al*. (1988). Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature* **334**, 676.
88. Nemazee, D. (2000). Receptor selection in B and T lymphocytes. *Annu. Rev. Immunol.* **18**, 19.
89. Klein, L., Klugmann, M., Nave, K. A., Tuohy, V. K., and Kyewski, B. (2000). Shaping of the autoreactive T-cell repertoire by a splice variant of self protein expressed in thymic epithelial cells. *Nature Med.* **6**, 56.
90. Kamradt, T., and Mitchison, N. A. (2001). Tolerance and autoimmunity. *N. Engl. J. Med.* **344**, 655.

91. Saoudi, A., Seddon, B., Fowell, D., and Mason, D. (1996). The thymus contains a high frequency of cells that prevent autoimmune diabetes on transfer into prediabetic recipients. *J. Exp. Med.* **184**, 2393.
92. Seddon, B., and Mason, D. (2000). The third function of the thymus. *Immunol. Today* **21**, 95.
93. Amital, H., Levy, Y., Davidson, C., *et al*. (2001). Catastrophic antiphospholipid syndrome: Remission following leg amputation in 2 cases. *Semin. Arthritis Rheum.* **31**, 127.
94. Casetta, I., and Granieri, E. (2000). Clinical infections and multiple sclerosis: Contribution from analytical epidemiology. *J. Neurovirol*. **6**(Suppl. 2), S147.
95. von Herrath, M. G., Coon, B., Lewicki, H., *et al*. (1998). In vivo treatment with a MHC class I-restricted blocking peptide can prevent virus-induced autoimmune diabetes. *J. Immunol.* **161**, 5087.
96. Schiffenbauer, J., Soos, J., and Johnson, H. (1998). The possible role of bacterial superantigens in the pathogenesis of autoimmune disorders. *Immunol. Today* **19**, 117.
97. Teplizki, H., Buskila, D., Isenberg, D. A., *et al*. (1987). Low serum anti-TB glycolipid antibodies titer in the sera of patients with SLE as an indicator of CNS involvement. *J. Rheumatol.* **14**, 507.
98. Abu Shakra, M., Buskila, D., and Shoenfeld, Y. (1999). Molecular mimicry between host and pathogen: Examples from parasites and implication. *Immunol. Lett.* **67**, 147.
99. Sasazuki, T., Kikuchi, I., Hirayama, K., *et al*. (1989). HLA-linked immune suppression in humans. *Immunol. Suppl.* **2**, 21.
100. Gibbon, C., Smith, T., Egger, P., Betts, P., and Phillips, D. (1997). Early infection and subsequent insulin dependent diabetes. *Arch. Dis. Child.* **77**, 384.
101. Lahat, N., Miller, A., and Rahat, M. (2000). Significance and regulation of the expression of MHC class II molecules on autoimmune and neoplastic thyroid cells. *In* "Cancer and Autoimmunity" (Y. Shoenfeld and M. E. Gershwin, eds.), pp. 317–335. Elsevier, Amsterdam.
102. Kingston, A. E., Bergsteinsdottir, K., Jessen, K. R., *et al*. (1989). Schwann cells co-cultured with stimulated T cells and antigen express major histocompatibility complex (MHC) class II determinants without interferon-gamma pretreatment: Synergistic effects of interferon-gamma and tumor necrosis factor on MHC class II induction. *Eur. J. Immunol.* **19**, 177.
103. Neufeld, D. S., Platzer, M., and Davies, T. F. (1989). Reovirus induction of MHC class II antigen in rat thyroid cells. *Endocrinology* **124**, 543.
104. Khoury, E. L., Pereira, L., and Greenspan, F. S. (1991). Induction of HLA-DR expression on thyroid follicular cells by cytomegalovirus infection *in vitro*: Evidence for a dual mechanism of induction. *Am. J. Pathol.* **138**, 1209.
105. Fisfalen, M. E., Franklin, W. A., DeGroot, L. J. *et al*. (1990). Expression of HLA ABC and DR antigens in thyroid neoplasia and correlation with mononuclear leukocyte infiltration. *J. Endocrinol. Invest.* **13**, 41.
106. Redondo, M. J., Rewers, M., Yu, L., *et al*. (1999). Genetic determination of islet cell autoimmunity in monozygotic twin, dizygotic twin, and non-twin siblings of patients with type 1 diabetes: Prospective twin study. *Br. Med. J.* **318**, 698.
107. Dexhage, H. A., Botazzo, G. F., and Doniach, D. (1980). Evidence for thyroid-growth-stimulating immunoglobulins in some goitorous thyroid diseases. *Lancet* **2**, 287.
108. Shechter, Y., Maron, R., Elias, D., and Cohen, I. R. (1982). Autoantibodies to insulin receptor spontaneously develop as anti-idiotypes in mice immunized with insulin. *Science* **216**, 542.
109. Erlanger, B. F., Cleveland, W. L., Wassermann, N. H., *et al*. (1987). Autoantibodies to receptors by an auto-anti-idiotypic route. *Ann. N.Y. Acad. Sci.* **505**, 416.
110. Homcy, C. J., Rockson, S. G., and Haber, E. (1982). An anti-idiotypic antibody that recognizes the beta-adrenergic receptor. *J. Clin. Invest.* **69**, 1147.
111. Korn, I. L., and Abramsky, O. (1981). Myasthenia gravis following viral infection. *Eur. Neurol.* **20**, 435.
112. Lentz, T. L., Burrage, T. G., Smith, A. L., Crick, J., and Tignor, G. H. (1982). Is the acetylcholine receptor a rabies virus receptor? *Science* **215**, 182.
113. el Roiey, A., Sela, O., Isenberg, D. A., *et al*. (1987). The sera of patients with *Klebsiella* infections contain a common anti-DNA idiotype (16/6) Id and anti-polynucleotide activity. *Clin. Exp. Immunol.* **67**, 507.
114. Shoenfeld, Y., and Mozes, E. (1990). Pathogenic idiotypes of autoantibodies in autoimmunity: Lessons from new experimental models of SLE. *FASEB J.* **4**, 2646.
115. Shoenfeld, Y., Blank, M., Mendlovic, S., and Mozes, E. (1990). The role of anti-DNA antibody idiotypes in SLE. *J. Immunol.* **51**, 313.
116. Bakimer, R., Fishman, P., Blank, M., *et al*. (1992). Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anticardiolipin antibody (H-3). *J. Clin. Invest.* **89**, 1558.
117. Blank, M., Tomer, Y., Stein, M., *et al*. (1995). Immunization with anti-neutrophil cytoplasmic antibody (ANCA) induces the production of mouse ANCA and perivascular lymphocyte infiltration. *Clin. Exp. Immunol.* **102**, 120.
118. Tomer, Y., Gilburd, B., Sack, J., *et al*. (1996). Induction of thyroid autoantibodies in naive mice by idiotypic manipulation. *Clin. Immunol. Immunopathol.* **78**, 180.
119. Shoenfeld, Y., Gilburd, B., and Hojnik, M. (1996). Induction of Goodpasture antibodies to noncollagenous domain (NC1) of type IV collagen in mice by idiotypic manipulation. *Hum. Antibodies Hybridomas* **6**, 122.
120. Roncarolo, M. G., and Levings, M. K. (2000). The role of different subsets of T regulatory cells in controlling autoimmunity. *Curr. Opin. Immunol.* **12**, 676.
121. Mosmann, T. R., and Sad, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* **17**, 138.
122. Amital, H., Levy, Y., Blank, M., *et al*. (1998). Immunomodulation of murine experimental SLE-like disease by interferon-Y. *Lupus* **7**, 445.
123. Sinigaglia, F., D'Ambrosio, D., Panina-Bordignon, P., and Rogge, L. (1999). Regulation of the IL-12/IL-12R axis: A critical step in T-helper cell differentiation and effector function. *Immunol. Rev.* **170**, 65.

124. Swain, S. L. (1999). Helper T cell differentiation. *Curr. Opin. Immunol.* **11**, 180.
125. Barak, V., and Shoenfeld, Y. (1999). Cytokines in autoimmunity. *In* "The Decade of Autoimmunity" (Y. Shoenfeld, ed.), pp. 313–323. Elsevier, Amsterdam.
126. Romagnani, S. (1995). Biology of human Th1 and Th2 cells. *J. Clin. Immunol.* **15**, 121.
127. Segal, R., Bermas, B. L., Dayan, M., *et al*. (1997). Kinetics of cytokine production in experimental systemic lupus erythematosus: Involvement of T helper cell 1/T helper cell 2-type cytokines in disease. *J. Immunol.* **158**, 3009.
128. Amital, H., Levi, Y., Blank, M., *et al*. (1998). Immunomodulation of murine experimental SLE-like disease by interferon-gamma. *Lupus* **7**, 445.
129. Llorente, L., Richaud Patin, Y., *et al*. (2000). Clinical and biologic effects of antiinterleukin-10 monoclonal antibody administration in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 1790.
130. Alcocer-Varela, J. (2001). Main role of cytokines in autoimmunity. *Isr. Med. Assoc. J.* **3**, 374.
131. Llorente, L., Richaud-Patin,Y., Fior, R., *et al*. (1994). In vivo production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus: A potential mechanism of B lymphocyte hyperactivity and autoimmunity. *Arthritis Rheum.* **37**, 1647.
132. Ramanthan, S., de Kozak, Y., Saoudi, A., *et al*. (1996). Recombinant IL-4 aggravates experimental autoimmune uveoretinitis in rats. *J. Immunol.* **157**, 2209.
133. Tang, H., Mignon-Gdefroy, K., Garotta, G., Charreire, J., and Nicoletti F. (1993). The effects of a monoclonal antibody to interferon-γ on experimental autoimmune thyroiditis (EAT): Prevention of disease and decrease of EAT-specific T cells. *Eur. J. Immunol.* **23**, 275.
134. Boissier, M. C., Chiocchia, G., Bessis, N., *et al*. (1995). Biphasic effect of interferon-γ in murine collagen-induced arthritis. *Eur. J. Immunol.* **25**, 1184.
135. Inobe, J., Slavin, A. J., Komagata, Y., *et al*. (1998). IL-4 is a differentiation factor for transforming growth factor-beta secreting Th3 cells and oral administration of IL-4 enhances oral tolerance in experimental allergic encephalomyelitis. *Eur. J. Immunol.* **28**, 2780.
136. Blank, M., George, J., Barak, V., *et al*. (1998). Oral tolerance to low dose β2-glycoprotein-I: Immunomodulation of experimental antiphospholipid syndrome. *J. Immunol.* **161**, 5303.
137. Weinblatt, M. E., Kremer, J. M., Bankhurst, A. D., *et al*. (1999). A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N. Engl. J. Med.* **340**, 253.
138. Maini, R. N., Breedveld, F. C., Kalden, J. R., *et al*. (1998). Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* **41**, 1552.
139. Sonderstrup, G., and McDevitt, H. O. (2001). DR, DQ, and you: MHC alleles and autoimmunity. *J. Clin. Invest.* **107**, 871.
140. Wang, C. Y., Davoodi-Semiromi, A., Huang, W., *et al*. (1998). Characterization of mutations in patients with autoimmune polyglandular syndrome type 1 (APS1). *Hum. Genet.* **103**, 681.
141. Walport, M. J. (2001). Complement—Second of two parts. *N. Engl. J. Med.* **344**, 1140.
142. Becker, K. G. (1999). Comparative genetics of type 1 diabetes and autoimmune disease: Common loci, Common pathways? *Diabetes* **48**, 1353.
143. Molokhia, M., and McKeigue, P. (2000). Risk for rheumatic disease in relation to ethnicity and admixture. *Arthritis Res.* **2**, 115.
144. Casciola-Rosen, L. A., Anhalt, G., and Rosen, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317.
145. Rosen, A., and Casciola-Rosen, L. (2001). Clearing the way to mechanisms of autoimmunity. *Nature Med.* **7**, 664.
146. Verhoven, B., Schlegel, R. A., and Williamson, P. (1995). Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. *J. Exp. Med.* **182**, 1597.
147. Mevorach, D., Zhou, J. L., Song, X., and Elkon, K. B. (1998). Systemic exposure to irradiated apoptotic cells induces autoantibody production. *J. Exp. Med.* **188**, 387.
148. Rodenburg, R. J., Raats, J. M., Pruijn, G. J., and van Venrooij, W. J. (2000). Cell death: A trigger of autoimmunity? *Bioessays* **22**, 627.
149. Greidinger, E. L. (2001). Apoptosis in lupus pathogenesis. *Front. Biosci.* **6**, 1392.
150. Botto, M. (2001). Links between complement deficiency and apoptosis. *Arthritis Res.* **3**, 207.
151. Mehrian, R., Quismorio, F. P., Jr., Strassmann, G., *et al*. (1998). Synergistic effect between IL-10 and bcl-2 genotypes in determining susceptibility to systemic lupus erythematosus. *Arthritis Rheum.* **41**, 596.
152. D'Alfonso, S., Rampi, M., Bocchio, D., *et al*. (2000). Systemic lupus erythematosus candidate genes in the Italian population: Evidence for a significant association with interleukin-10. *Arthritis Rheum.* **43**, 120.
153. Restifo, N. P. (2000). Building better vaccines: How apoptotic cell death can induce inflammation and activate innate and adaptive immunity. *Curr. Opin. Immunol.* **12**, 597.
154. Nagata, S., and Golstein, P. (1995). The Fas death factor. *Science* **267**, 1449.
155. Suzuki, N., Ichino, M., Mihara, S., Kaneko, S., and Sakane, T. (1998). Inhibition of Fas/Fas ligand-mediated apoptotic cell death of lymphocytes *in vitro* by circulating anti-Fas ligand autoantibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **41**, 344.
156. Kim, Y. T., Puntillo, E., DeBlasio, T., Weksler, M. E., and Siskind, G. W. (1987). Regulation of antibody production by hybridoma cultures. I. Anti-idiotype antibody-

mediated down- regulation of anti-DNA antibody production by hybridoma cells. *Cell. Immunol.* **105**, 65.
157. Roubaty, C., Bedin, C., and Charreire (1990). Prevention of experimental autoimmune thyroiditis through the anti-idiotypic network. *J. Immunol.* **144**, 2167.
158. Brown, C. A., Carey, K., and Colvin, R. B. (1979). Inhibition of autoimmune tubulo-interstitial nephritis in guinea by heterologous antisera containing anti-idiotypic antibodies. *J. Immunol.* **123**, 2102.
159. Rauch, J., Murphy, E., Roths, J. B., Stollar, B. D., and Schwartz, R. S. (1982). A high frequency idiotypic marker of anti-DNA autoantibodies in MRL-Ipr/Ipr mice. *J. Immunol.* **129**, 236.
160. Mahana, W., Guilbert, B., and Avrameas, S. (1987). Suppression of anti-DNA antibody production in MRL mice by treatment with anti-idiotypic antibodies. *Clin. Exp. Immunol.* **70**, 538.
161. Blank, M., Mansori, J., Tomer, Y., *et al*. (1994). Suppression of experimental systemic lupus erythematosus (SLE) with specific anti-idiotypic antibody-saporin conjugate. *Clin. Exp. Immunol.* **98**, 434.
162. Fishman, P., Bar-Yehuda, S., and Shoenfeld, Y. (2002). IVIG to prevent tumor metastases (review). *Int. J. Oncol.* **21**, 875.
163. Fishman, P., and Shoenfeld, Y. (2001). Intravenous immunoglobulin (IVIg) as an inhibitor of tumor growth: From autoimmunity to cancer. *In* "Old Herborn University Seminar Monograph" (P. J. Heidt, V. D. Rusch, and D. van der Waaij, eds.), pp. 93–107. Herborn Litterae.
164. Shulman, J., Pierce, M. I., and Lukens, A. (1960). Studies on thrombopoiesis. I. A factor in normal human plasma required for platelet production. Chronic thrombocytopenia due to its deficiency. *Blood* **16**, 343.
165. Imbach, P., Barandun, S., d'Apuzzo, V., *et al*. (1981). High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. *Lancet* **1**, 1228.
166. Hughes, R. A. (1996). Intravenous IgG in Guillain–Barre syndrome. *Br. Med. J.* **313**, 376.
167. Gajdos, P., Chevret, S., Clair, B., Tranchant, C., and Chastang, C. (1997). Clinical trial of plasma exchange and high-dose intravenous immunoglobulin in myasthenia gravis. *Ann. Neurol*. **41**, 789.
168. Dalakas, M. C., Illa, I., Dambrosia, J. M., *et al*. (1993). A controlled trial of high-dose intravenous immune globulin infusions as treatment for dermatomyositis. *N. Engl. J. Med.* **329**, 1993.
169. Dalakas, M. C. (1998). Mechanism of action of intravenous immunoglobulin and therapeutic considerations in the treatment of autoimmune neurologic diseases. *Neurology* **51**, S2.
170. Newburger, J. W., Takahashi, M., Burns, J. C., *et al*. (1986). The treatment of Kawasaki syndrome with intravenous gamma globulin. *N. Engl. J. Med.* **315**, 341.
171. Jayne, D. R., Chapel, H., Adu, D., *et al*. (2000). Intravenous immunoglobulin for ANCA-associated systemic vasculitis with persistent disease activity. *QJM* **93**, 433.
172. Rosenbaum, J. T., George, R. K., and Gordon, C. (1999). The treatment of refractory uveitis with intravenous immunoglobulin. *Am. J. Ophthalmol.* **127**, 545.
173. Rauova, L., Rovensky, J., and Shoenfeld, Y. (2003). Immunomodulation of autoimmune diseases by high-dose intravenous immunoglobulins. *Spring. Semin. Immuopathol.*
174. Giannini, E. H., Lovell, D. J., Silverman, E. D., *et al*. (1996). Intravenous immunoglobulin in the treatment of polyarticular juvenile rheumatoid arthritis: A phase I/II study. *J. Rheumatol.* **23**, 919.
175. Corvetta, A., Della Bitta, R., Gabrielli, A., Spaeth, P. J., and Danieli, G. (1989). Use of high dose intravenous immunoglobulin in systemic lupus erythematosus: Report of three cases. *Clin. Exp. Rheumatol.* **7**, 295.
176. Levy, Y., Sherer, Y., George, J., *et al*. (2000). Intravenous immunoglobulin treatment of lupus nephritis. *Semin. Arthritis Rheum.* **29**, 321.
177. Levy, Y., Sherer, Y., Ahmed, A., Langevitz, P., *et al*. (1999). A study of 20 SLE patients with intravenous immunoglobulin: Clinical and serologic response. *Lupus* **8**, 705.
178. Said, P. B., Martinuzzo, M. E., and Carreras, L. O. (1992). Neutralization of lupus anticoagulant activity by human immunoglobulin "*in vitro*." *Nouv. Rev. Fr. Hematol.* **34**, 37.
179. Krause, I., Blank, M., Kopolovic, J., *et al*. (1995). Abrogation of experimental systemic lupus erythematosus and primary antiphospholipid syndrome with intravenous gamma globulin. *J. Rheumatol.* **22**, 1068.
180. Panto, F., Giordano, C., and Amato, M. P. (1990). The influence of high dose intravenous immunoglobulins on immunological and metabolic pattern in newly diagnosed type I diabetic patients. *J. Autoimmun.* **3**, 587.
181. Baschieri, L., Antonelli, A., Nardi, S., *et al*. (1997). Intravenous immunoglobulin versus corticosteroid in treatment of Graves' ophthalmopathy. *Thyroid* **7**, 579.
182. Comi, G., Nemni, R., Amadio, S., Galardi, G., and Leocani, L. (1997). Intravenous immunoglobulin treatment in multifocal motor neuropathy and other chronic immune-mediated neuropathies. *Mult. Scler.* **3**, 93.
183. Sherer, Y., Levy, Y., Langevitz, P., *et al*. (2001). Adverse effects of intravenous immunoglobulin therapy in 56 patients with autoimmune diseases. *Pharmacology* **62**, 133.
184. Friedman, A., and Weiner, H. (1994). Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc. Natl. Acad. Sci. USA* **91**, 6688.
185. Whitacre, C. C., Gienapp, I. E., Orosz, C. G., and Bitar, D. (1991). Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. *J. Immunol.* **147**, 2155.
186. Chen, Y., Inobe, J., Marks, R., *et al*. (1995). Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature* **377**, 257.
187. Shoenfeld, Y., and Cervera, R. (1999). Innovations in autoimmunity in the last decade. *In* "The Decade of

Autoimmunity" (Y. Shoenfeld, ed.), pp. 7–29. Elsevier, Amsterdam.
188. Benson, J. B., Campbell, M. K., Guan, Z., *et al*. (2000). T-cell activation and receptor downmodulation precede deletion induced by mucosally administered antigen. *J. Clin. Invest.* **106**, 1031.
189. Chen, Y., Kuchroo, V. K., Inobe, J. I., Hafler, D. A., and Weiner, H. L. (1994). Regulatory T-cell clones induced by oral tolerance: Suppression of autoimmune encephalomyelitis. *Science* **265**, 1237.
190. Thompson, H. S. G., and Staines, N. A. (1986). Gastric administration of type II collagen delays the onset of severity of collagen-induced arthritis in rats. *Clin. Exp. Immunol.* **64**, 581.
191. Lieder, O., Santos, L. B. M., and Lee, C. S. Y. (1989). Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. II. Suppression of disease and *in vitro* immune responses is mediated by antigen-specific $CD8^+$ lymphocytes. *J. Immunol.* **142**, 748.
192. Myers, L. K., Higgins, G. C., and Finkel, T. H., *et al*. (2001). Juvenile arthritis and autoimmunity to type II collagen. *Arthritis Rheum.* **44**, 1775.
193. Choy, E. H., Scott, D. L., Kingsley, G. H., *et al*. (2001). Control of rheumatoid arthritis by oral tolerance. *Arthritis Rheum.* **44**, 1993.
194. Whitacre, C. C., Gienapp, I. E., Meyer, A., Cox, K. L., and Javed, N. (1996). Treatment of autoimmune disease by oral tolerance to autoantigens. *Clin. Immunol. Immunopathol.* **80**, S31.
195. Faria, A. M. C., and Weiner, H. L. (1999). Oral tolerance: Mechanisms and therapeutic applications. *Adv. Immunol.* **73**, 153.
196. Pozzilli, P., and Gisella Cavallo, M. (2000). Oral insulin and the induction of tolerance in man: Reality or fantasy? *Diabetes Metab. Res. Rev.* **16**, 306.
197. Yin, J. A., and Jowitt, S. N. (1992). Resolution of immune-mediated diseases following allogeneic bone marrow transplantation for leukaemia. *Bone Marrow Transplant.* **9**, 31.
198. Sherer, Y., and Shoenfeld, Y. (1998). Stem cells transplantation: A cure for autoimmune disease. *Lupus* **7**, 137.
199. Traynor, A. E., Schroeder, J., Rosa, R. M., *et al*. (2000). Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: A phase I study. *Lancet* **356**, 701.
200. Hiepe, F., Rosen, O., Thiel, A., *et al*. (1999). Successful treatment of refractory systemic lupus erythematosus (SLE) by autologous stem cell transplantation (ASCT) with in vivo immunoablation and ex vivo depletion of mononuclear cells. *Arthritis Rheum.* **42**, S170.
201. Tyndall, A., and Gratwohl, A. (2000). Immune ablation and stem-cell therapy in autoimmune disease: Clinical experience. *Arthritis Res.* **2**, 276.
202. Teitelbaum, D., Aharoni, R., Sela, M., and Arnon, R. (1991). Cross-reactions and specificities of monoclonal antibodies against myelin basic protein and against the synthetic copolymer 1. *Proc. Natl. Acad. Sci. USA* **88**, 9528.
203. Aharoni, R., Teitelbaum, D., Sela, M., and Arnon, R. (1997). Copolymer 1 induces T cells of the T helper type 2 that crossreact with myelin basic protein and suppress experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* **94**, 10821.
204. Johnson, K. P., Brooks, B. R., Cohen, J. A. *et al*. (1998). Extended use of glatiramer acetate (Copaxone) is well tolerated and maintains its clinical effect on multiple sclerosis relapse rate and degree of disability. *Neurology* **50**, 701.
205. Singh, R. R., Ebling, F. M., Sercarz, E. E., and Hahn, B. H. (1995). Immune tolerance to autoantibody-derived peptides delays development of autoimmunity in murine lupus. *J. Clin. Invest.* **96**, 2990.
206. Kaliyaperumal, A., Michaels, M. A., and Datta, S. K. (1999). Antigen-specific therapy of murine lupus nephritis using nucleosomal peptides: Tolerance spreading impairs pathogenic function of autoimmune T and B cells. *J. Immunol.* **162**, 5775.
207. Waisman, A., Ruiz, P. J., Israeli, E., *et al*. (1997). Modulation of murine systemic lupus erythematosus with peptides based on complementarity determining regions of a pathogenic anti-DNA monoclonal antibody. *Proc. Natl. Acad. Sci. USA* **94**, 4620.
208. Blank, M., Shoenfeld, Y., Cabilly, S., *et al*. (1999). Prevention of experimental antiphospholipid syndrome and endothelial cell activation by synthetic peptides. *Proc. Natl. Acad. Sci. USA* **96**, 5164.
209. McNeeley, P. A., Iverson, G. M., Furie, R. A., *et al*. (2001). Pre-treatment affinity for LJP 394 influences pharmacodynamic response in lupus patients. *Lupus* **10**, 526.
210. Krause, I., Blank, M., and Shoenfeld, Y. (2000). Peptide immunotherapy in autoimmune diseases. *Drug News Perspect.* **13**, 78.
211. Harper, J. M., and Cook, A. (2001). Beneficial effects of non-depleting anti-CD4 in MRL/Mp-lpr/lpr mice with active systemic lupus erythematosus and microscopic angiitis. *Autoimmunity* **33**, 245.

2

OVERVIEW OF CELLULAR IMMUNE FUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

George C. Tsokos

INTRODUCTION

Systemic lupus erythematosus (SLE) is considered a prototypic human autoimmune disease, which manifests itself with a variety of fascinating clinical and immunological features. Although the causes of SLE, and autoimmunity in general, remain unknown, considerable evidence has been accumulated on the pathophysiologic mechanisms, which lead to the failure of distinction between self and self +X and the production of autoantibodies. Genetic, hormonal, and environmental factors have been attributed certain roles in the etiology of autoimmunity. Both cellular and hormonal abnormalities have been identified and participate in the pathogenesis of SLE. Our understanding of the nature of these abnormalities has advanced along with our perception of the complexity of the immune system. In human autoimmune disease, immune aberrations vary from the production of a single autoantibody in the absence of any other detectable immune abnormalities to the production of multiple autoantibodies accompanied by universal derangement of the immune system. An example of the former case is membranoproliferative glomerulonephritis, which is characterized by the production of a unique antialternative pathway C3 convertase [1]. The latter is typified by SLE.

Our concept of autoimmunity has evolved gradually over the past century. The immune response against self was considered harmful ("horror autotoxicus") at the turn of the century [2]. In an effort to explain the prevention of self-reactivity, Burnet formulated a hypothesis according to which autoreactive clones ("forbidden clones") are eliminated during the ontogenic process [3]. Data have provided evidence that the autoimmune response is part of the normal immune response [4].

Current concepts on the cellular basis of autoimmunity include a deficit in the active process of immune regulation [5–7], participation of oligoclonal (restricted) T-cell populations [8], selection or exclusion of parts of the T-cell receptor (TCR) repertoire, which leads to the expression of "forbidden," autoreactive TCR genes [9, 10], genetic factors [11–13], defects in apoptosis [14, 15], aberrations in costimulation [16–19], abnormalities in lymphokine production [7, 20–23], and abnormalities in immune cell signaling and gene transcription [24–26].

Our efforts to understand the contribution of cellular abnormalities in the immunopathogenesis of SLE are further complicated by (a) the clinical heterogeneity of the disease; (b) patient sampling, which is particularly true when most reports are made on one or two dozens of patients; (c) the heterogeneity in the nature of the prevailing autoantibodies and their presumed ability to alter immune cell function; (d) the fact that the deranged immune response may become normalized during periods of disease inactivity; and (e) drugs that are frequently used in the treatment of SLE patients modulate the immune response and thus complicate patient sampling and interpretation of the results that are obtained from *in vitro* studies.

This chapter attempts to provide the reader with a complete systematic description of the cellular immune defects and aberrations that have been reported in SLE and discusses their relative contribution to the pathophysiology of the disease.

MONOCYTE/MACROPHAGE FUNCTION

Macrophages/monocytes serve, along with B lymphocytes and dendritic cells, as antigen-presenting cells (APC). Macrophages express major histocombatibilty genes (MHC) class II molecules that are recognized by the helper/inducer T cells in conjunction with processed antigen fragments.

Monocytes from patients with SLE display diminished phagocytic activity that does not increase upon stimulation *in vitro* with lipopolysaccharide [27]. Serum factors and particularly circulating immune complexes may be responsible for the deficient phagocytic function in lupus patients.

Monocytes from patients with SLE produce decreased amounts of interleukin (IL)-1 [28]. This defect can be corrected by indomethacin, indicating that excessive prostaglandin production suppresses the production of IL-1. Production of IL-1β by stimulated and nonstimulated SLE monocytes is decreased regardless of disease activity [29]. Activated monocytes produce prostaglandins (PGE2), which inhibit IL-1 production [30], mononuclear cells produce a glycoprotein inhibitor of the action of IL-1 [30], and immune complexes stimulate monocytes to produce IL-1 and the IL-1 inhibitor [31]. It is unclear why in the presence of huge amounts of circulating immune complexes SLE monocytes do not produce the IL-1 receptor antagonist. Molecular data are needed to interpret the role or the cause of deficient IL-1 production in monocyte dysfunction in SLE.

The percentage of monocytes in Ficoll-isolated peripheral mononuclear cells (MNC) from patients with SLE is higher than that from normals (detected by staining with α-naphthylesterase, unpublished experiments [27]). Removal of monocytes from culture systems that were set up to test a number of cellular immune functions *in vitro* has led to the amelioration of defective responses [32]. Reduction of the percentage of monocytes improves the proliferative response of lupus MNC to PHA [33, 34], the proliferative response in autologous mixed lymphocyte reaction (AMLR) [35], the pokeweed mitogen-induced immunoglobulin (Ig) synthesis by SLE MNC [36], the mixed lymphocyte reaction proliferative response and T lymphocyte-mediated lympholysis [37], the pokeweed mitogen-induced plaque-forming cell responses of peripheral SLE MNC [38], the anti-DNA antibody synthesis [39, 40], and the Ig production by lupus B cells [41]. SLE monocyte-derived culture supernatants inhibit profoundly normal T-cell responses [34]. These experiments indicate that *in vivo*-preactivated monocytes secrete prostaglandins and/or other inhibitory molecules that interfere with the *in vitro* function of lymphocytes. Adjustment for optimal numbers of monocytes in various culture systems is hence mandatory to avoid improper evaluation of experimental data. Indeed, the production of eicosanoids such as PGE_2 and thromboxane B_2 by SLE monocytes is increased [42].

Peripheral blood monocytes from patients with active disease exhibit decreased expression of surface human leukocyte antigen (HLA)-DR antigens and decreased accessory function for T-cell activation [43–46]. *In vitro*, SLE sera reduce the accessory function of normal monocytes. This suggests the existence of circulating factors (anti-macrophage antibodies [47], immune complexes, or other) that are responsible for the impaired macrophage function in patients with SLE. Does SLE serum affect the function of monocytes? Some reports indicate that SLE serum is toxic to monocytes and prohibits its function and differentiation [46], whereas others have suggested that SLE serum promotes monocytes differentiation to dendritic cells [48]. The latter report attributed the effect of the SLE serum on the presence of interferon (INF)-α. The production of INF-α has been claimed to be increased by earlier reports [49] and the previously mentioned report and decreased by others [50]. Reasons for the diversity in the reported levels of interferon-α are many, including technical and patient cohort differences. Reasons for the reported increased differentiation or lack thereof are unclear. The most obvious explanation may relate to the fact that the report by Blanco *et al*. [48] included only pediatric patients with SLE.

SLE monocytes cultured in optimal percentages with allogeneic or autologous T lymphocytes express a decreased ability to induce a proliferative response. Prothymosin-α, a thymic preparation, normalizes these responses by direct action on monocytes [51]. A similar defect, also correctable by prothymosin-α, has been described in patients with multiple sclerosis. Monocytes from patients with multiple sclerosis have decreased numbers of surface membrane HLA-DR molecules. Decreased HLA-DR expression on the surface membrane of monocytes from patients with multiple sclerosis is associated with decreased AMLR [52]. Preculture of monocytes from multiple sclerosis patients with prothymosin-α enhances DR expression and normalizes the AMLR response [53]. More definitive data are needed to establish the status of expression of DR

TABLE 1 Macrophage Function in SLE

Decreased MHC class II surface expression
Decreased expression of costimulatory molecules (CD80)
Decreased expression of CD44 and Fcγ receptors
Increased production of eicosanoids
Decreased production of IL-1β and receptor antagonist
Increased spontaneous apoptosis
Decreased ability to stimulate in AMLR
Increased propensity to differentiate to dendritic cells
Increased suppressor cell function of *in vitro* immune cellular responses
Decreased phagocytosis

molecules on the surface of lupus monocytes and their role in the development of the disease. Defects in the expression of costimulatory molecules (CD80, B7-1) are discussed later. Peripheral blood monocytes from lupus patients display increased rates of spontaneous apoptosis [54]. Apoptosis of lupus cells is also discussed later.

In conclusion (Table 1), lupus monocytes produce less IL-1 and express decreased amounts of surface HLA-DR antigens and possibly costimulatory molecules. These abnormalities may interfere with their ability to process and present antigen and provide appropriate costimulation to lymphocytes. Decreased phagocytic function may contribute to the established [55] increased susceptibility of lupus patients to infections.

The expression of CD44, a molecule believed to participate in the clearance of apoptotic cells, is decreased in patients with SLE [56]. Decreased clearance of apoptotic debris can contribute to excessive stimulation of immune cells in SLE. Decreased expression of Fcγ receptors (II and III) may also account for the decreased clearance of immune complexes [57] and antibody-covered apoptotic bodies.

DENDRITIC CELLS

The numbers and function of dendritic cells have been studied in patients with SLE. Two studies present opposing results. In the first one, dendritic cell numbers, defined as $Lin^{-}DR^{+}CD4^{+}$ cells, and function, evaluated in a mixed lymphocyte reaction, were decreased in patients with SLE. In addition, the surface expression of B7 and CD40 was reduced in SLE dendritic cells [58]. In the second study, both the numbers and function of dendritic cells were found to be increased [48]. Besides methodological differences, the fact that the first study used adults whereas the second one used children with SLE may account for the differences. If dendritic cell function proves to differentiate pediatric and adult lupus SLE, then more studies will be needed to understand the differences between the two disease subsets.

TABLE 2 Overview of T Cell Abnormalities in Patients with SLE

T-cell numbers
T lymphopenia
Increased numbers of $DR^{+}CD4^{+}$ and DR^{+} $CD8^{+}$
Increased numbers of $CD3^{+}CD4^{-}CD8^{-}$ cells
$CD4^{+}$ T-cell epitope deficiency
T-cell function
Increased helper cell activity expressed by both $CD4^{+}DR^{+}$ and $CD3^{+}CD4^{-}D8^{-}$ cells
Normal concanavalin A suppressor cell function of polyclonal B-cell responses
Deficient concanavalin A-induced suppressor cells of antigen-specific responses
Deficient EBV-associated suppressor/cytotoxic cell function
Anti-T-cell antibodies
Anti-$CD4^{+}$ antibodies
Anti-$CD8^{+}$ antibodies
Antiblast T-cell antibodies
Anticytotoxic T-cell antibodies
Anti-NK cell antibodies
Anti-lupus-associated membrane protein (LAMP)
Anti-CD45 antibodies

T LYMPHOCYTES

T-cell abnormalities are multiple and prominent in patients with SLE. T-cell abnormalities are crucial in the pathogenesis of the disease because T cells regulate B-cell function and the production of most of the pathogenic autoantibodies is T cell dependent. T lymphocyte abnormalities in patients with lupus are summarized in Table 2.

Lymphocyte Numbers

Lymphopenia is common in SLE and its severity relates to disease activity [59–61]. Peripheral B lymphocytes are present in normal numbers, whereas the absolute number of T lymphocytes is decreased. The decrease is often marked in the suppressor/cytotoxic lymphocyte subpopulation as defined by the presence of cell surface markers recognized by monoclonal antibodies or appropriate functional assays. The number of circulating helper T lymphocytes (Tμ, $OKT4^{+}$, $Leu\text{-}3A^{+}$, $CD4^{+}$) is less affected. A preferential loss of a subset of

T lymphocytes with the higher density of cell surface markers has been reported [62]. Anti-T lymphocyte antibodies have been found in SLE sera and may be responsible for the elimination of T cells [63–66]. The ratio of helper/suppressor cells in SLE has been reported to be increased in a nonconsistent manner. In one of the studies, low, normal, and high ratios were found in 25, 50, and 25% of patients, respectively [67]. Other investigators have reported that the most commonly observed abnormality in untreated patients with active SLE is a relative decrease in $CD4^+$ (helper/inducer) lymphocytes, whereas decreased percentages of $CD8^+$ (suppressor/cytotoxic) cells were observed in less than 10% of more than 100 patients with SLE [68].

The OKM1 monoclonal antibody recognizes an epitope of the CR3 (receptor binding iC3b). CR3 is expressed on macrophages, monocytes, glanulocytes, and natural killer (NK) cells [69, 70]. A subpopulation of $CD3^+$ lymphocytes also carries an antigen recognized by the OKM1 monoclonal antibody [71]. Ficoll-prepared MNC from healthy individuals that express CR3 may be classified in those carrying a receptor for the Fc portion of IgG (FcR^+) and those carrying CD3. In healthy individuals, 60% of the $CR3^+$ cells are FcR^+ and 18% are $CD3^+$ [71]. In patients with SLE, only 33% are FcR^+ and 50% coexpress CD3 antigen. Of the $CD3^+CR3^+$ cells, only 14% were $CD4^+$ and 44% were $CD8^+$. This expanded $CD3^+CR3^+$ lymphocyte subpopulation in SLE does not contain precursors for NK cells because stimulation of this subpopulation with either IL-2 or IFN-γ does not induce the generation of NK activity [71]. Interestingly, $CD3^+CR3^+$ cells do not fluctuate with the degree of lymphopenia and disease activity in patients with SLE. In contrast, $CD3^+CD4^+CR3^+$ cells increase during periods of disease activity and might provide help to B cells to produce Ig [72].

In conclusion, various T-cell subsets have been described to be decreased in lupus patients. Lymphopenia correlates with disease activity. The major controversy regarding lymphocyte subset numbers in SLE relates to whether they contribute to immunoregulatory imbalances or whether they represent secondary effects of autoantibodies against various cellular antigens.

T Lymphocyte CD4 Epitope Deficiency

Expression of the CD4 molecule on the surface of T cells demonstrates heterogeneity. Expression of CD4 molecules on the surface membrane of T cells from African-Americans but not Caucasian individuals may be normal, intermediate, or deficient. The mode of inheritance of the expression of CD4 molecules is autosomal dominant [73, 74]. Stohl and Kunkel [75] described three black Jamaicans with SLE who lacked $CD4^+$ T cells. A culture of peripheral MNC cells from these patients in the presence or absence of antigens did not facilitate CD4 expression among T cells. Lymphadenopathy was part of the lupus syndrome [75]. Subsequently, another patient with thymoma, hypogammaglobulinemia, and red blood cell aplasia was described who lacked the $CD4^+$ epitope from peripheral blood T cells [76]. Ten more patients with CD4 epitope deficiency were reported from Saudi Arabia [77]. Eight patients had SLE and two had rheumatoid arthritis. Of the patients with SLE, two had IgA deficiency and one had C4 deficiency. The CD4 epitope deficiency is not associated with any HLA haplotype. The CD4 epitope deficiency in the rare patients who were described earlier implies genetic basis for the disease.

Functional Features of Anti-T-Cell Antibodies

Antilymphocyte antibodies found in SLE patient sera are heterogeneous because they recognize different T-cell subsets, B cells, monocytes, and NK cells. Some lymphocytotoxic antibodies are directed against thymocytes [64]. Circulating anti-T-cell antibodies were first found to inhibit concanavalin A-induced suppressor T cells [80]. A T-cell subset, which was identified by gradient centrifugation, was found to be missing from patients whose sera contained IgM anti-T-cell antibodies [62]. Subsequently, Sakane *et al*. [81–83] reported studies that documented that anti-T-cell antibodies were responsible for the decreased suppressor cell function in these patients. In further studies in which murine monoclonal antibodies were used to identify human T-cell subsets, anti-T-cell antibodies in SLE sera were further characterized and were found to target the suppressor–inducer CD4 subpopulation [84]. However, other studies using highly purified peripheral $T4^+$, $T8^+$, autologous rosette-forming cells, or $T4^+$ and $T8^+$ clones as targets in cytotoxicity assays showed almost no difference in killing between SLE and normal sera [85–87].

More definitive studies [79, 88] have shown that SLE sera-derived IgM antilymphocyte antibodies recognize different isoforms of the CD45 molecule (molecular mass ~200 Da). CD45 is pivotal in T-cell signaling during stimulation because it has protein tyrosine phosphatase activity [89, 90]. Indeed, the protein tyrosine phosphatase activity of anti-CD45 antibody precipitates from lupus lymphocytes was found to be

decreased significantly. Decreased phosphatase activity correlated with disease activity but not with the dose of prednisolone. The CD45 protein content was not decreased, suggesting that enzymatic activity was affected [91].

IgM antilymphocyte antibodies have also been shown to recognize separate specificities on intact or desialylated peripheral T cells [92]. Other experiments demonstrated that antibodies distinct from conventional cold-reactive cytotoxic antibodies target T-cell blasts and interfere with the expression of interleukin 2 receptors [92]. These antibodies might interfere with the development of IL-2-dependent immune responses in patients with SLE. Anti-T-cell antibodies have long been known to inhibit mixed lymphocyte reactions [65] and cytotoxic responses to xenoantigens [93] and alloantigens [37], as well as NK cell activity [94]. IgG antilymphocyte antibodies potentiate antibody-dependent cell cytotoxicity [95]. Antilymphocyte antibodies may bind to lymphocytes by a non-Fc-mediated mechanism and by Fc to antibody-dependent cell-mediated cytotoxicity effector cells and cause lymphopenia. This explains why the presence of antilymphocyte antibodies correlates with both lymphopenia and decreased antibody-dependent cell-mediated cytotoxicity among SLE-derived MNC. The aforementioned discussion indicates that circulating anti-T lymphocyte antibodies in patients with SLE are heterogeneous.

Antilymphocyte antibodies have been found to cross-react with tissue antigens, including nuclear antigens [96–98], human brain tissue [99], and trophoblastic antigens [100]. This information suggests potential cross-reactivity between anti T-cell and other autoantibodies, including anti-DNA.

A murine monoclonal anti-DNA antibody was found to bind to a surface membrane structure (lupus-associated membrane protein, LAMP) of Raji cells [101] and other cell types, including normal human lymphocytes [102]. Similar antibodies were found in the sera of patients with SLE [103]. These findings have led to the formulation of a hypothesis whereby LAMP rather than DNA may trigger the autoimmune response.

Activated T Lymphocytes

Activated but not resting lymphocytes express markers such as DR antigens, IL-2, transferrin, insulin receptors, and other surface molecules that can be identified by monoclonal antibodies. Patients with SLE have been reported to have increased percentages of circulating DR^+ T lymphocytes. We have found increased expression of HLA-DR antigens on T cell from patients with active SLE. Both $CD4^+$ and $CD8^+$ T cells express increased levels of DR antigens. DR^+ cells are slightly larger than DR^- cells and provide help to autologous B cells to secrete immunoglobulin [104]. Another study demonstrated that DR^+ T cells are distributed preferentially among the $CD8^+$ subpopulation [105]. More recently, $CD8^+DR^+$ cells were found to correlate with disease activity [106].

The finding that T cells from patients with SLE express increased amounts of mRNA for protooncogenes (c-*myc,* c-*myb, and* c-*raf*) further supports the presence of activated T cells in these patients and implies a possible role for activated T cells in the expression of SLE [107–109]. Although lupus T cells express more surface DR antigens, they express fewer numbers of IL-2 receptors. The number of intermediate affinity IL-2-binding molecules p70/75 is decreased on the surface of freshly isolated T cells from lupus patients [110]. Lupus T cells do not proliferate *in vitro* in response to IL-2 [110]. Furthermore, stimulation of lupus T cells *in vitro* with phytohemagglutinin causes a decreased expression of high-affinity IL-2 receptors [111]. The cause for the discrepancy between the high levels of DR expression and the low levels of IL-2 receptor expression on the surface of lupus T cells is unknown. It may simply represent an activational arrest of lupus T cells at a phase between cells expressing DR antigens and cells expressing receptors for IL-2 or a basic defect of the transcription and expression of surface IL-2 receptors. Interpretation of decreased IL-2 receptor expression is complicated by the finding (see later) of increased levels of circulating soluble forms of the IL-2 receptors.

T Suppressor Lymphocyte Function

Numerous studies have been performed to test the hypothesis that deficient suppressor cell activity is responsible for the uncontrolled production of autoantibodies by B cells and the subsequent development of autoimmunity. Concanavalin A-induced suppressor cell function in patients with SLE has been reported to be deficient [112–117] or normal [118, 119]. Concanavalin A-induced suppressor cell activity has been tested in a number of systems, including proliferative responses [117, 120, 121] and pokeweed mitogen-induced plaque-forming cell responses [114, 116, 122–124]. Deficient suppressor cell activity has been shown in the aforementioned studies to correlate with disease activity, serum DNA binding, low serum C3 levels, and treatment status. In procainamide and diphenylhydantoin-induced SLE, suppressor cell function is normal [125, 126]. Spontaneous and mitogen-induced nonspecific suppressor cell activity becomes

normal during pregnancy in certain SLE patients [127].

Concanavalin A is a potent stimulus of T cells and, if not used under proper conditions (concentration, cell density, etc.), may not be sensitive enough to detect small differences among responding subpopulations. A number of studies have used more specific stimuli known to induce suppressor cell activity. EBV-associated suppressor/cytotoxic cell function is defective in patients with SLE who were seropositive to EBV [128]. Similarly, concanavalin A-activated T cells in some patients fail to suppress antitrinitrophenyl–*Brucella abortus* responses of autologous B cells [129].

Patients with SLE have severely impaired autologous mixed lymphocyte reaction responses (reviewed in Tsokos and Balow [32]). Deficient responses can be restored to normal levels if the responding cells are treated with exogenous IL-2 [130] or if the stimulatory non-T cells are pretreated with formalin-treated *S. aureus* or phorbol myristate acetate, suggesting that deficient responses may represent in part a failure of SLE non-T cells to present an appropriate stimulus for the generation of T-cell responses [131]. These studies indicate that (a) cellular abnormalities in patients with SLE can be detected when subtle stimuli (EBV, autoantigens) are used instead of more potent mitogens and (b) suppressor cell defects might be involved in the pathogenesis of SLE along with other cellular aberrations [32].

In one study, deficient concanavalin A-induced suppressor cell activity was found not only among SLE patients, but also in one-third of their relatives [115]. A major cross-reactive idiotype (16/6) among monoclonal anti-DNA antibodies has been identified. Antibodies carrying the 16/6 idiotype are increased in sera of patients with SLE and are deposited in the glomeruli and the skin. Schatner *et al*. [132] reported that some patients with SLE and their first-degree relatives have simultaneously defective suppressor cell function and increased levels of the 16/6 idiotype. They also found a correlation between the severity of the suppressor cell defect and the level of 16/6 idiotype carrying serum antibodies. However, increased 16/6 levels were commonly found in the presence of normal suppressor T-cell function. Despite these limitations, which imply that other factors must operate to cause tissue damage and clinical disease, these experiments strongly indicate a genetic input in the expression of cellular abnormalities in patients with SLE.

Impairment of $CD8^+$ function in SLE patients has been attributed to an impaired secretion of IL-6 and IFN-γ and an increased production of IL-12 [133]. In the coculture system that was used to reach this conclusion, IL-6 and IFN-γ are responsible for the suppressor function of the $CD8^+$ cells. These experiments have instigated a line of investigations that may lead to the validation of the earlier described defective suppressor cell function in SLE.

T Helper Lymphocyte Function

Increased helper cell activity has been considered a possible alternative explanation to the defective T suppressor cell function for increased B-cell function. Increased helper cell activity was first shown in the MRL *lpr/lpr* autoimmune murine strain [134]. In human lupus, a number of T cell subpopulations have been identified to provide help to B cells to produce Ig. $CD4^+DR^+$ cells from lupus patients provide help *in vitro* to autologous and allogeneic B cells to secrete more Ig [104]. A newly recognized $CD3^+$ subpopulation in the peripheral blood of patients with SLE, which lacks surface CD4 and CD8 molecules, provides help to autologous B cells to secrete cationic anti-DNA antibodies, which are considered nephritogenic [135]. Linker-Israeli *et al*. [136] showed that $CD8^+DR^+$ T lymphocytes provide substantial help *in vitro* to autologous B cells to produce anti-DNA antibodies. Optimal help to autologous B cells was provided by mixtures of $CD4^+$ and $CD8^+$ cells, $CD4^+$ and $CD16^+$ (NK cells), and $CD8^+$ and $CD16^+$ cells. Although $CD8^+$ and $CD16^+$ [137] are known to act as suppressor cells, they may have a special role in the pathogenesis of human lupus.

It should be noted that defective T helper cell activity in patients of SLE has been reported. SLE T cells have been shown not to support IgM anti-TNP responses [138] and autologous and allogeneic *in vitro* B-cell colony formation [139]. Other studies have reported normal T helper cell activity [140, 141].

Double Negative T Cells in SLE

CD4 and CD8 negative cells populate the thymus and the lymph nodes of MRL lpr/lpr mice and have been shown to provide helper signals to B cells to secrete autoantibodies [134]. Studies by Datta and colleagues [142] have shown that double negative cells ($CD4^-CD8^-$) from the spleens of MRL lpr/lpr, (NZBxSWR)F1 and (NZBxNZW)F1 murine strains provide help to B cells to produce cationic IgG anti-DNA antibodies. These antibodies are considered nephritogenic because they bind to and form immune complexes with positively charged sites of the glomeruli [143–145]. Furthermore, $CD4^-CD8^-$ T cells lines from these animals similarly provide help to syngeneic B cells to produce nephritogenic antibodies that share the cross-reactive idiotype called Id^LNF1 (lupus nephritis idiotypes) [146].

Peripheral lymphocytes from patients with SLE also produce highly cationic IgG class anti-DNA antibodies when cultured *in vitro* [135]. $CD4^+$ cells as well as $CD4^-$ and CD8-depleted peripheral $CD3^+$ cells provide help to syngeneic B cells to produce cationic anti-DNA antibodies. Only T cells from patients with inactive disease provide help, whereas T cells from patients with inactive disease and normal individuals failed to do so. Patients with active disease exhibited a striking expansion of the $CD4^-$ $CD8^-$ lymphocytes that were also $TCR\alpha\beta^+$ (TCR) and $CD3^+$. Two-thirds of these double negative cells were $Dw29^+$ (helper, 4B4), whereas one-third were $CD45R^+$ (suppressor/inducer, 2H4). A similar distribution of $CDw29^+$ and $CD45R^+$ cells has been described previously in $CD4^+$ lupus cells [147]. This $CD4^-CD8^-CD3^+$ $TCR\alpha\beta^+$ subpopulation is novel in humans and was detected by virtue of its expansion in the peripheral blood of patients with SLE [135]. The origin and expansion of this subpopulation in patients with SLE remain to be elucidated. Nevertheless, it represents a novel subpopulation, which is committed to provide help to B cells to produce pathogenic autoantibodies.

Rajagopalan *et al*. [148] established IL-2-dependent T-cell lines from T cells from patients with active lupus nephritis. Only 15% of these lines had the ability to selectively augment the production of pathogenic anti-DNA autoantibodies that were cationic in charge, IgG in class, specific for DNA, and clonally restricted in spectrotype. The majority (5 out of 6) of these lines was $CD4^+$ and expressed the TCRaβ. The remaining 10 were $CD4^-CD8^-$; 3 of them expressed the TCRaβ while 7 expressed TCRγδ. On addition to providing specific help to autologous B cells, all lines responded by proliferation to some endogenous antigen presented by autologous B cells. The DN T-cell repertoire includes cells restricted by CD1 Ag-presenting molecules. Indeed, it was shown that CD1c-restricted $CD4^-$ and $CD8^-$ T cells from SLE patients can provide help to CD1c(+) B cells for IgG production and could therefore promote pathogenic autoantibody responses in SLE [149]. Because neutralizing Abs to CD1c inhibited the ability of DN T cells to induce IgG production from CD1c(+) B cells, CD1c may represent a therapeutic target.

Heat Shock Proteins in Lupus Immune Cell Biology

Autoreactive responses of the $CD4^+$ helper T cell lines, which were discussed in the preceding section, were restricted by MHC class II antigens, whereas the double negative T-cell lines were not. Endogenous heat shock proteins (HSP) of the HSP60 family, which were shown to be expressed by lupus B cells, were responsible for stimulating the autoreactive proliferation of $\gamma\delta^+$ T helper cells. The fact that lupus sera contain IgM and IgG antibodies against the HSP70 family of proteins [150] strengthens the notion that HSP may serve as pathogenic antigens in SLE [151].

HSP have been highly conserved during evolution because of their fundamental importance in cell biology and responses to stressful assaults [152]. HSP proteins have gained interest because they are found in organisms that commonly afflict humans. These organisms elicit immune responses that may later evolve into autoimmune responses and overt autoimmune diseases [153, 154]. HSP from *M. lepra*, *tuberculosis*, *bovis*, and other bacteria are closely homologous to HSP of mammals. TCRγδ-bearing lymphocytes recognize HSP epitopes by a mechanism similar to that of $a\beta^+$ T lymphocytes, i.e., in the form of small processed protein fragments bound to antigen-presenting molecules [155]. HSP proteins have been shown to play a role in the pathogenesis of inflammatory arthritis in animals and human rheumatoid arthritis. Adjuvant arthritis in rats can be initiated by T-cell lines and clones specific for the mycobacterial HSP65 [156]. $a\beta^+$ or γδ T-cell clones from rheumatoid synovial fluid recognize HSP65 [157] and specifically the first 15 amino acid peptide of the NH2 terminus of this protein [158]. Although HSP epitopes may not represent the original antigens involved in rheumatoid arthritis or SLE, HSP certainly contain crucial cross-reactive elements. Research along these lines may identify the "pathogenic" T cells, characterize the circumstances that permit their development and expansion, and finally develop vaccination strategies against the development of T cells that recognize HSP [153].

Studies show that HSP-70 alters the function of kinases and phosphatases that are involved in cell signaling. Using HSP-70-transfected Jurkat T cells, we demonstrated that HSP-70 overexpression offers thermoprotection but enhances TCR/CD3- and Fas antigen-induced apoptotic cell death. This phenomenon was associated with downregulation of the Ag receptor-initiated early signal transduction pathways, including increases in $[Ca2^+]_i$ and protein tyrosine phosphorylation, and with upregulation of Fas-mediated early metabolic events [159]. In addition, HSP-70 inhibits stress kinases that are activated following the exposure of cells to heat shock, strong oxidants, UV irradiation, and other stressors and prevents the programmed cell death that ensues exposure to these stressors [160]. If programmed cell death and exposure to physical, chemical, or psychological stressors are of pathogenic importance in lupus, then a better understanding of the molecular and cellular biology of the HSP-70 is warranted.

TABLE 3 T-Cell Clones in the Study of the Pathogenesis of SLE

A. Expansion of peripheral T cells with IL-2		
	Phenotype:	$CD4^+$, $CD3^+$ $\alpha\beta^+$ $CD4^-$, $CD8^-$, $CD3^+\gamma\delta^+$ $CD4^-CD8^-$
	Function:	Help autologous B cells to produce cationic anti-DNA antibodies
	Genotype:	Vγ1, Vδ5,1,3
B. Expansion of peripheral T cells with IL-2 and autoantigen		
a. snRNP antigen		
	Function:	Proliferate in response to snRNP antigen
	Origin:	$HLA\text{-}DR2^+$ or $HLA\text{-}DR4^+$ patients with SLE, mixed connective tissue disease and normal individuals
b. Proliferation of T cells in response to autoantigen		
	Function:	Respond to P antigen; drive B cells to produce anti-P antibodies
C. Expansion of peripheral T cells in the presence of IL-2-containing medium and 6-thioguanine		
	Frequency:	Increased frequency in the peripheral blood of patients with SLE, multiple sclerosis, and systemic sclerosis. Correlation with disease activity and duration
	Function:	Preferential proliferative response to basic myelin protein (multiple sclerosis) or help B cells produce anti-DNA antibodies

T-Cell Clones in the Study of the Pathogenesis of Lupus

T cells are considered to be responsible for B-cell hyperactivity and autoantibody production, to contribute to the initiation and progression of tissue pathology, and to the inability of patients with SLE to successfully fend off infectious agents [161]. Three major technical approaches have been used to characterize the nature of the specific T cells that are involved in the pathogenesis of SLE. In the first, T cells are being expanded by continuous stimulation with IL-2; in the second, T cells are stimulated *in vitro* by the presumptive antigen; and in the third, T cells are expanded in selective media (Table 3).

One-seventh of the T-cell lines that have been established from patients with lupus nephritis following *in vitro* expansion with IL-2 can help autologous B cells to produce cationic anti-DNA antibodies. The majority of the T helper cell lines were $CD4^+$, whereas a small fraction were $\alpha\beta$ TCR^+ $CD4^-CD8^-$ and $\gamma\delta$ TCR^+ [135, 148]. $\gamma\delta$ TCR^+ clones were able to augment anti-DNA antibody production dramatically and in a non-MHC-restricted fashion [148].

Clones reactive with the presumptive autoantigen small nuclear ribonucleoprotein (snRNP) have been established from both patients with SLE and normal individuals who carry the HLA-DR2 or HLA-DR4 genotypes following stimulation of peripheral blood MNC with IL-2 and snRNP [162]. All clones responded in an MHC-restricted fashion to snRNP. Clones derived from normal individuals frequently used the Vβ6 TCR gene [162].

Study of anti-Sm antigen-derived clones from patients with SLE has revealed that T cells recognize a highly restricted set of Sm peptides, with three epitopes on Sm-B and two in Sm-D. Longitudinal studies revealed that the response is stable and support the concept that T-cell immunity to the Sm antigen is antigen driven [163]. In line with these observations is the fact that these clones use TCR CDR3 in a restricted manner [164].

One-third of lupus peripheral blood MNC responded to recombinant ribosomal P2 protein, despite the fact that the response to the recall antigen tetanus toxoid was diminished. The majority of the responding patients had circulating anti-P antibodies, whereas none of the seronegative patients or normal controls responded. The proliferative response was mediated by $CD4^+$ cells and was MHC class II restricted [165]. Apparently these autoantigen-responsive T cells are responsible for the production of anti-P autoantibodies in patients with lupus.

The identification of putative autoantigens may prove useful in reestablishing tolerance. Indeed, myelin basic protein (MBP) disease-inducing T cells have been deleted successfully by a high dose of MBP antigen in a murine model of autoimmune encephalomyelitis [166]. Additionally, oral administration of chicken collagen II in a double-blinded clinical study to patients with rheumatoid arthritis resulted in significant clinical improvement [167].

Studies of T-cell mutations in the *hprt* gene have provided one approach to better understanding *in vivo* immune activity in patients with autoimmune disorders [168–170]. It is based on the principle that random mutation in any gene would be expected to occur more frequently in proliferating than in resting T cells. This approach was used initially to show that patients with multiple sclerosis have increased numbers of mutated T cells that react preferentially to myelin basic protein *in vitro* [171]. It has been found that many patients with SLE have increased numbers of 6-thioguanine-resistant T cells, presumably due to mutations in the *hprt* gene of those T cells [172]. In that study of 18 patients, it was suggested that patients with many mutant T cells were those with the greatest amount of lupus disease. More recently, it has been found that there is a strong correlation between the number of mutant T cells and the duration and magnitude of past lupus disease activity [173]. In another study, it was determined that the T cells that carry the *hprt* gene mutation are phenotypically similar to wild T cells and may help autologous B cells

produce the anti-DNA antibody in a MHC-restricted fashion [174]. Although T cells that carry the *hprt* gene mutation do not exclusively contain the ability to help the production of autoantibody, they may be useful in producing "autoreactive" T-cell clones in the absence of any antigenic stimulation. An increased frequency of *hprt*-mutated T cells has been observed in the peripheral blood of patients with other systemic rheumatic diseases, such as systemic sclerosis [175] and anti-U1 70-kDa autoantibody-positive connective tissue disease [176]. Systemic analysis of the T-cell receptor repertoire of *hprt*-mutated T cells shall examine the clonal origin of these cells, whereas further testing of their function is needed to test whether they have a pathogenic role in the disease process.

T-Cell Antigen Receptor Gene Repertoire in SLE

Autoreactive T cells that bear TCR gene products recognizing self-antigens are eliminated in the thymus by either positive or negative selection. Deletion of the variable region of the β chain of TCR has been reported in various autoimmune murine strains that develop lupus spontaneously [12, 13]. The relevance of these deletions to the development of the disease is under discussion. An early study of the restriction fragment polymorphism of the a, β, and γ chains of TCR in Australians did not reveal any association with lupus [177]. Also, antibody-recognized epitopes of the variable region of the β chain in peripheral T cells was also similarly distributed between lupus patients and normal individuals [178]. In contrast, another study demonstrated an association between anti-Ro antibody production in American patients with lupus and a constant region of the β chain restriction fragment. The majority of these patients were also DQw1 positive [179]. Tebib *et al.* [180] demonstrated that a restriction fragment (1.3 and 3.0-kb band pairs) of the constant region of the α chain of TCR was predominant among American Caucasian patients with SLE but not among Mexican patients with SLE. The same study showed that in three out of five families with SLE the 1.3/3.0-kb band patern was partially concordant with disease transmission. Differences in these cited data may be due either to ethnic heterogeneity or to differences in the restriction enzymes that were used. Vα24JαQ positive T cells are decreased in patients with active disease and reemerge following treatment [181].

T-cell lines that had been established from the peripheral blood of patients with lupus nephritis and had been shown to provide help to autologous B cells to produce anti-DNA antibodies (see earlier discussion) [148] were further studied to determine the molecular structure of the T-cell receptor chains. Sequencing of α and β TCR chain genes from 42 $CD4^+$ lines from five lupus patients revealed recurrent motifs of highly charged residues in their CDR3 loops. Four of the five patients displayed the Vα8 gene family. Some of the studied clones responded to the nonhistone chromosomal protein HMG and nucleosomal histone proteins [182]. These results are in keeping with previous studies from the same group in murine lupus [183] and suggest that autoimmune T helper cells in patients with lupus expand in response to charged antigens.

Indiscriminate sequencing of γ and δ chains of TCR from the peripheral blood of patients with lupus showed oligoclonal expansion of γδ T cells in lupus [184] similar to that observed by the same group in patients with sarcoidosis and rheumatoid arthritis. Sequencing of γ and δ chains of TCR of T-cell lines from patients with lupus nephritis showed certain biases in the selection of these genes [185].

Clones reactive with the presumptive autoantigen small nuclear ribonucleoprotein (snRNP) derived from normal individuals frequently used the Vβ6 TCR gene [162]. T-cell lines from patients with mixed connective tissue disease that expanded in response to snRNP displayed Vβ1, Vβ3, Vβ5.2, and Vβ14 families at increased frequencies [186] and use the CDR3 gene in a restricted fashion [187]. Characterization of TCR profiles from different patients and different sources (blood and kidney tissues) identified that T-cell clonotypes expand at different times and at different sites [188]. These findings suggest that the sensitizing antigens are changing over time and may be different in various inflammatory sites. Obviously, these studies contribute to our understanding of the pathogenesis of lupus and generate a rationale for specific immunologic interventions (Table 4) [189]. More than a decade ago it was shown that the response to certain autoantigens was highly restricted in the usage of TCR V genes. Specific treatments of autoimmune diseases by targeting the TCR were initiated. At this point the enthusiasm has been decreased

TABLE 4 T-Cell Receptor Structure in SLE

A. T cells that facilitate the production of anti-DNA antibodies	
Vγ1, Vδ5, Vδ1, Vδ3	
Cationic motif in CDR3; Vα8[a]	
B. Indiscriminate lupus peripheral γδ T cells	
Vδ1, Vδ2 Vγ9	
B. T cells that respond to the snRNP antigen	
Vβ6	
Vβ1, Vβ3, Vβ5.2, Vβ14	

[a] These data follow the murine lupus paradigm and stress the importance of charge in the production of anti-DNA antibodies that can bind to the charged glomerular basement membrane.

and the approach is criticized because the autoimmune response spreads intra- and intermolecularly over time, the autoimmune infiltrate (in diseases such as thyroiditis and multiple sclerosis) is extremely diverse, and autoimmune cells represent only a small fraction and do not regulate the function of the vast majority of nonspecific cells [190].

LYMPHOKINES

IL-2

SLE T cells have been shown to produce decreased amounts of interleukin-2 [191, 192]. Both $CD4^+$ and $CD8^+$ cells are responsible for the deficiency [193]. This is in agreement with the inability to detect IL-2 receptors on the surface of activated (DR^+CD3^+) lupus cells [104]. The fact that lupus T cells proliferate *in vitro* spontaneously more than normal T cells, particularly in the presence of the phytohemagglutinin-stimulated normal T-cell supernatant, suggests the presence of functionally activated T cells and indicates that DR^+CD3^+ lupus T cells carry low-affinity IL-2 receptors or receptors for other growth factors [104].

Decreased IL-2 secretion, *in vitro*, by lupus cells correlates with increased disease activity, lack of previous treatment, and increased numbers of spontaneously immunoglobulin-secreting B cells [191, 192]. Despite the fact that lupus T cells fail to produce IL-2 *in vitro,* the serum levels of IL-2 were increased, indicating the presence of activated cells *in vivo* [194]. A decreased production of IL-2 by lupus MNC *in vitro* is the product of regulatory action exercised by cells or soluble factors. This statement is supported by several lines of experimental evidence. (A) Removal of $CD8^+$ cells increases IL-2 production by *in vitro*-stimulated SLE MNC to normal or above normal levels [195, 196]. These $CD8^+$ cells, which suppress IL-2 production by lupus MNC, are radiosensitive [197]. In addition to $CD8^+$ T cells, which suppress IL-2 production by SLE MNC, another cell, which is $CD3^-CD8^-CD16^+$, also suppresses IL-2 production [198]. (B) Phorbol myristate acetate, which activates protein kinase C directly, restores IL-2 production to near normal levels. This indicates that the IL-2 production defect is distal to protein kinase C [197, 199]. Phorbol myristate acetate restores IL-2 production in both $CD4^+$ and $CD8^+$ T cells [200, 199]. (C) Culture of SLE MNC *in vitro* without a stimulus for 2–3 days restores their ability to produce normal amounts of IL-2 in response to mitogens [194, 201, 202, 203] and increases significantly the IL-2-producing precursor frequency *in vitro* [204]. Although *in vivo* exhaustion of T cells due to continuous activation may be responsible for this observation, elimination of suppressor cells during the preculture period may also contribute. (D) Culture of lupus MNC under proper cell concentration conditions affects IL-2 production. Lupus MNC cultured at low cell densities overproduced IL-2 [205]. (E) Circulating autoantibodies directed against IL-1-producing adherent cells or IL-2-producing cells may interfere with IL-2 production [206].

Evidence that is discussed later in this chapter has revealed that the two sets of transcriptional defects, i.e., decreased expression of the enhancing transcription factors NF-κB [207] and AP1 [208] and increased expression of the transcriptional repressor CREM [209], are responsible for the decreased expression of IL-2 in patients with SLE.

IL-2 Inhibitor

The activity of IL-2 *in vivo* is regulated by the presence of a 60–70,000 MW, heat-stable, IL-2 inhibitor [210]. Patients with SLE and other autoimmune disorders lack this inhibitor, leaving thus uncontrollable the action of the increased IL-2 amounts [210, 211].

Soluble IL-2 Receptors

Activated T cells produce a soluble form of IL-2 receptor. Sera from patients with SLE have increased levels of circulating IL-2 receptors that correlate with disease activity and several immunologic parameters [212–216]. Increased levels of circulating soluble IL-2 receptors are present in the sera of autoimmune murine strains [217] and other autoimmune human diseases [216, 218]. Because soluble IL-2 receptors bind IL-2, circulating soluble IL-2 receptors may regulate IL-2 levels in patients with SLE. The type and the location of the IL-2 receptor-producing cell, which is responsible for elevated serum IL-2 receptor levels, are unknown. Freshly isolated T cells from peripheral MNC from patients with SLE have decreased levels of the intermediate affinity IL-2-binding molecules p70/75 [110].

The levels of IL-2 are regulated at different levels.

1. Regulation at the level of production: decreased numbers of IL-2-producing precursor cells, unresponsiveness to exogenous antigens, or action of regulatory cells. Because the promoter region of the IL-2 gene is known along with the various transcription factors that bind to it, it should be easy to characterize completely the molecular basis of the decreased production of IL-2 in lupus patients.

2. Regulation at the level of circulation: neutralization by binding to the soluble IL-2 receptor and neutralization by binding of IL-2 inhibitors.
3. Regulation at the level of action: mitogen-activated lupus B and T cells express decreased amounts of IL-2 receptors, which will interfere with the action of IL-2, or *in vivo*, lupus lymphocytes may express more IL-2 receptors [219] and facilitate the action of IL-2. Deficient interleukin production accompanies and at least partly explains the obviously decreased levels of various forms of cellular cytotoxicity in SLE patients [220].

IFN-γ

Peripheral MNC from patients with SLE exhibit deficient production of IFN-γ upon stimulation with PHA or IL-2 [221]. Stimulation of SLE MNC with Newcastle disease virus, polyinosinic, propionolactone-inactivated measles virus [222], and Sendai virus [223] also resulted in diminished IFN-γ production. Another study has shown normal IFN-γ production by mitogen-stimulated SLE MNC [224]. The significance of IFN-γ in the pathogenesis of SLE relates to its antiviral activity and to its ability to enhance cytotoxic activity and suppress antibody production [129]. Decreased IFN-γ may contribute to a higher incidence of infections and autoantibody production in patients with SLE.

These findings contrast reports of patients with rheumatoid arthritis who developed lupus following a systemic administration of IFN-γ [225, 226], suggesting that IFN-γ may promote autoimmunity. Lymphocytes from lupus-prone mice express increased levels of IFN-γ mRNA [227], and IFN-γ may promote autoimmunity by enhancing the expression of autoantigens [228]. Additional studies have shown clearly that IFN-γ promotes autoimmunity. Specifically, NZB/NZW mice treated with an anti-IFN-γ antibody [229] and MRL/lpr mice deficient in IFN-γ (IFNγ-/-) [230, 231] or MRL/lpr [232, 233] and NZB/NZW lacking the IFN-γ receptor (MRL/lpr γR -/-) [234] have less or no glomerulonephritis and other autoimmune manifestations.

IL-6

IL-6 is produced by different cell types, including monocytes and T cells. The most important function of IL-6 is the promotion of Ig production by activated B cells and EBV-transformed B cells [235]. SLE MNC were found to express high levels of mRNA for IL-6 [191]. IL-6 mRNA is also increased in patients with SLE because its half-life is increased, indicating decreased decay [236]. Although the possibility that this is the result of increased transcription has not been tested formally, promoter polymorphisms that are more prevalent in SLE patients may contribute to increased transcription [237]. The production of IL-6 is not autonomous and it can be downregulated in the presence of exogenous IL-10, which increases the decay of the IL-6 mRNA [236].

Increased levels of Il-6 have been found in the cerebrospinal fluid of patients with SLE and central nervous system involvement [238]. Successful treatment of the central nervous system disease in these patients resulted in significant reduction of the IL-6 levels [238]. The possible role of IL-6 in the pathogenesis of SLE is further supported by the finding that mesangial cells from rats with proliferative glomerulonephritis express increased levels of mRNA for IL-6. Because recombinant IL-6 promotes the *in vitro* growth of mesangial cells, it can be speculated that IL-6 acts as an autocrine factor in amplification of the proliferative process of glomerulonephritis [239]. IL-6 is also present in involved kidneys of SLE patients [239]. Factors leading to the constitutive expression of IL-6 in SLE have not been elucidated yet, but they may involve other regulatory cytokines. B cells from patients with SLE express constitutively high numbers of receptors for IL-6 and secrete spontaneously large amounts of IL-6 [240]. These observations establish an autocrine pathway of B-cell activation that may further promote the production of autoantibodies. Finally, IL-6 has been shown to promote disease expression on NZB/NZW F1 mice [241].

IL-10

Lymphokines are responsible for driving or prohibiting various B-cell activation and differentiation phases. The relative role of IL-10 in driving human autoantibody production was studied. IL-10 is produced by monocytes and B lymphocytes and it has been shown to suppress monocyte function on the one hand and promote antibody production on the other [242, 243]. Llorente *et al*. [244] showed that recombinant IL-10 enhanced the ability of peripheral blood lupus mononuclear cells to produce spontaneously IgA, IgM, and IgG. The role of IL-10 in the production of anti-DNA antibodies was also tested in mice with severe combined immunodeficiency that were reconstituted with peripheral MNC from lupus patients. In these chimeric animals, production of the human anti-DNA antibody was blocked by anti-IL-10, but not by anti-IL-6 antibodies. Although IL-10 was given the leading role in the aforementioned experimental system, we should consider that other lymphokines, particularly IL-6, may play significant roles [245, 246]. An autocrine pathway similar to that of IL-6 can be claimed for IL-10, which

is produced by B lymphocytes and promotes autoantibody production [244].

Single nucleotide polymorphisms (SNP) of the IL-10 promoter determine the levels of IL-10 production in humans. These SNP are significantly associated with SLE in African-Americans and may define one component of genetic susceptibility to SLE in this group [247]. It should be noted that IL-10 promoter polymorphisms were not found in a Japanese cohort of SLE patients [248]. It is still possible, although not yet tested, that IL-10 promoter polymorphisms are responsible for the transcriptional regulation of the *IL-10* gene.

Neutralization of IL-10 may present another therapeutic option for lupus and other systemic autoimmune diseases. IL-10 is produced at increased levels by non-T cells from patients with rheumatoid arthritis lupus and Sjogren's syndrome [249, 250]. Both serum IL-10 levels and the message for IL-10 correlated with IgM rheumatoid factor levels in patients with rheumatoid arthritis [250]. Continuous infusion of the anti-IL-10 antibody of (NZB/W) F1 mice from birth on improved survival dramatically, indicating either that IL-10 is directly in promoting disease activity by acting on B lymphocytes and other cells or that elimination of IL-10 reversed the cytokine ratio in favor of the type 2 group of cytokines (see later) [251].

IL-12

IL-12, another cytokine in the Th1 group, has also been found to be decreased in the sera of patients with active disease [252]; mononuclear cells from patients with new onset disease [253] and macrophages from NZB/NZW and MRL/lpr mice [254] also produce decreased amounts of IL-12 [255]. Interestingly, IL-12, delivered by means of gene therapy, prevented autoimmune disease development in the graft-versus-host (DBA into F1) model [256], opening the possibility of utilizing IL-12 in the treatment of human disease.

Tumor Necrosis Factor

Jacob and McDevitt [257] presented evidence that the tumor necrosis factor (TNF) gene may be involved in the pathogenesis of murine lupus nephritis. Specifically, they showed that a restriction fragment length polymorphism in the TNF gene correlated with decreased production of TNF and that treatment of these mice with TNF delayed the development of nephritis significantly. Peripheral blood lymphocytes from HLA-DR2- and DQw1-positive normal individuals produce low amounts of TNF upon stimulation with antigens. In contrast, lymphocytes from DR3- and DR4-positive individuals produce high levels TNF. SLE patients who are DR2, DQW1 positive and are associated with an increased frequency of lupus nephritis produce very low amounts of TNF under similar conditions. DR2- and DR4-positive SLE patients, who are associated with a low frequency of glomerulonephritis, produce high levels of TNF [257, 258]. This study presents TNF as the causative link between HLA-DR2, DQw1 positivity and clinical expression of lupus nephritis. Mitogen and phorbol myristate acetate-induced TNF production was found decreased in another study in all patients with untreated lupus. Decreased TNF production was associated with decreased polymorphonuclear cell-mediated phagocytosis. Because decreased phagocytosis can be improved by the addition of exogenous TNF, an association between decreased TNF production and decreased susceptibility to bacterial infections was speculated [259]. Serum levels of TNF in patients with SLE are comparable to those of normal individuals [260]. Lupus patients with bacterial infections have elevated serum TNF levels, as normal individuals do [260]. The similarity between murine and human data strengthens the possibility that TNF may participate in the expression of the disease. The possible role of TNF-α in defective activation-induced cell death (AICD) is discussed later.

TGF-β

Although the phenomenon of immunosuppression is well established, mechanisms involved in the generation of lymphocytes with downregulatory activity are poorly understood. Stimulation of PBL with anti-CD2, but not anti-CD3, generated substantial amounts of active TGF-β. The presence of active TGF-β coincident with $CD8^+$ T-cell activation can condition these cells to mediate downregulatory activity [261]. TGF-β can induce certain $CD4^+$ T cells in the naive ($CD45RA^+RO^-$) fraction in human peripheral blood to develop powerful, contact-dependent suppressive activity [262]. Anti-CD2 antibody-stimulated SLE T cells produced a decreased production of TGF-β in comparison with healthy controls [263]. These experiments have shed light on the claimed decreased "suppressor" cell activity in SLE patients and its role in the downregulation of the autoimmune response [7].

Other Cytokines

IL-4, a Th2 cytokine, cytoplasmic content is increased in NK and double negative SLE T cells [264]. The serum levels of IL-16 are increased in patients with active

TABLE 5 Lymphokines in Patients with SLE

Definite abnormality	
IFN-γ	Decreased production
IL-1	Decreased production
IL-2	Decreased production certain patients; role of stimulus and culture condition
IL-6	Increased levels in sera and cerebrospinal fluid; increased mRNA for IL-6 in SLE peripheral MNC
IL-10	Increased production responsible for decreased APC function and B-cell overactivity in SLE patients
IL-12	Decreased production
Possible abnormality	
IL-3	Treatment with IL-3 prevents fetal loss in animals with antiphospholipid syndrome
Side effects of treatment with lymphokines	
IL-2 or IFN-α	thyroiditis, SLE Anti-DNA antibodies Multiple symptoms usually found in patients with autoimmune rheumatic diseases IFN-γ induction of expression of autoantigens

disease [265]. Serum levels of IL-15, which is functionally related to IL-2, are decreased in SLE [266]. These new preliminary reports add to the complexity of abnormal cytokine production in SLE.

Polarization of the Lymphokine Production Response

The aforementioned studies (Table 5) indicate that SLE is characterized by an imbalance in the ratio of type 1 (IFN-γ, IL-2):type 2 (including IL-4, IL-5, IL-6, and IL-10) cytokine-secreting PBMC. In one study, type 1 and type 2 lymphokines were estimated in the same group of patients. T cells were the major source of IL-2, IL-4, and IFN-γ, whereas monocytes were the primary source of IL-6 and IL-10 in the peripheral blood of lupus patients. Significantly fewer PBMC spontaneously secreted IFN-γ and IL-2, whereas significantly more PBMC produced IL-6 and IL-10 in lupus patients versus controls. The disease severity in lupus patients correlated with an elevated ratio of IL-10:IFN-γ-secreting cells [267]. In contrast, another study, which included only patients with lupus nephritis, a Th1 predominance was found [268, 269]. An imbalance between type 1 and type 2 cytokines is increasingly being recognized in autoimmune disorders, including RA [270].

PROLIFERATIVE RESPONSES

Antigens and Mitogens

Proliferative responses of SLE MNC to a variety of antigens and mitogens are decreased [33, 271–276]. The magnitude of the proliferative response does not always correlate to disease activity. SLE serum factors suppress the proliferative response of normal cells. Some studies have reported normal proliferative responses [277–280]. Antilymphocyte antibodies [274], increased monocytic suppression [33], altered dose kinetics to PHA [281], and treatment of patients with corticosteroids [282] may all contribute to the decreased proliferative responses. One study showed that lupus $CD4^+$ cells do not respond to self-antigen-presenting cells presenting tetanus toxoid or influenza virus [283]. Defects in antigen-presenting cell function (see later) and T cells are responsible for the decreased proliferative responses.

Alloantigens

Proliferative responses of SLE MNC to alloantigens are moderately decreased [37, 65, 284], or normal [279]. Antilymphocyte antibodies are probably responsible for the decreased responses [65, 284]. Decreased responses did not correlate with clinical disease activity and immunologic parameters [37]. Alloantigens may stimulate $CD4^+$ or $CD8^+$ T cells [283]. The pathways of interaction between alloantigen-presenting cells and $CD4^+$ or $CD8^+$ are relatively intact. SLE $CD4^+$ and $CD8^+$ cells stimulated with alloantigen-presenting cells produce normal amounts of IL-2 [283].

Autologous Mixed Lymphocyte Reaction

AMLR is decreased in patients with SLE [285–288]. AMLR is considered important in homeostasis of the normal immune response. Studies of AMLR in identical twins with discordant disease activity have shown that cells from patients with active disease fail both to stimulate and to respond; in contrast, cells from patients with inactive disease may stimulate but they fail to respond in AMLR [289]. In another similar study, the defect was limited to the stimulating cells [290]. The CD45R antigen characterizes a $CD4^+$ subset that is responsible for the induction of suppressor/effector cells. $CD4^+CD45R^+$ cells proliferate maximally during AMLR, and anti-CD45R antibodies (2H4) block the suppressor/inducer function of AMLR-activated $CD4^+CD45R^+$ cells [291]. Patients with SLE, specifically those with renal disease, have reduced percentages of $CD4^+CD45R^+$ cells [147]. Expression of the CD45R

antigen does not increase on the surface of $CD4^+$ T cells from SLE patients following stimulation in AMLR [291].

Skin Tests

Reactivity to a variety of antigens placed intradermally in patients with SLE is decreased. The antigens used include streptokinase-streptodornase [282, 292–294], purified protein derivative [277, 282, 292, 293], mumps, dermatophytin, monilia, trichophytin [295], and candida [277, 282, 293, 294]. Normal skin reactivity was found in a number of the studies [277, 296–298].

Responses to Anti-CD3 Antibody

T cells from SLE patients display increased proliferative responses when stimulated with the anti-CD3 monoclonal antibody (mAb) [299–301]. This increased response is not associated with increased IL-2 production or increased IL-2 receptor expression but rather to a not proven increase in the binding of IL-2 to the IL-2 receptors that are expressed on the surface membrane of SLE T cells [299]. Furthermore, monocytes from these SLE patients expressed normal amounts of receptors that bind the Fc portion of the anti-CD3 mAb (FcRII) [299] . In contrast, many other studies (discussed under IL-2 production and APC function) have shown that the anti-CD3 mAb-mediated proliferative and IL-2 production responses are low in at least half of the SLE patients.

BIOCHEMICAL AND MOLECULAR ABNORMALITIES OF SLE T CELLS

Capping of Surface Molecules

Primary biochemical and molecular defects that affect the signal transduction pathway in lupus lymphocytes could offset the importance of immunoregulatory abnormalities in the pathogenesis of SLE, although the former may give rise to the latter.

Cross-linking of surface membrane molecules with polyvalent ligands forces them to move laterally, accumulate in patches, and finally form a cap at one pole of the cell. Capping of CD3, CD4, and CD8 molecules on the surface membrane of lupus lymphocytes is impaired significantly as compared to normal subjects. Lymphocytes, of both helper/inducer and suppressor/cytotoxic subsets, from patients with active lupus disease fail to initiate the capping process. Furthermore, of the cells that are able to cap, only part of them can regenerate and reexpress new molecules. Patients with inactive disease have a lesser but still significant capping defect. This capping defect is not due to the inhibitory defect of anti-T-cell antibodies [302]. The lateral mobility of CD3, CD4, and CD8 molecules on the surface membrane of T cells is mediated in part by the cAMP-dependent pathway [303, 304]. Cross-linking of surface molecules induces a rapid rise of intracytoplasmic cAMP concentrations. This process is inhibited by antagonists of the cAMP-dependent protein kinase, whereas treatment of cells with agents that increase the intracellular cAMP concentration enhances the rate of capping [304, 305]. Inhibition and induction studies of protein kinase A (cAMP-dependent) in lymphocytes from patients with inactive disease have shown that the activity of this enzyme is defective. This defect was further confirmed by showing that cAMP-dependent protein kinase A-induced phosphorylation of intracellular proteins is defective in SLE [306]. A defect in the adenylate cyclase-dependent mechanisms in SLE lymphocytes has been suggested [307] in view of deficient responses to aminophylline and prostaglandin E_2.

Kammer *et al.* [308] have shown elegantly that T cells from patients with active disease but not from patients with rheumatoid arthritis and normal individuals display impaired protein kinase A-catalyzed protein phosphorylation. Although the T-cell membrane proteins that are phosphorylated routinely by PKA were intact, the amount of the PKA-I isozyme was decreased. Patients with mild or moderate disease activity also displayed, albeit to a lesser extent, PKA-I isozyme deficiency. The importance of these observations is enormous because alterations in the cellular protein phosphorylation pattern may alter cellular homeostasis and eventually disturb immune function.

Oncogenes in SLE

Oncogenes play a major role in the control of proliferation of animal cells. Protooncogenes are harbored in all vertebrate cells and have the potential to become dominant transforming genes. Certain oncogenes have structural similarities with growth factors, i.e., the *v-sis* protein product is almost identical to the β chain of the platelet-derived growth factor, whereas the *k-fgf* is related to fibroblast growth factors. Cells overexpressing *v-sis* and *k-fgf* grow in the absence of growth factors. Other oncogenes are related to growth factor receptors, i.e., *c-kit* to the platelet-derived growth factor receptor, *c-erb* to the epidermal growth factor receptor, *c-fms* to the colony-stimulating factor-1 receptor, and *c-ros* to the insulin receptor. Cellular activation leads to the accumulation of mRNA for a number of oncogenes

known as early growth-regulated *genes* (*c-myc*, *c-fos*) or late growth-regulated genes (*c-myb*, *c-mos*) [309].

Low but detectable levels of the oncogenes *c-myc*, *c-myb*, and *c-raf* have been found in B and T lymphocytes from normal individuals. In contrast, patients with SLE and other autoimmune diseases express significantly increased amounts of mRNA for these protooncogenes. B lymphocytes from these patients express three- to sixfold higher levels of protooncogene RNAs than T lymphocytes. Increased levels of oncogene mRNA expression in SLE cells are due to increased nuclear transcription rates and prolonged degradation rates. The mechanisms that lead to these two abnormalities remain unknown [108]. We and others have explored the possibility that gene amplification, gene rearrangement, or retroviral (HTLV family) infection might cause this oncogene activation, but results have been negative so far [108, 109, 310, 311]. Whether this oncogene activation in patients with SLE is caused by intrinsic and/or extrinsic factors has yet to be determined. The exact role of oncogene activation in lymphocyte activation in autoimmune diseases, as well as its relation to the increased incidence of lymphoreticular malignancies [312–319] observed in these diseases, is not clear at the present time.

T-Cell Signaling Abnormalities

Immune cells respond to external antigens following their engagement to the antigen receptor through a series of biochemical processes involving protein tyrosine phosphorylation, calcium mobilization, and activation of transcription factors known as cell signaling. The antigen receptor, TCR/CD3, is a multisubunit complex consisting of α and β chains, CD3γ, δ, and ε chains, and a ζ–ζ homodimer [320–322] or heterodimer of ζ with the η chain or γ chain of the high-affinity IgE receptor FcεRI . The disulfide-linked α–β heterodimer is responsible for antigen recognition, whereas CD3ε–δ, CD3ε–γ, and ζ–ζ homodimers couple with the α–β chains to initiate intracellular signal transduction. Each subunit of the ζ chain homodimer possesses three immune receptor tyrosine-activated motifs (ITAMs), whereas other CD3 chains contain one ITAM each. Upon TCR activation, the tyrosine residues within the ITAMs become phosphorylated by Lck and Fyn, leading to the association and activation of ZAP-70. Once activated, Fyn, Lck, Syk, and ZAP-70 cooperate in the tyrosine phosphorylation, activation, and juxtaposition of downstream signal transducers that contribute to the initiation of MAP kinase cascades, PI 3-kinase activation, Ca^{2+} flux, and activation of transcription factors. Eventually these transcription factors translocate to the nucleus and modulate the expression of genes that regulate lymphocyte activation, cellular proliferation, anergy or apoptosis, secretion of soluble mediators, and effector functions.

TABLE 6 Molecular Defects of the T-Cell Signaling Pathways in Systemic Lupus Erythematosus

Molecular defect	Functional effect
CD45 tyrosyl phosphatase	Deficient
TCR ζ chain	Deficient, increased lipid raft association
FcεRIγ	Increased
Lck tyrosyl kinase	Deficient
Inositol trisphospate	Increased
Calcium	Increased and more sustained
PKA-I and PKA-II isozymes	Deficiencies
Protein kinase C	Deficiency
NF-κB, p65 Rel A subunit	Deficiency
Elf-1	Deficiency
CREM	Upregulation and increased nuclear binding to—180 site of the IL-2 promoter
Ras-MAP kinase	Deficiency
CD40 L	Increased

Following engagement of the antigen receptor, T and B lymphocytes from SLE patients respond rapidly by hyperphosphorylating a number of cytosolic signaling protein intermediates and increasing their concentration of free calcium [323, 324] (Table 6, Fig. 1). The increased calcium response occurs in patients with SLE in a disease-specific manner and independently of disease activity and treatment [324]. All major T-cell subsets, including $CD4^+$, $CD8^+$, and $CD4^-CD8^-$ cells, display increased responses. Interestingly, short-term T-cell lines [324] and autoantigen-specific T-cell clones [325] display increased calcium responses, suggesting that this molecular defect is central to the pathogenesis of the disease. Increased calcium responses may account for the increased expression of CD40L, FasL, and c-*myc* whose expression is calcium dependent [326]. It should be noted here that the expression of CD40L is inhibited by the calcineurin inhibitor cyclosporin [10]. The substrates that are hyperphosphorylated in SLE T cells following stimulation with anti-CD3 antibodies have not characterized. It appears though that the hyperphosphorylated proteins should be distinct, whereas others are underphosphorylated. An example of underphosphoryalted substrates in *cbl* [10].

In SLE T cells, the more sustained and increased calcium response has faster kinetics and is observed under deficient TCR ζ chain expression [48, 225, 323,

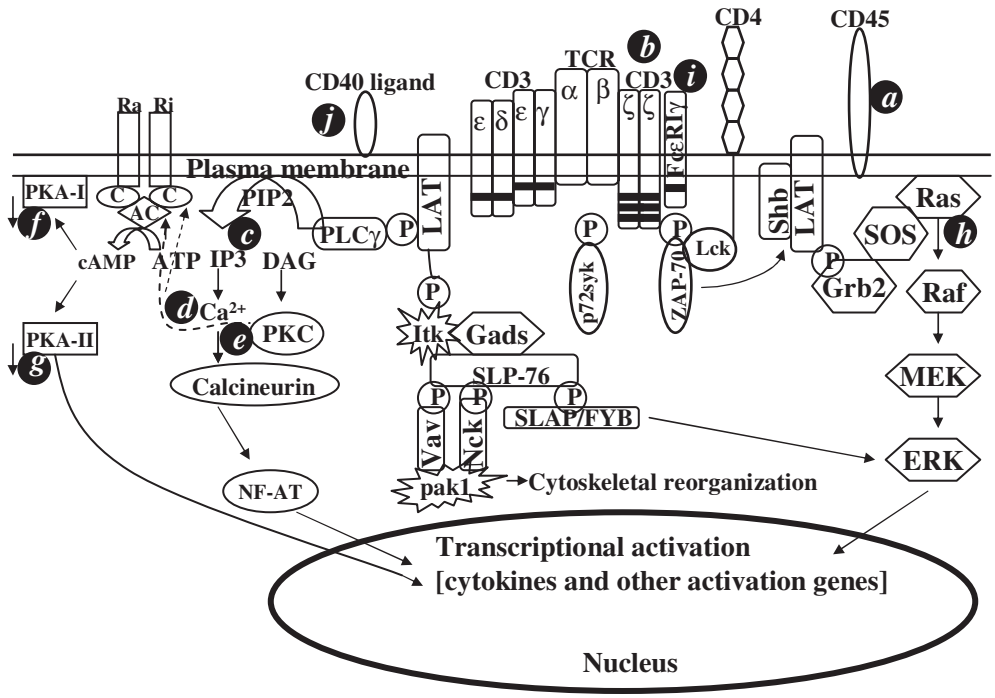


FIGURE 1 Current model of T-cell signal transduction in T lymphocytes depicting the molecular defects identified in SLE. Upon TCR activation by antigen, tyrosine residues within the three ITAMs (-) become phosphorylated by Lck and Fyn, leading to the association and activation of ZAP-70. Once activated, Fyn, Lck, Syk, and ZAP-70 cooperate in tyrosine phosphorylation, activation, and juxtaposition of downstream signal transducers that contribute to the initiation of MAP kinase cascades, PI 3-kinase activation, Ca^{2+} flux, and activation of transcription factors. Eventually these transcription factors translocate to the nucleus and modulate the expression of genes that regulate lymphocyte activation, cellular proliferation, anergy or apoptosis, secretion of soluble mediators, and effector functions. Signaling abnormalities currently identified in SLE T cells are indicated in black circles. *a*, Deficient CD45 phosphatase; *b*, TCR ζ chain deficiency; *c*, moderate increase in inositol trisphosphate; *d*, increased and sustained intracellular calcium levels; *e*, reduced PKC phosphorylation; *f*, deficient protein kinase A I activity; *g*, impaired protein kinase A II activity and increased nuclear translocation of free RIIβ subunit; *h*, reduced Ras-MAP kinase signaling; *i*, increased expression of the FcεRIγ chain that replaces the deficient TCR ζ chain; *j*, increased expression of CD40 ligand.

327]. The deficient TCR ζ chain can explain a number of cellular aberrations that have been observed in patients with SLE, including the defective cytotoxic responses that were described earlier and the reported decreased activation-induced cell death in SLE T cells [328]. The TCR ζ chain is considered to be the limiting factor in T-cell receptor assembly, transport, and surface expression and is crucial to receptor signaling function [320–322]. The TCR ζ chain has also been implicated in the selection of the TCR repertoire and in the prevention of autoimmunity. A vast majority of the SLE patients also display decreased expression of TCR ζ chain mRNA. The TCR ζ chain transcript is generated as the spliced product of eight exons that are separated by distances of 0.7 kb to more than 8 kb [329]. The TCR ζ chain gene is located in chromosome 1q23 [330, 331]. Genes encoded in chromosome 1q have been suggested to contribute to genetic predisposition and susceptibility to SLE by a genome-wide scan of multiplex SLE families [24, 332–334]. Genetic linkage of the TCR ζ chain gene to the FcγRII and FγRIII gene cluster, a candidate locus implicated in genetic susceptibility to SLE, suggests that the TCR ζ chain might play an important role in genetic predisposition to SLE.

Although the precise molecular mechanisms underlying ζ chain deficiency are still being examined, current evidence supports the possibility of a transcriptional defect. In SLE T cells that expressed low levels of TCR ζ chain transcripts, cloning and sequencing revealed more frequent heterogeneous polymorphisms/mutations and alternative splicing of the TCR ζ chain [225, 226, 335, 336]. Most of these mutations are

localized to the three ITAMs or GTP-binding domain and could functionally affect the ζ chain, providing a molecular basis to the known T-cell signaling abnormalities in SLE T cells. Absence of the mutations/polymorphisms in the genomic DNA suggests that these are the consequence of irregular RNA editing. SLE patients also showed a significant increase in the splice variants of the ζ chain. The splicing abnormality included two insertion splice variants of 145 and 93 bases between exons I and II and also several deletion splice variants of the TCR ζ chain resulting from the deletion of individual exons II, VI, and VII or a combined deletion of exons V and VI; VI and VII; II, III, and IV; and V, VI, and VII in SLE T cells [337]. Transfection of a mouse ζ-deficient cell line, MA5.8, with the alternatively spliced isoforms of the TCR ζ chain and TCR/CD3 activation showed functional variation among the splice variants and the wild-type TCR ζ chain. However, the surface expression of the TCR remained similar in MA5.8 cells transfected with the alternatively spliced isoforms of the TCR ζ chain, suggesting that the splice variation does not impair assembly, transport, and surface expression of TCR.

RT-PCR analysis of the TCR ζ chain 3′-untranslated region showed an alternatively spliced 344-bp product with both splicing donor and acceptor sites, resulting from the deletion of nucleotides from 672 to 1233 of TCR ζ chain mRNA. Unlike the normal TCR ζ chain, the expression of TCR ζ chain with the alternatively spliced 344-bp 3′-untranslated region was higher in SLE T cells compared to non-SLE controls. Preliminary studies show that the stability of the TCR ζ chain with the alternatively spliced 3′-untranslated region is more unstable, leading to its downregulation in SLE T cells (G. C. Tsokos *et al.*, unpublished data). Because many of the principal antinuclear antibodies in SLE are directed against spliceosome components, it is not clear whether these autoantibodies play any role in defective mRNA synthesis, splicing, and processing in SLE T cells. Obviously, such a possibility would presume that antibodies can enter the live cells and reach the nucleus. Certain antibodies have been claimed to enter live cells and alter mRNA translation and the production of protein [338].

Abnormalities of Elf-1 Expression

Analysis of the TCR ζ chain transcription factor Elf-1 indicates that the p98 form of the protein was decreased in the majority of lupus patients, whereas the p80 form was increased. Elf-1 is a member of the Ets (E twenty-six specific) transcription factor family. Elf-1 has a calculated molecular mass of 68 kDa; it exists in T cells as both 80- and 98-kDa forms. We determined that the 80-kDa Elf-1 was located mainly in the cytoplasm, whereas the 98-kDa Elf-1 was the major nuclear form. Immunoprecipitation disclosed consistently that the retinoblastoma protein (Rb), which retained Elf-1 in the cytoplasm, displayed higher affinity for the 80-kDa form than the 98-kDa form, indicating that conversion from the 80- to 98-kDa form allows Elf-1 to evade the tethering of Rb and facilitates its nuclear translocation. We found the molecular basis for this conversion involving dual posttranslational processes, i.e., glycosylation and phosphorylation. Specifically, *N*-acetylglucosaminidase, but not the endoglycosidase H, digested the 98-kDa Elf-1 efficiently, whereas actin was not affected. The 98-kDa form was further resolved into three bands, and all bands were either enhanced or suppressed following treatment with okadaic acid or bacteriophage λ phosphatase, respectively. In contrast, only a minor portion of the 80-kDa Elf-1 was in the phosphorylated state subjected to the similar regulation by okadaic acid and phosphatase as the 98-kDa form, indicating that the 98-kDa Elf-1 is the active form. Electrophoretic mobility shift assays and ultraviolet cross-linking studies indicated that the phosphorylated 98-kDa Elf-1 is the functional form that binds to the TCR ζ chain promoter. Nuclear proteins from approximately 40% of SLE T cells displayed decreased production of the 98-kDa form but not the 80-kDa form, which correlates well with their defective TCR ζ chain promoter binding. Another 20% of SLE T-cell nuclear proteins are defective in TCR ζ chain promoter binding, despite normal expression of both 98 and 80 forms of Elf-1. The defective formation of functional 98-kDa Elf-1 thus underlies the defective TCR ζ chain expression in SLE patients [339].

The TCR ζ chain exists in multiple forms and membrane fractions with a distinct function in the Ag-mediated signaling process. Studies on the complete spectrum of expression of various molecular forms of the TCR ζ chain have shown that the phosphorylated 21- and 23-kDa forms of the TCR ζ chain are decreased significantly in SLE T cells compared to normal T cells. In contrast, major ubiquitinated forms of the TCR ζ chain were increased in SLE T cells, suggesting that the TCR ζ chain undergoes an enhanced ubiquitin-mediated degradation in SLE T cells. The level of the TCR ζ chain was also decreased significantly in the detergent-insoluble membranes in SLE T cells. Similarly, expression of the TCR η chain, an alternatively spliced form of the ζ chain, was diminished in SLE T cells. Upregulation of a novel 14-kDa form of the ζ chain, a potential alternatively spliced or degraded species of the TCR ζ chain in SLE T cells, has been identified [340].

Mechanisms of Increased TCR/CD3-Mediated $[Ca^{2+}]_i$ Response in SLE

How the TCR engagement induces hyperphosphorylation of cytosolic proteins and a increased $[Ca^{2+}]_i$ response in the milieu of deficient TCR ζ chain has been a topic of intense research. In addition to the possible gain-of-function mutations of the TCR ζ chain, our investigation has proposed two mechanisms involving increased expression of the FcεRIγ chain and increased membrane lipid-raft association of the residual TCR ζ chain, which could explain the supranormal TCR/CD-mediated $[Ca^{2+}]_i$ response in SLE T cells. Also, the protein tyrosine phosphatase activity of CD45 on peripheral blood lymphocytes is reduced in SLE [91].

FcεRIγ Chain Replaces the Defective TCR ζ Chain

The hypothesis that other members of the ζ chain family may substitute for the deficient TCR ζ chain was first investigated by Enyedy *et al.* [341]. Immunoprecipitation/immunoblotting and confocal microscopy demonstrate that a large proportion of SLE T cells express very high levels of FcεRIγ that functionally associates with the TCR and takes part in antigen receptor-mediated signal transduction. Expression of FcεRIγ *in lieu* of the TCR ζ chain has been reported in mouse large granular lymphocytes [342]. T lymphocytes from tumor-bearing mice expressed TCR that completely lacked the TCR ζ chain and was replaced by the FcεRIγ chain [343, 344]. Also, TCR ζ-deficient mice have been shown to express FcεRIγ as part of the TCR–γδ complex [205, 345, 346]. Unlike the TCR ζ chain, which mediates signaling through ZAP-70, FcεRIγ mediates signaling by associating with the 100-fold more potent phosphorylated protein kinase Syk [347, 348]. At present it is unknown whether Syk is upregulated in SLE T cells and whether FcεRIγ transduces the signal by associating with Syk or other downstream signaling molecules. A high-level expression of Syk and alternative antigen receptor-mediated signaling has been described in T cells of patients with ZAP-70 deficiency [347]. Overexpression of FcεRIγ in normal T cells also shows an increased TCR/CD3-mediated $[Ca^{2+}]_i$ response, suggesting that the single ITAMs in the FcεRIγ compared to the three in the TCR ζ chain do not hinder the level of $[Ca^{2+}]_i$ response (unpublished results). The high-level expression of the FcεRIγ chain could replace the defective TCR ζ chain and contribute to the aberrant antigen receptor-initiated signaling in SLE T cells.

Increased Membrane Lipid Raft Association of the Residual TCR ζ Chain

The TCR ζ chain associated with the detergent-insoluble fraction is distributed between the cytoskeleton and lipid-rich membrane microdomains, composed primarily of sphingolipids and cholesterol, and an enriched subset of proteins that float laterally as "rafts" within the plasma membrane [349]. Lipid rafts are preformed functional modules that serve as platforms for signal transduction and membrane trafficking. Data indicate that lipid rafts are crucial for affecting TCR signal transduction [350–352]. TCR engagement leads to translocation and concentration of the tyrosine-phosphorylated TCR ζ chain and downstream signal transduction molecules within lipid rafts [353]. Conversely, perturbation of the structural integrity of lipid rafts inhibits TCR-induced protein tyrosine phosphorylation and Ca^{2+} flux [351, 354].

Dissociation of the lipid rafts by cholesterol depletion using methyl-β-cyclodextrin showed an increased percentage of the residual membrane-bound TCR ζ chain in lipid rafts in resting SLE T-cell membranes [340]. Fluorescence microscopy indicated that the residual TCR ζ chain is more clustered on the cell membranes of SLE T cells compared to normal cell membranes. Faster kinetics of the TCR/CD3-mediated $[Ca^{2+}]_i$ response in SLE T cells also support the clustering or cross-talk between signaling pathways in SLE. Upon TCR/CD3 activation, ζ chain clusters became more prominent in SLE T cells and they superimpose with LAT, suggesting that they are colocalized to lipid rafts. Increased lipid raft association and surface clustering of the residual TCR ζ chain may explain the supernormal TCR/CD3-mediated $[Ca^{2+}]_i$ response in SLE T cells. Based on these data, a model has been proposed that suggests that although there is a deficiency in SLE T cells, the residual TCR ζ chain is more associated with membrane lipid rafts, resulting in more preformed TCR clustering. Increased membrane TCR clustering, as well as replacement of the deficient TCR ζ chain by the FcεRIγ chain, could lead to a supranormal $[Ca^{2+}]_i$ response under TCR ζ chain deficiency and decreased tolerance in SLE T cells. In support of this view, it has been reported that mice with *N*-acetylglucosaminyltransferase deficiency show decreased glycosylation of T-cell membrane proteins that prevent galectin binding, thereby disrupting the galectin-glycoprotein lattice, increased clustering of TCR [355]. Increased TCR clustering in these autoimmune mice had a very similar phenotype of human SLE, with lowered T-cell activation thresholds and increased TCR signaling [355]. Similarly, it has also been sug-

gested that genetic remodeling of protein glycosylation by mutation of α mannosidase II induces autoimmune disease [356]. As the association among the residual ζ chain with the lipid rafts, TCR clustering, and T-cell effector functions is further explored, it is likely that important new insights will emerge that will explain ζ chain abnormalities and autoreactivity of T cells.

Deficient Protein Kinase A Activity

Studies on the adenyl cyclase/cAMP/protein kinase A (AC/cAMP/PKA) system, a key metabolic pathway integral to cellular homeostasis, identified impaired cAMP-dependent protein phosphorylation in SLE T cells [357]. An abnormal cAMP-dependent signaling pathway is associated with deficient CD8 uppressor T-cell function and altered cytoskeletal regulation of CD3, CD4, and CD8 receptor mobility within the plane of the plasma membrane [302, 358]. It has been demonstrated that the abnormal cAMP-dependent signaling pathway reflects a profound reduction in PKA-I isozyme [308]. Deficient PKA-I activity is the consequence of a reduced type I regulatory subunit. Deficient T-cell PKA-I activity reflects reduction of both holoenzymes $RI\beta_2C_2 > RI\alpha_2C_2$ [359]. Deficient PKA-I activity possibly contributes to altered T-cell effector function by altering the protein phosphorylation that regulates cellular pathways that promote cell growth and differentiation. Similar to the TCR ζ chain, increased mutation/polymorphism of protein kinase A I regulatory subunit α has been reported in a SLE patient [360]. The kinase activity of protein kinase C [361] and Lck is also impaired in SLE T cells [362]. The activity of other kinases, such as protein kinase PKR, which is involved in the phosphorylation of translation initiation factors, is increased in SLE T cells [363].

An interrelationship between the Ca^{2+} and AC/cAMP/PKA pathway raises the possibility that defective PKA activity in part contributes to the impaired Ca^{2+} homeostasis. The TCR/CD3-mediated increase in IP_3 and $[Ca^{2+}]_i$ is downregulated by the inactivation of PLC-γ1 through PKA-dependent phosphorylation [364]. Deficient PKA-catalyzed phos-phorylation may retain the activity of PLC-γ1 and contribute to the supranormal and sustained $[Ca^{2+}]_i$ response in SLE T cells.

Ras-MAP kinase activity is also impaired in SLE T cells and has been connected to decreased DNA methylation (see section on drug-induced lupus).

Abnormalities in Transcription Factor Expression

Early signaling abnormalities are followed by the altered activation of transcription factors and abnormal gene transcription. It is notable that while certain genes are transcribed at low rates (the T-cell receptor ζ chain and interleukin-2 genes), others are transcribed at increased rates (genes for the γ chain of the Fc receptor for IgE and the CD40 ligand) [24]. In addition to the TCR ζ chain, transcription factor Elf-1, described earlier, defects have been identified both in the expression/activation of other transcription enhancers and repressors, including the NF-κB p65-Rel A subunit and p-CREM.

Deficient p65-Rel A Subunit of NF-κB in SLE T Cells

The transcription factor NF-κB plays a profound role in immune and proinflammatory responses and IL-2 production [365]. The possibility that reduced IL-2 production by SLE T cells may be a product of altered NF-κB activity has been analyzed by electrophoretic mobility shift assays (EMSA) [207]. NF-κB activity in the nuclear extracts is decreased significantly in SLE T cells. In the group of SLE patients with decreased NF-κB activity, the transcriptionally active, heterodimeric p65/p50 complex was not formed in the cytosol. The deficiency of the NF-κB heterodimeric complex could be responsible for the downregulation of IL-2 and may have extensive pathophysiological significance in the expression of the disease.

Increased CREM Binding to the –180 Site of the IL-2 Promoter

SLE T cells stimulated *in vitro* in response to antigens or mitogens proliferate significantly less than T cells from normal donors. Activated SLE T cells also secrete low IL-2 *in vitro* that may reflect increased T-cell anergy. Another mechanism for the low IL-2 production by SLE T cells is inhibition of IL-2 enhancer/promoter transcriptional activation. Cyclic AMP response element modulator (CREM) and inducible camp early repressor (ICER) are two transcriptional repressors that bind to camp response elements (CREs) and downregulate genes containing this binding site. It has been demonstrated that phosphorylated CREM (p-CREM) binds to the –180 region of the IL-2 enhance/promoter and contributes to T-cell anergy [209]. This raised the question that p-CREM upregulation may contribute to the downregulation of IL-2 secretion and contribute T-cell anergy. Nuclear extracts from resting SLE T cells showed significantly increased binding of p-CREM/p-CREB to the –180 site of the IL-2 enhancer/promoter [209]. Some patients revealed both p-CREM and p-CREB, although p-CREM was the

main factor. We have found that activated normal T cells increase p-CREB binding by 10-fold in contrast to negligible p-CREM binding to the –180 site. In contrast, activated SLE T cells bound both p-CREM and p-CREB in a 2:1 ratio. Thus, preferential phosphorylation and predominant occupancy of the –180 site of the IL-2 enhancer/promoter by p-CREM during activation may hamper optimum transcriptional activation by p-CREB binding, resulting in very low IL-2 production in SLE T cells. In contrast to the heteromeric complex formation between p-CREB to two nuclear coactivators, CREB-binding protein (CBP) and p300 that initiate transcription in normal T cells [366], SLE T cells mainly form p-CREM/CBP/p300 complexes that may suppress the IL-2 synthesis.

Abnormal Nuclear Translocation of RIIβ Subunit

The RIIβ subunit of PKA II is not classified currently as a nuclear factor, although its translocation to nucleus and binding to CREB support the role of a transcription factor. Upon activation of the PKA-II holoenzyme, RIIα/β_2C_2, by reversible cAMP binding to the RII subunits, releases RIIβ that reversibly translocates to the nucleus. Although it has been suggested that nuclear RIIβ binds CREB, at present its function in the nucleus still remains uncertain. The RIIβ subunit can be constitutively in the nucleus, suggesting an ongoing activation of RIIβ_2C_2 during homeostasis. The mechanism of deficient PKA-II activity in SLE T cells differs from PKA-I and involves increased nuclear translocation of the RIIβ to the nuclear fraction, diminishing the capacity to form a RIIβ_2C_2 holoenzyme [367].

Impaired Protein Translation

PKR is a serine/threonine protein kinase that regulates protein translation [368]. As an inactive holoenzyme, the molecule exists in its dephosphorylated form. However, binding of dsRNA to PKR results in a conformational change, causing autophosphorylation and homodimer formation [368, 369]. Once activated, PKR can phosphorylate several substrates, including those regulating protein synthesis. The initiation of protein translation is regulated at two steps: (a) eukaryotic initiation factor 4e (eIF4e)-mediated binding of the 40S ribosomal subunit to the 5′ end of the mRNA and (b) eIF2α-mediated binding of the initiating methionine (met)-tRNA to the 40S ribosomal subunit [369]. Activated PKR phosphorylates eEF2α, preventing formation of the eEF2α–met-tRNA complex and thereby inhibiting protein synthesis and subsequent cellular proliferation. Interestingly, mice heterozygous for a PKR null mutation exhibit decreased signaling through the p38 MAPK pathway [369]. Stimulation of normal T cells with PMA/IO increased protein translation approximately 90-fold. In contrast, similar treatment of T cells from subjects with active SLE increased protein synthesis only 5-fold, whereas T cells from people with inactive SLE only increased protein synthesis ~20-fold. A comparison of PKR levels revealed that stimulation of SLE T cells caused more rapid and larger increases in PKR than controls, resulting in increased eEF2α phosphorylation. Further, overexpressing PKR in T cells by transfection suppressed protein synthesis. Comparison of PKR mRNA levels in stimulated SLE and control T cells demonstrated no differences, indicating that increased PKR activity in SLE T cells reflects posttranscriptional regulatory differences [363]. The precise mechanism underlying PKR overexpression in SLE T cells is currently unknown.

Abnormal Immune Cell Signaling and Autoimmunity

The aforementioned data, along with those that will be discussed in subsequent sections (B lymphocytes and apoptosis), establish that antigen receptor-initiated signaling is abnormal in SLE. As of now there is no information on the signaling events that are initiated by cytokine receptors or other lymphocyte receptors. It is probable that more abnormalities will be revealed. Aberrant cell signaling is initiated after antigen binds the lymphocyte receptor and is further modulated by signaling events that are initiated by cytokine receptors and other cell membrane receptors. When the quantity and/or the quality of the produced signaling events cross a certain "threshold", then autoimmunity ensues (Fig. 2). Data generated from the study of autoimmune mice concur with this proposition. Break of tolerance in transgenic mice is associated with abnormal (increased) lymphocyte signaling [370], and the expression of cytochrome TCR in MRLlpr mice is associated with increased responses [371].

ANTIGEN-PRESENTING CELL FUNCTION

Abnormal APC function was further suggested by studies that evaluated the response of SLE T cells to the recall antigen influenza virus and alloantigens [372, 373]. Indeed, one-half of the studied patients failed to respond to the MHC self-restricted antigen influenza-A virus while they responded to an alloantigen that can use both MHC self-restricted or unrestricted T-cell activation pathways [372]. Previous reports have shown

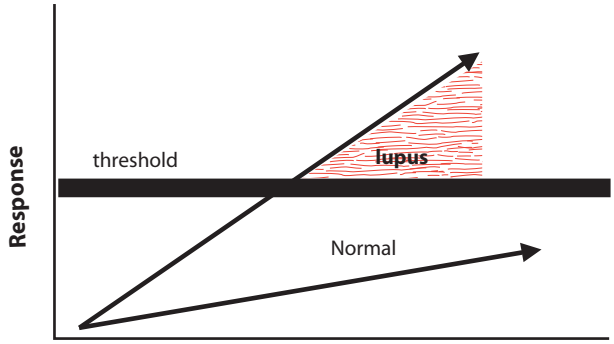


FIGURE 2 Abnormal immune cell signaling and autoimmunity. Aberrant cell signaling is initiated after antigen binds the lymphocyte receptor and it is further modulated by signaling events that are initiated by cytokine receptors and other cell membrane receptors. When the quantity and/or the quality of the produced signaling events crosses a certain "threshold," autoimmunity ensues.

defective IL-1 production [28] and expression of HLA-DR antigens on the surface membrane of monocytes from patients with SLE [43, 44]. Collectively, these studies strongly suggest that the APC function is deficient in the majority of patients with SLE. Defective APC function has also been implied by studies where the need for APC-delivered costimulation is bypassed by effectively cross-linking the CD28 [374] or the CD26 [375] T-cell surface molecules.

Indeed, a defect was found in the induction of B7-1 (CD80) expression by lupus APCs [373]. Resting normal APC do not express B7-1, but treatment with IFN-γ induces the expression of surface B7-1. Lupus APC failed to express surface B7-1 following stimulation with IFN-γ. Interestingly, replenishment of B7-1 in the culture environment by means of adding B7-1-transfected P815 murine mastocytoma cells increased significantly the response of lupus T cells to tetanus toxoid and the anti-CD3 monoclonal antibody. These experiments also revealed that the Fcγ receptor present on the surface of lupus APC is unable to cross-link efficiently the murine monoclonal anti-CD3 antibody and initiate T-cell proliferation (Fig. 3). This function was effectively served by the Fc receptor that is expressed on the surface of P815 murine mastocytoma cells. Reduced expression of CD80 by non-B-cell APC *in vivo* has also been reported in patients with rheumatoid arthritis [376, 377]. In contrast, CD86 was expressed by an increased percentage of lupus B cells [378, 379] and is clearly upregulated in the rheumatoid synovium [377]. Differences in the expression of these two molecules in autoimmune diseases may reflect the fact that each one of them may have different functional attributes or that their molecular requirements for upregulation in disease states are different.

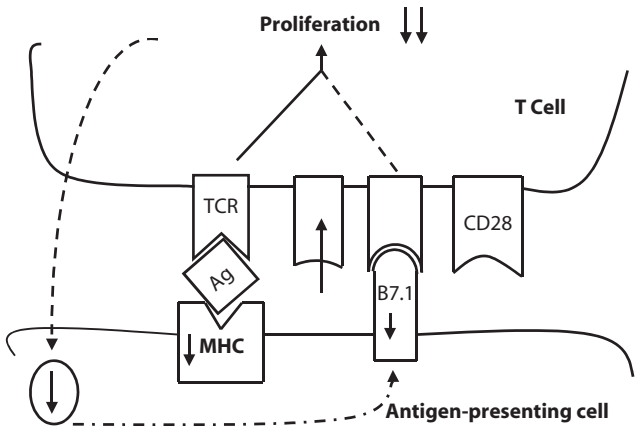


FIGURE 3 Defective upregulation of the B7.1 molecule on the cell surface of antigen-presenting cells (APC) in patients with SLE. Soluble or surface molecules of T-cell origin induce the expression of B7.1 on the surface of APC. An additional signal is thus delivered via the counterreceptor CD28 (constitutively present on T cells), avoiding the induction of a state of anergy, which otherwise would ensue, for the T cell. Recall antigens presented to lupus T cells in a MHC-restricted fashion and in the presence of APC produce subphysiological proliferative responses compared to normal T cells. This is because the important upregulation of B7.1 expression on APCs is decreased significantly.

COSTIMULATION IN LUPUS

B7-CD28

The role of B7-CD28 interaction in T-cell stimulation has been studied extensively. The CD28/CTLA4-B7 family [16] is crucial in IL-2 production and tolerance induction [18]. The CD28/CTLA4-B7 pathway determines tolerance induction and activation of T cells from MRL *lpr/lpr* mice [380]. A B7 binding construct (CTLA4 molecule fused with the heavy chain of Ig to provide decreased plasma clearance rates) was given to mice that develop lupus spontaneously and the rate of survival increased along with a lower titer of autoantibodies and lesser kidney pathology [381]. Because CTLA4 can block both B7-1 and B7-2, effectively it is not known as of yet whether blockage of one or the other or both molecules is necessary for the therapeutic effect. Additionally, it is not known whether B7-1 or B7-2 is the primary molecule involved in the expression of murine lupus. Treatment of animal models for EAE and nonobese diabetic mouse with anti-B7-1 and/or anti-B7-2 antibodies produced opposing results. These observations indicate that (a) the various autoimmune animal models are different diseases with different immunoregulatory requirements; (b) the availability of B7 molecules, and probably others, is mandatory for the

response to the autoantigen; and (c) the autoantigen may be ignored if the appropriate milieu is not available. This proposal is further supported by the following experimental information.

First, constitutive expression of B7-1 alone by keratinocytes does not induce $CD4^+$-mediated autoimmunity but greatly increases and prolongs cutaneous delayed-type hypersensitivity reactions to exogenously applied antigens [382, 383]. Of note, keratinocytes from patients with psoriasis display increased levels of B7 [384]. Transgenic mice that express B7-1 on the surface membrane of pancreatic cells and the appropriate T-cell receptor (double transgenes) develop diabetes [385]. Also, animals that express B7-1 and antigens of the lymphocytic choriomengitis virus (double transgenes) in pancreatic B cells develop anti-self(viral) cytotoxic T lymphocytes and diabetes [386].

Second, endothelial cells from nonsun-exposed areas from lupus patients overexpressed E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) [387]. Tissue overexpression of adhesion molecules is in accordance with the reported increased levels of soluble adhesion molecules in the sera of patients with lupus (reviewed in Tsokos [388]) and infer their involvement in the autoimmune pathogenic process. Nevertheless, they have not addressed whether the expression of adhesion molecules by APCs or lymphocytes has a primary role in the initiation of the pathologic process. This question has been addressed in a definitive experiment [389]: D10 cloned helper T cells were transfected successfully with the LFA1 (CD18) gene and injected weekly (six times) into syngeneic normal AKR mice. Four weeks after the last injection, the mice produced anti-dsDNA antibodies and pathologic features of glomerulonephritis. Therefore, it can be assumed that circulating T cells that are programmed to recognize DNA pause their ignorance of the autoantigen in the presence of LFA-1. Drugs that cause the overexpression of LFA-1 on the surface of cells [390, 391] can initiate an autoimmune process [392].

CD40-CD40 Ligand

Pathogenic antibodies require cognate interaction between T and B cells. CD40 (expressed on B cells) and gp39 (CD40 ligand, expressed on T cells) have been shown to play important roles in the production of antibody. Patients with CD40 ligand deficiency display increased levels of IgM and deficient responses to antigens. Experiments have shown that signaling through the CD40 ligand (gp39) is necessary for the expansion of antigen-specific helper T cells because mice that lack the CD40 ligand fail to produce an antigen-specific antibody [393, 394]. Interruption of the CD40–gp39 interaction SNF1 autoimmune mice with and anti-gp39 antibody resulted in a decreased expansion of autoimmune memory B cells and long-term improvement of the clinical disease [395]. Experiments that show therapeutic effects in autoimmune mice following interruption of the B7-CD28 and CD40-gp39 paths indicate that these paths are involved in a serial manner in the production of antibody.

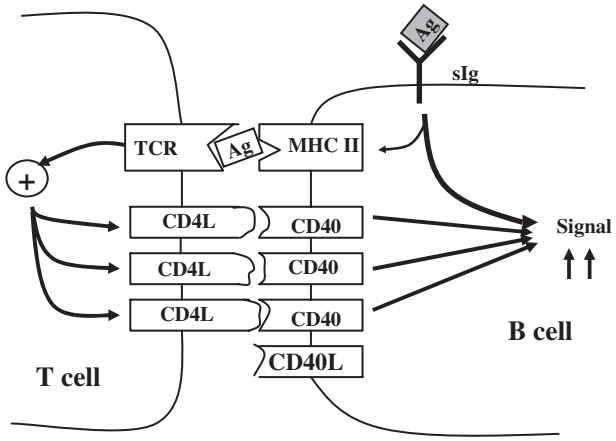


FIGURE 4 Dysregulation of a CD40-CD40L pair of molecules. Antigen presentation to T cells induces the transient expression of CD40L on their surface. This in turn, via its counterreceptor CD40 (constitutively expressed on B cells mainly), delivers an important costimulatory signal to the CD40-bearing cell. In SLE, the upregulation of CD40L on T cells is significantly more intense and more prolonged. This can deliver excessive help to lupus B cells for the production of autoantibodies. B cells in SLE also become $CD40L^+$ upon activation, a finding with unclear significance as of yet.

The CD40-CD40 ligand (CD40L) also appears to malfunction in lupus. The number of circulating $CD40L^+$ T cells of lupus patients is not increased compared to normal individuals; however, the induction of this molecule on lupus $CD4^+$ and $CD8^+$ T cells is both enhanced and prolonged [396]. Moreover, it has been reported that lupus B cells unexpectedly express $CD40L^+$ upon stimulation in an equally intense manner to the T cells. This abnormally regulated molecule on lupus lymphocytes is still functional, as it was shown in two sets of elegant experiments: (1) anti-CD40L mAb was shown to inhibit the production of anti-DNA antibodies [397] and (2) CD40L positive cells induced the expression of CD80 (B7-1) in a B-cell line [396] (Fig. 4).

Although unclear it has become an emerging theme that SLE patients have abnormal RNA splicing processes. Abnormal splicing was discussed earlier for the TCR ζ chain. Abnormal splicing involving the transmembrane region of a molecule will result in the pro-

duction of a soluble form. Both CD40 [398, 399] and CD40L [398] soluble forms have been detected in patients with SLE and they obviously interfere with the interaction of the surface-bound counterparts. Abnormal splicing and increased levels of Fas and FasL are discussed later.

LYMPHOCYTE TRAFFICKING

T lymphocytes that home in various tissues and initiate the pathologic process may express abnormally high levels of receptors that facilitate attachment to endothelial or other cells. Other pairs of molecules are involved exclusively in providing the costimulatory signal to T cells, whereas other pairs of molecules are involved in both cell costimulation and adhesion/homing of cells [16–18].

Several sets of surface molecules participate in the cell–cell and cell–substitium adhesion process. Members of the β1 integrin family of adhesion molecules are major adhesive receptors for the extracellular matrix. Members of the β2 integrin family, VLA4 and VLA3, are involved in both cell–cell and cell–extracellular matrix adhesion [400]. The expression of these molecules is regulated under normal conditions and upregulated in various disease conditions such as atherosclerosis [401], inflammation [402], and rheumatoid arthritis [403]. VLA4 and LFA1 were found to be overexpressed on the surface membrane of lymphocytes from patients with SLE. Interestingly, VLA4 was overexpressed only in lymphocytes from patients who had vasculitis. Lymphocytes from these patients showed increased adhesion to cord vein endothelial cells [58]. Neutrophils from patients with active lupus displayed increased surface membrane expression of β2 integrin CD11b/CD18 [404]. Obviously, these findings shed light on the pathogenesis of vasculitis [405] and other tissue injury in patients with lupus. Increased expression of the VLA4 partner on the surface of endothelial cells may also be present and further facilitates the attachment of lymphocytes to endothelial cells and the initiation of the inflammatory process.

CYTOTOXIC RESPONSES

Cytotoxic mechanisms can be separated into natural cytotoxicity, antibody-mediated cytotoxicity, and specific cytotoxicity. Cytotoxic mechanisms in SLE have been reviewed extensively [32, 406]. Decreased cytotoxic responses are the least disputed abnormalities in SLE. All aspects of cytotoxic responses have always been described to be abnormally low (Table 7).

TABLE 7 Cellular Cytotoxic Function in SLE

Decreased NK cell activity
Decreased allogeneic cytotoxicity
Decreased xenogeneic cytotoxicity
Decreased antibody-dependent cell cytotoxicity
Decreased cytotoxicity function against virus-modified self targets
Decreased IL-2-induced killer cells (LAK cells)
Decreased antigen-nonspecific cell cytotoxicity

Natural Killer Cells

NK cell activity has been described to be low in patients with SLE [407–412]. Low NK activity is associated with disease activity [408, 411, 412]. Serum factors [411], antilymphocyte antibodies [407], and IgM and/or IgG anti-NK antibodies [94] may be responsible for decreased NK activity in lupus patients. In our study, half of the patients with SLE had decreased NK cell activity. Exogenous IFN did not enhance NK activity in the group of patients with low NK activity, although it enhanced NK activity in the group of patients with normal NK activity [412]. Thus, it seems that both decreased IFN production and unresponsiveness to IFN are responsible for low NK activity in SLE patients. Exogenous IL-2 can restore NK activity to normal levels *in vitro* [220, 413, 414].

The importance of decreased NK activity in SLE is severalfold. First, NK cells are known to suppress B-cell function [137] and, therefore, lupus B lymphocytes function in the absence of a suppressor mechanism. Second, NK cells are responsible for carrying out immune surveillance against tumor cells [415]. Patients with lupus have an increased frequency of lymphoreticular malignancies [312–319]. Third, NK cells recognize and kill virus-infected cells [415, 416]. Decreased NK activity may be responsible for the increased frequency of infections in SLE. Deficient elimination of virus-infected cells in lupus patients may be of pathogenic importance should the virus prove to be important in the pathogenesis of the disease. It can be speculated that failure to eliminate EBV-infected B cells may lead to continuous polyclonal B-cell activation. EBV has been considered of pathogenic importance in both rheumatoid arthritis [417–419] and SLE [128, 417]. Because EBV has been implicated, probably among other factors, in the development of non-Hodgkins lymphoma in human immunodeficiency virus-positive individuals [420] and because the frequency of non-Hodgkins lymphoma has been shown clearly to be increased among patients with lupus [421, 422], the significance of deficient cytotoxic responses in the expression of the disease pathology cannot be underestimated.

Other forms of nonspecific cytotoxicity have been found decreased in patients with SLE. Phytohemagglutinin-induced cellular cytotoxicity [423, 424] and IL-2-activated killer (LAK) cell activity [425] are decreased.

Specific Cytotoxicity

Cell-mediated lympholysis directed against xenoantigens (which is considered to be class II MHC antigen mediated) has been found severely depressed in patients with SLE [93]. Cell-mediated lympholysis against alloantigens is also decreased [37]. Cytotoxicity levels correlate with disease activity parameters.

Antibody-Dependent Cell-Mediated Cytotoxicity

Antibody-dependent cell-mediated cytotoxicity is a lytic mechanism mediated by MNC that carries receptors for the Fc portion of IgG [426–429]. Obviously, the specificity of the killing is determined by the specificity of the involved IgG antibody and not the antibody-dependent cell-mediated cytotoxicity effector cell. Killing may be mediated by certain subclasses of IgG and not by others [430].

Antibody-dependent cell-mediated cytotoxicity of SLE MNC has been tested against a variety of targets (Chang cells, chicken erythrocytes, Burro erythrocytes) and found to be decreased [116, 424, 431–434]. Decreased antibody-dependent cell-mediated cytotoxicity correlated with disease activity [435, 436]. Circulating immune complexes and cytophilic IgG molecules may occupy the Fc receptor of the effector cells and inhibit its ability to exercise antibody-dependent cell-mediated cytotoxicity. Overnight treatment of SLE MNC with pronase enhances antibody-dependent cell-mediated cytotoxicity. Also, the SLE-derived IgG antibody suppresses antibody-dependent cell-mediated cytotoxicity exercised by normal MNC [432]. Although antibody-dependent cell-mediated cytotoxicity expressed by SLE MNC *in vitro* is decreased, antibody-dependent cell-mediated cytotoxicity may be enhanced *in vivo*. Antilymphocyte antibodies many bind to lymphocytes through the $F(ab')_2$ fragment and to antibody-dependent cell-mediated cytotoxicity effector cells through their Fc portion. Antilymphocyte antibodies, lymphopenia, and low antibody-dependent cell-mediated cytotoxicity *in vitro* may thus be casually related [95]. Destruction of other cellular elements via antibody-dependent cell-mediated cytotoxicity may be an ongoing process in patients with SLE.

Nonantigen-Specific Cell Cytotoxicity

Finally, nonantigen-specific T-cell-mediated cytotoxicity is decreased in lupus patients [437]. Lupus T cells stimulated with an anti-CD3 monoclonal antibody in the presence of IL-2 displayed decreased cytotoxicity against Daudi target cells. The defect was limited to lupus patients and did not extend to patients with other rheumatic diseases. The cytolytic defect was not affected by disease activity or treatment. Interestingly, the proliferative responses of these expanded cells (in culture for 10–12 days) were normal in lupus cells, which makes the cytolytic defect more prominent in terms of significance [438]. Nonantigen-specific cytotoxicity is mediated by $CD56^+$ cells that include both natural killer and T cells. The percentages and absolute numbers of $CD56^+$ cells are decreased significantly in lupus patients in a disease activity and treatment-independent manner [439]. Subsequent experiments by the same group showed that the cytolytic defect was associated with a low expression of perforin. The Fas antigen-mediated killing was found to be normal [440]. Deficient nonantigen-specific cytotoxic activity could participate in the promotion of the autoimmune response by failing to eliminate autoantigen and polyclonally stimulated B-cells.

B LYMPHOCYTES

B-Cell Repertoire in Lupus

The main immunological event in the pathogenesis of lupus is B cell overactivity. Patients with active disease and mice with lupus have increased numbers of cells that produce anti-DNA and other autoantibodies [32, 441, 442]. Also, the number of B cells that produce antibody against nonself antigens such as TNP-KLH and ovalbumin is increased [443]. Experiments using the chamber ELIspot assay capable of determining the isotype and cross-reactivity of individual *in vivo*-activated lymphocytes [444, 445] in autoimmune mice showed that cross-reactive B cells were utilized at increased frequency in very old animals, which was associated with the production of pathogenic autoantibodies [446]. In patients with SLE, B cells secreting IgM antibodies against DNA or TNP-KLH were no more cross-reactive than those from normal controls. In contrast, IgG anti-DNA antibody-secreting cells from patients with SLE were significantly more cross-reacting than normals. The degree of cross-reactivity correlated with disease activity and serum anti-DNA antibody levels [447]. Epstein–Bar virus-transformed B cells from patients with SLE produce antibodies that do

not differ with respect to antigenic specificities, avidity, isotype, or titer. However, anti-DNA antibodies bearing the anti-DNA-associated idiotype 8.12 [448] were found only in SLE [449]. These findings are in agreement with those of Klinman *et al*. [447] in that they show that the SLE B-cell repertoire is closer to the normal B-cell repertoire than the serum antibody profile. Moreover, SLE anti-DNA antibodies are different with respect to cross-reactivity and idiotype expression.

B Lymphocyte Subsets

Although the number of spontaneously Ig-secreting cells is increased in both peripheral blood and bone marrow in patients with SLE [32], the number of phenotypically defined B cells appears to be within normal limits.

Expression of the T-cell marker CD5 by B cells defined a subset that has been assigned an important role in autoimmunity. NZB and (NZBxNZW)F1 mice have increased numbers of $CD5^+$ B cells [450, 451]. $CD5^+$ B cells are elevated in patients with Sjögren's syndrome [450, 452] and rheumatoid arthritis [453, 454]. In the majority of patients with chronic lymphocyte leukemia, the malignant clone is $CD5^+$ [455, 456]. $CD5^+$ B cells produce most of the polyreactive antibodies with antiself and anti-idiotypic reactivity [450, 451, 457, 458]. In the NZB mouse, $CD5^+$ B cells produce antierythrocyte antibodies. In the (NZBxSWR)F1 mouse, $CD5^+$ cells produce cationic anti-DNA antibodies [10, 459]. In patients with Sjögren's syndrome and rheumatoid arthritis, $CD5^+$ B cells produce IgM rheumatoid factor [452, 458, 460].

Sporadic patients with SLE may have increased numbers of circulating $CD5^+$ B cells [461, 462]. The average percentage of $CD5^+$ $CD20^+$ B cells is comparable to that of healthy individuals [463]. Both $CD5^+$ and $CD5^-$ B cells from patients with SLE produced equal amounts of anti-ssDNA. IgM polyreactive anti-ssDNA antibodies are produced primarily by $CD5^+$ B cells, whereas IgG and IgA anti-ssDNA antibodies are produced by $CD5^-$ B cells [463]. In another study [464], both $CD5^+$ and $CD5^-$ SLE B cells were found to produce comparable amounts of IgG and IgM anti-DNA antibodies. It is unclear why SLE patients do not follow the example of other murine and human autoimmune disorders.

Phenotypic Changes

Desai-Mehta *et al.* [397] described for the first time the appearance of a costimulatory molecule, CD40 ligand (CD40L, CD154), on the surface membrane of lupus B cells, a marker considered to be characteristic of activated T cells. This finding was disease specific. In the peripheral blood of patients with active SLE $CD40L^+$, B cells were 20.5-fold more when compared to healthy donors. In patients with inactive SLE the number of $CD40L^+$ B cells was comparable to those of controls. Nevertheless, activation-induced CD40L expression on the surface of lupus B cells was 17-fold the baseline levels compared to a 7.6-fold increase recorded in control subjects. CD40L was synthesized actively within the lupus B cell as supported by data of CD40L mRNA detection in lupus B cells.

CD40L expressed on the surface of lupus B cells retains full functional properties. The addition of a neutralizing anti-CD40L mAb in *in vitro* cell culture settings decreased cationic anti-dsDNA production. Because not only T cells but also B cells from SLE patients express functional CD40L on their cell surface and because both kinds of lymphocytes express CD40, a bidirectional cognate stimulatory loop may function between lupus T and B cells [465]. Moreover, it was reported that CD40L expressed on the surface of B cells costimulates other B cells as well [466].

In addition to CD40L, CD80 and CD86 are aberrantly expressed on the surface of lupus B cells. Fresh, small, resting, peripheral B cells but also large, activated B cells from patients with SLE were studied for the expression of CD80 and CD86. While CD80 expression was increased only slightly on the surface of large, activated lupus B cells, the expression of CD86 was 2.5- and 7-fold increased on the surface of activated and resting lupus B cells, respectively, when compared to B cells obtained from patients with allergic disorders [378]. While patients in remission only were studied, and the functional integrity of the costimulatory molecules was not assessed, this study provides evidence that B cells from patients with SLE overexpress molecules that belong to the B7 family of costimulatory molecules. Expression of B7 family molecules has been shown to be a prerequisite for the disruption of immune tolerance toward self-antigen [467].

Spontaneously Ig-Secreting B Cells

The number of spontaneously Ig-secreting B cells is increased significantly in patients with SLE [60, 410, 468–471]. Spontaneously activated SLE B cells correlate with disease activity, serum DNA binding, low levels of serum C3, treatment with immunomodulatory drugs [38, 470, 471] and a high RNA/DNA ratio in nuclear chromatin [472]. Unlike B cells from NZB mice, which secrete primarily the IgM antibody [442], B cells from adult patients with SLE secrete Ig of IgG and IgA

classes [38, 473, 474]. It has been reported that B cells from children with SLE secrete primarily IgM [475]. Bone marrow from patients with active or inactive SLE contains large numbers of B cells secreting spontaneously Ig [476].

Mitogen-Induced Ig Secretion

Although the number of spontaneously Ig-secreting B cells is increased in SLE, stimulation of MNC *in vitro* with pokeweed mitogen causes a rapid decrease of Ig production [38, 114, 468, 476–479]. Removal of monocytes from the culture system increases significantly the pokeweed mitogen-induced polyclonal response [38] and the dsDNA-induced specific response [35]. Because pokeweed mitogen is a T-cell-dependent B-cell polyclonal stimulator and anti-DNA production is T cell dependent, these studies do not indicate that B cells from SLE patients have a primary defect. Indeed, stimulation of B cells with the T-cell-independent antigens *S. aureus* Cowan strain I [480] and EBV [128] *in vitro* induces the secretion of normal amounts of Ig.

Lymphokines in Lupus B-Cell Function

Lymphokines are responsible for driving or prohibiting various B-cell activation and differentiation phases. This section addresses the contribution of lymphokines secreted by T cells and macrophages (endocrine or paracrine action) and those secreted by B cells themselves (autocrine action).

B cells from autoimmune mice demonstrate hyperresponsiveness to activating signals and lymphokines promoting B-cell growth and differentiation [481]. Human lupus B cells similarly overrespond to factors produced by T cells [482]. Percoll gradient-separated small resting human SLE B cells show a stronger proliferative response to *S. aureus* Cowan strain I [483]. Addition of the 10- to 12-kDa B-cell growth factor (BCGF) further enhances their proliferative response. *S. aureus* -activated lupus B cells respond with excessive production of Ig if T-cell-derived factors are present [464, 483]. *S. aureus* -preactivated lupus B cells also demonstrate more vigorous responses to IL-1 [484]. Activated B cells express the receptor for IL-2. One-half of *S. aureus* -activated normal B cells express the IL-2 receptor; *S. aureus* -activated SLE B cells express less IL-2 receptors on their surface [485]. This is further corroborated by the finding that *S. aureus* -activated SLE B cells do not respond to exogenous IL-2 [484]. Even in the absence of mitogen SLE B cells proliferate vigorously in the presence of the 50-kDa BCGF, but not in the presence of IL-2 and the 20-kDa BCGF [486]. In an other study [487], enriched SLE B-cell populations were found to respond excessively to recombinant 12-kDa BCGF. Notably, only one-half of the patients responded excessively regardless of disease activity. IL-4 enhanced and transforming growth factor-β suppressed, at expected levels, the BCGF-induced B-cell proliferation. Although IFN-γ suppresses the *in vitro* response of lupus cells to TNP–*B. abortus* [129], IFN-γ increases dramatically the ability of freshly isolated lupus non-T cells to produce Ig [488]. In summary, resting and *S. aureus* -activated lupus cells respond excessively to BCGF-mediated stimulation. IL-2 does not seem to participate in the regulation of proliferation of at least resting SLE B cells, whereas IL-4 and transforming growth factor B regulate the response normally.

EBV-transformed B cells and normal B cells produce lymphokines that may regulate B cell function. SLE lymphocytes [489] and purified B cells [490–494] produce factors that are able to promote proliferation and differentiation in B cells. In one of the studies [494], the factor that was produced by B cells and was acting on B cells had an estimated molecular mass of approximately 18 kDa. This factor promoted both proliferation and differentiation of SLE B cells and did not identify with IFN or IL-2. In contrast, it contained IL-1, IL-4, and IL-6 activity. Lupus B cells respond to IL-1, Il-4, and IL-6 [494].

The relative role of IL-10 in driving human anti-DNA antibody production was shown in mice with severe combined immunodeficiency that were reconstituted with peripheral MNC from lupus patients. In these chimeric animals, production of the human anti-DNA antibody was blocked by anti-IL-10, but not by anti-IL-6 antibodies [244]. A role for IL-10 in the expression of human lupus was inferred by other data, which showed that increased disease activity was associated with a preferential (over IFN-γ) expansion of peripheral cells producing IL-10 [267]. IL-10 may suppress APC function and may be responsible for the defective B7-1 expression [373].

The action of excessive amounts of stimulatory lymphokines is probably facilitated by deficient negative feedback. IgG Fc receptor-mediated signaling delivers a negative signal to B cells [495]. In autoimmune mice, this signaling is defective [496]. There is some indication, yet not direct, that Fc-mediated negative signaling is defective in SLE [497, 498] (see also section on cell signaling). The combination of positive feedback exercised via B cell stimulatory factors as described earlier and the lack of negative feedback provided by Fc receptor ligands contribute to the perpetuation of autoantibody production by lupus B cells.

Aberrant B-Cell Antigen Receptor Signal Transduction

The studies discussed earlier present evidence supporting the view that B cells from patients with SLE have functional as well as phenotypic abnormalities that are at least in part independent of the activity of the underlying disease. It is thus possible that such aberrations represent intrinsic lupus B-cell defects. It is also possible that the heterogeneous defects described previously may have a common underlying central biochemical abnormality. Crucial aspects of lymphocyte function, such as activation, proliferation, cytokine production, effector functions, and apoptosis, are determined by the signaling biochemical pathway initiated following ligation of the surface antigen receptor. Physiologically, the ligand for BCR is the relevant antigen, and for the autoreactive B cell it is the autoantigen.

We currently know that the biochemical cascade triggered in the B cell following the interaction between BCR and antigen can be closely mimicked by anti-BCR antibodies. The question of possible antigen–receptor-mediated signaling aberrations in B cells of lupus patients using anti-Ig antibodies has been addressed [499]. Stimulation of circulating B cells from patients with SLE through their sIgM BCR produced significantly higher fluxes of free intracytoplasmic Ca^{2+} when compared to similarly induced responses of B cells from patients with other systemic rheumatic diseases or to the responses obtained from normal B cells. This phenomenon was not limited to signaling through the sIgM BCR, but was also observed when the sIgD BCR was used for triggering the signaling process. The elevated Ca^{2+} responses were contributed significantly from the intracellular calcium stores. Nevertheless, the production of inositol 1,4,5-trisphosphate (the principal mediator of free calcium release from the intracellular compartment) was elevated only slightly, raising the possibility of either a hypersensitive Ca^{2+} release machinery or of a dominant inositol trisphosphate-independent pathway(s) of Ca^{2+} release.

The earliest known BCR-mediated signaling event is the activation of protein tyrosine kinases, which results in the tyrosyl phosphorylation of cellular proteins [500]. The tyrosyl phosphorylation reaction has numerous substrates; it is only a few of such substrates that have been identified. In lupus B cells, the overall level of sIgM-initiated protein tyrosyl phosphorylation was significantly enhanced and correlated with the augmented BCR-mediated free calcium responses. More specifically, at least four cellular proteins with molecular sizes between 36 and 64 kDa were significantly hyperphosphorylated in anti-IgM-treated lupus B cells compared to the response of B cells from normal controls [499]. The aberrant BCR-mediated signaling process was not associated with disease activity, treatment status, and specific clinical manifestations. Moreover, enhanced tyrosyl protein phosphorylation was disease specific, implying a possible intrinsic lupus B-cell defect, which may have a pathogenic impact. Furthermore, the increased Ca^{2+} responses could represent a biochemical and molecular basis for the enhanced expression of CD40L on the surface of lupus B cells upon stimulation. It was reported previously that CD40L upregulation on the cell surface is predominantly NFAT, and hence Ca^{2+} dependent [25]. Signaling abnormalities encountered in SLE B lymphocytes may provide a biochemical and molecular background for such diverse abnormalities as lymphocyte activation, anergy, and cell death.

It is interesting that similar to the human lupus BCR-initiated signaling, abnormalities were encountered in a study of experimental animal lupus. Feuerstein *et al*. [370] induced systemic lupus-like autoimmunity by inducing graft-versus-host disease in a background of a double transgenic sIg/sHEL murine tolerance model. The induction of autoimmunity correlated with phenotypical as well as with signal transduction changes of B cells similar to the human disease. The B-cell surface expression of CD21 (part of the complement receptor 2, CR2, complex) was decreased significantly in the autoimmune state. In addition, changes in the BCR-mediated protein tyrosyl phosphorylation pattern in B cells developed following the induction of autoimmunity. In the autoimmune but not in the tolerant state, two substrates with apparent molecular masses of 78 and 60 kDa were hyperphosphorylated following BCR cross-linking. The similarity of BCR-induced signaling in patients with SLE and in this particular autoimmunity model underscore the potentially central pathogenic role of aberrantly functioning B cells in both conditions.

B-Cell Surface Receptors That Provide Negative Regulation

There are several different B-cell surface signaling inhibitory receptors. Among them, the functions of CD5, CD22, and FcγRIIB1 are better understood [500]. The ligand for CD5 has been shown to be CD72. The role of $CD5^+$ B cells in SLE and in experimental animal lupus was discussed previously. The role of coreceptor CD22 was incompletely understood until a study using CD22 knockout mice clearly disclosed that CD22 is a signaling inhibitory molecule. Young CD22 knockout mice displayed autoimmunity, increased BCR-initiated cytoplasmic calcium responses, hypergammaglobulinemia, and circulating IgM autoAb [501]. Adult CD22

knockout mice displayed glomerulonephritis, circulating IgG anti-dsDNA, and anticardiolipin autoAb [502]. This is a particularly interesting model of systemic lupus-like autoimmunity because it is monogenic and because a B-cell molecular defect created an autoAb response that included isotype switching and affinity maturation of the (auto)antibody, characters very reminiscent of a T-cell-dependent immune response. Nevertheless, CD22 expression on the surface of B cells from patients with either active or inactive SLE is similar to that of normal controls [503].

A B-cell surface inhibitory receptor with a role that is well appreciated is the one for the Fc fraction of IgG type IIB1 (FcγRIIB1, CD32). When antigen bound to IgG is presented to the BCR, then BCR and FcγRIIB1 are cocross-linked. The net signal is of smaller magnitude than the signal generated by antigen alone. The cytoplasmic-free Ca^{2+} response is of shorter duration and the B-cell response is incomplete, not resulting in full B-cell activation and proliferation. Previous studies have reported that the system of receptors for the Fc fraction of IgG is overall malfunctioning in SLE, resulting perhaps in the production of excess antibodies and the accumulation of immune complexes [504]. Genetically manipulated mice having a B-cell surface FcγRIIB1 deficiency manifested hypergammaglobulinemia [505].

Engagement of CD32 in human SLE B cells fails to suppress the BCR-mediated signaling response by failing to engage SHIP, a molecule that is needed to inhibit the signaling process [341]. Therefore, defective negative regulation may be, at least partially, responsible for the increased B-cell response in SLE. The coreceptors CD22 and FcγRIIB1 bear a signaling inhibitory domain in their cytoplasmic tail called the Immunoreceptor tyrosine-based inhibitory motif (ITIM). ITIM become functional when the tyrosyl residues lying in its context become phosphorylated under the influence of protein tyrosine kinases. Tyrosyl-phosphorylated ITIMs become the docking and activating sites for SH2 domain-containing protein tyrosine phosphatases, particularly SHP-1 and SHIP [150–152]. It is interesting that SHP-1 is absent or dysfunctional in other autoimmunity models, the *motheaten* and the *viable motheaten* mouse, respectively [500].

Phosphorylation of ITIM tyrosyl residues is accomplished by protein tyrosine kinases. The ITIM lying in the cytoplasmic tail of FcγRIIB1 and CD22 coreceptor undergoes tyrosyl phosphorylation by the *src* family kinase *lyn*. In the absence of *lyn* the CD22-initiated signaling inhibitory pathway is not triggered, despite adequate amounts of B-cell surface CD22 expression. In $lyn^{-/-}$ animals, immature B cells developed normally but mature B cells were decreased. Rather unexpectedly it was shown that BCR-initiated signaling events were not only propagated, but in fact were enhanced. $lyn^{-/-}$ mice develop autoimmunity with features quite reminiscent of lupus: they have hypergammaglobulinemia, an increased sensitivity to IL-4, and develop autoAb and glomerulonephritis [506].

It is interesting that one study reported a defective expession of mRNA and protein lyn [507] and another one an abundant presence [508] of lyn in B cells from patients with SLE. It is likely that decreased lyn in SLE B cells contributes to B-cell overactivity by deficiently regulating the BCR-initiating response.

What Causes B-Cell Hyperactivity?

There are several possible explanations, which either alone or in concert may be involved in B-cell overactivity and antibody production.

B cells may be intrinsically abnormal: (a) B-cell subsets secreting autoantibodies may be expanded as discussed earlier. (b) B cells may overrespond after cross-linking of the surface antigen receptor by antigen. (c) B cells may overrespond to lymphokines as discussed earlier. (d) B cells receive aberrant regulatory messages through other cell surface receptors: Deficient negative signaling through the Fcγ receptors, as discussed previously, or excessive help through complement receptors (see discussion under complement receptors).

B cells may receive inappropriate external stimulation: (a) B cells become overactive in the presence of excessive quantities of lymphokines or, more importantly, unbalanced levels of type 1 and type 2 lymphokines, as discussed in the lymphokine section. (b) Excessive help by abnormally expanded helper T cells obviously contributes to B-cell overactivity.

B cells may undergo excessive polyclonal activation. As discussed earlier, lupus patients are characterized by the presence of a wide range of antibodies and generalized hypergammaglobulinemia. In addition, it has been well established that polyclonal activators can break B-cell tolerance. From the clinical point of view it has been well established that infections may exacerbate disease activity.

Autoantigen-specific B-cell stimulation has been classically accepted to drive B cells to produce autoantibodies. The most important antibodies such as anti-DNA (see Chapter 10) undergo isotype switching and affinity maturation.

Hypogammaglobulinemia in SLE

SLE is characterized by hypergammaglobulinemia. Over the past decade 20 SLE patients have been

described who during the course of their disease developed hypogammaglobulinemia. Hypogammaglobulinemia was not associated necessarily by decreased levels of anti-DNA antibodies [509–517]. A number of causes have been claimed in the aforementioned reports to be responsible for the complication, including prolonged treatment with cytotoxic drugs, the absence of phenotypically defined B cells, and increased T-cell suppressor cell activity.

REGULATION OF IMMUNE RESPONSES BY COMPLEMENT

Complement Receptor Expression

CR1 is a cell surface polymorphic glycoprotein present on a variety of cells, including B lymphocytes, certain T-cell subsets, monocytes, and glomerular podocytes [69, 70, 518–520]. It binds the C3b and C4b fragments of complement 3 and exhibits molecular weight polymorphism. CR1 participates in the phagocytic process and provides erythrocytes with the ability to carry and clear immune complexes. The number of CR1 was initially reported to be decreased on the surface membrane of SLE erythrocytes [521]. Genetic studies of the erythrocyte CR1 deficiency in SLE have produced controversial data with numerous studies reporting for or against genetic predisposition [522–532]. An occasional patient with SLE may have circulating antibodies directed against CR1 that are responsible for decreased numbers of erythrocyte CR1 [533, 534]. In addition, it seems that the levels of CR1 on the surface of erythrocytes correlate with disease activity and levels of circulating immune complexes [535–538], which supports that CR1 deficiency is not a primary defect. Conversely, studies have shown that the inheritance of certain alleles of the CR1 does not predispose to lupus [525, 539]. The number of CR1 of the surface membrane of lupus polymorphonuclear cells seems to reflect disease activity [540]. Patients with discoid lupus erythematosus who are completely asymptomatic have normal levels of erythrocyte CR1 [541].

A soluble form of CR1 is detected in the human plasma [542]. A larger form of CR1 is released in the urine bound to immune complexes thought to derive from podocytes and to represent a marker of glomerular injury [543].

On addition to decreased numbers of erythrocyte CR1, the number of CR2 (receptor for C3d and EBV) is decreased on the surface of lupus B lymphocytes [544–546].

Studies in lupus-prone mice have shown a decreased expression of CR on the surface of B cells prior to the clinical initiation of the disease [547]. It is unclear whether loss of CR accelerates or worsens the disease process. Data discussed earlier support either proposal. Treatment of MRL *lpr/lpr* mice with anti-CR anti-=bodies for up to 3 months failed to alter the disease process (G. Tsokos, T. Kinoshita, and F. Steinberg, unpublished results).

Complement Receptor Ligands

Abundant activation of the complement system takes place in patients with SLE. It is manifested by (a) decreased serum levels of several factors of both classic and alternative pathways (C2, C4, etc) [548, 549]; (b) increased levels of complement factor breakdown products, i.e., C3d and C3a [550, 551]; and (c) the formation and tissue deposition of immune complexes [536]. In addition to immune complexes, which clearly are responsible for the activation and consumption of the majority of complement factors, certain patients produce IgG antibodies that have one of the following characteristics: (a) bind to and stabilize the C3 convertase of the classical complement activation pathway (C4b2a), thus promoting a continuous consumption of C3 and C4 [552], or (b) bind to and activate C3 in the absence of antigen (C3-activating factor) [553]. Following any form of activation of the complement, complement factor breakdown products are generated that stay in the circulation for variable time periods, either in monomeric or polymeric (when bound to immune complexes) form [536]. It should be noted that polymorphonuclear cells from some lupus patients produce more C3 than normal cells, which may provide additional breakdown products [554]. Immune cells, particularly B lymphocytess, express receptors for various complement breakdown products, i.e., CR1, which binds C3b and C4b; CR2, which binds C3d; and CR3, which binds C3bi [69]. C3b and C3d can inhibit B-cell function when they are present in monomeric form [555, 556]. This action may serve as a negative "feedback" mechanism to the activated immune system. Polyvalent CR ligands may activate B cells [556, 557] and act synergistically with anti-IgM antibodies in stimulating B cells [555, 557]. Indeed, immunization of mice with genetically engineered molecules that are composed of antigen and multiple copies of C3d displayed a significantly enhanced immune response [558, 559]. A special association between surface immunoglobulin and CR on B cells is probably responsible for these effects [560]. Thus, activation of the complement pathway takes place in patients with SLE and the products of this process interfere with the function of immune cells only to further complicate the interpretation of the observed cellular aberrations. Complement fragments may stay in

the circulation as monomeric CR ligands or may attach to immune complexes [536] and form polyvalent CR ligands. As discussed earlier, polyvalent CR ligands act synergistically with B-cell antigens in amplifying B-cell function. Under this hypothesis, DNA-containing immune complexes will stimulate DNA-specific B cells to produce more anti-DNA antibodies. Monomeric CR ligands may suppress B-cell function and lead to a decreased production of "useful" antibodies. Further studies are needed to clarify the role of CR in the pathogenesis of human lupus.

APOPTOSIS

Biochemistry and Molecular Biology

Apoptosis is an active, signal-dependent cellular process. Cells dying by apoptosis present characteristic morphological changes and fragmentation of genomic DNA into oligonucleosomal bands of approximately 180 bp [561–563]. An endogenous endonuclease that is Ca^{2+}-dependent [564] is involved in this process. Several genes have been associated with apoptosis. The *bcl-2* gene encodes membrane-associated proteins of 26 and 22 kDa (members of the oncogene family), which are present in the nuclear envelope, the endoplasmic reticulum, and the inner mitochondrial membrane [565], and is considered to protect cells from apoptosis. In contrast to this protective function, the APO-1 (Fas) gene encodes a 319 amino acid protein that promotes apoptosis. Cytoplasmic and extracellular domains of the human APO-1 antigen consist of 145 and 157 amino acids, respectively. The proximal 68 amino acid region of the intracytoplasmic domain is responsible for mediating cell killing, whereas the C-terminal amino acid region contains negative regulatory elements [566, 567]. Deletions and/or mutations of the intracytoplasmic domain transfected in L919 cells prevent apoptosis. Apoptosis mediated by the APO-1 receptor is an active process where the action of the APO-1 antigen with its ligand induces apoptotic cell death. The APO-1 gene was identified to be equivalent to the murine Fas gene.

Organization of the human Fas gene is now known. The promoter region contains consensus sequences for AP-1, GF-1, NY-Y, CP-2, EBP 20, and c-myb. The gene consists of nine exons and spans more than 26 kb of DNA in the long arm of chromosome 10 [568]. The expression of Fas antigen gene is regulated by signaling mediators that are produced in response to cross-linking of the CD3 molecule [569]. Reversely, cross-linking of the Fas antigen suppresses the CD3-mediated signaling [570]. It is obvious that in any interaction between T cells and APC that express the Fas ligand a number of functional outcomes may ensue as a result of signaling through multiple cell surface receptors.

Apoptosis in Murine Lupus-Prone Mice

MRL *lpr/lpr* mice carry a mutation at the intracytoplasmic domain of the Fas gene [571, 572]. The mutation has been identified to be a 5.3-kb insertion of homology with a sequence from the 3′ long terminal repeat of the early transposon [573]. These animals develop a lupus-like syndrome with lymphadenopathy, production of large amounts of anti-DNA antibodies, immune complex nephritis, and synovitis. The significance of the *lpr* mutation was tested directly by constructing transgenic MRL-*lpr/lpr* mice using a full-length murine Fas cDNA under regulation of the T-cell-specific CD2 promoter and enhancer. Indeed, early correction of the lpr defect in T cells is sufficient to eliminate the acceleration of autoimmune disease even in the presence of other cells that express the mutant lpr gene [574]. A similar phenotype is also seen in *gld* mice, which have a mutation in the Fas ligand, indicating that the receptor/ligand interaction is necessary for the apoptotic process [575].

Apoptosis in Human Lupus

Freshly isolated B cells and both $CD4^+$ and $CD8^+$ T lymphocyte subpopulations expressed higher levels of Fas antigen when compared to normal cells. Despite the spontaneously elevated levels of Fas antigen, mitogenic stimulation of both T and B lymphocytes from SLE patients resulted in normal Fas antigen upregulation [576, 577]. Freshly isolated and cultured lymphocytes from patients with SLE display higher levels of apoptosis when compared with lymphocytes from normal individuals [578]. Although expression of the Fas antigen did not associate with any clinical parameter [576], the apoptotic rates of lymphocytes correlated significantly with disease activity but not with prednisone treatment [578]. In addition, peripheral blood macrophages display increased rates of apoptosis [54]. T cells but not B cells from patients with SLE were also reported to have increased levels of *bcl-2* protein [577, 579]. These findings were largely reproduced by Lorenz *et al*. [580], and the following useful two points were added: (1) the increased apoptotic rate of lupus cells vanished when the cells were cultured *in vitro* with lymphokines or mitogens or superantigens and (2) these abnormalities extended in patients with other autoimmune disorders (mixed connective tissue disease and various forms of vasculitis). The Fas ligand was also found to be expressed at increased amounts in lupus

T cells and it retains full functional features [581]. Increased apoptosis may contribute to (a) the development of cytopenias and (b) the release of nuclear material in the circulation that may, under certain conditions, stimulate the immune system to produce autoantibodies [582].

Apoptosis of Keratinocytes

Ultraviolet irradiation of cultured human keratinocytes induces changes consistent with apoptosis, and the autoantigens are clustered in two distinct populations of blebs at the surface of apoptotic cells. The smaller blebs contain endoplasmic reticulum, ribosomes, and autoantigen Ro. The larger blebs contain nucleosomal DNA, Ro, and La and the small ribonucleoproteins [583]. Apparently, photosensitivity, i.e., disease exacerbation following exposure to sun, may be explained by the flooding of the immune system with autoantigens that activate T and B cells. Although there is no direct translation of murine lupus data on apoptosis to the human analogue, it is obvious that there is dysregulation of the apoptotic process in human lupus. It remains to be seen whether certain lupus patients and their family members have molecular defects of the Fas gene, its ligand, or other cell proliferation-controlling genes such as *bcl-2*, p53, or *c-myc.*

Soluble Fas Antigen

As discussed earlier, the APO-1/Fas antigen is expressed on the cell surface membrane. Another APO-1 mRNA has been identified in which the exon that encodes for the transmembrane region has been deleted. The deletion does not affect the open reading frame but results in a Fas protein that lacks the 21 amino acid transmembrane region and is secreted in the serum. Patients with SLE have increased levels of soluble Fas protein. Two functions were attributed to the soluble Fas protein: (a) protection of cells from apoptosis by binding to the natural ligand of the cell surface membrane-expressed Fas protein and (b) disturbance of the immune system. The latter was concluded after it was shown that injection of murine-soluble Fas in mice produced redistribution of the thymocyte subsets and an increase in the number of splenocytes [584]. The disease specificity, the levels, and the physiologic significance of soluble Fas molecules in lupus patients have been debated extensively [585–587].

Canale–Smith Syndrome

The human "apoptosis deficiency" syndrome has been recognized as the Canale–Smith syndrome [588]. These apoptosis-deficient patients obviously cannot eliminate any possible autoreactive cells that arise as a "by-product" of all immune responses and display autoimmune manifestations, which nevertheless are not the cardinal features. Interestingly, some of these patients experience cytopenias that cannot be explained by the defective apoptotic cell death [589]. Therefore, it is possible that the lupus cytopenias cannot be explained by the reported increased spontaneous apoptotic cell death.

Structural Defects of Fas and Fas Ligand Molecules in Human Lupus

Lupus patients have been studied for mutations in the known components of the apoptotic machinery that might resemble those of the apoptosis-deficient murine models, and only rare patients with mutations in the apoptotic machinery have been found. One patient with lupus (out of 100 tested) had defective Fas ligand expression [590], whereas another one with treatment-resistant lupus had increased expression of a truncated form of Fas antigen that lacked the transmembrane region. Her lymphocytes were producing large amounts of soluble Fas molecules [591]. Obviously, structural abnormalities of the Fas and Fas ligand genes and their products cannot account for defects in apoptosis in humans as in mice.

Activation-Induced Cell Death

The aforementioned studies have not addressed whether activated T cells from patients with lupus that have been shown to play a crucial role in driving B cells to produce autoantibody display altered programmed death in response to antigen/autoantigen. Activation-induced cell death plays an important role in the regulation of the immune response by eliminating pre-activated and potentially autoreactive cells. To elucidate possible abnormalities of activation-induced cell death in human SLE, activation-induced cell death in activated T cells from patients with SLE was studied [328]. CD3-mediated cell death was determined in short-term T-cell lines by flow cytometry using propidium iodide staining and analysis of DNA subdiploid peak populations. It was found to be significantly lower in T cells from SLE patients compared to cells from normal controls. Anti-Fas mAb-mediated cell death was similar in SLE and control cell lines. CD3-mediated activation-induced cell death could be blocked in control and SLE T-cell lines by an IgG anti-Fas mAB. Indirect immunofluorescence analysis showed statistically significantly less intracellular TNF-α in SLE T cells than in control cells. These data show that activated T cells from

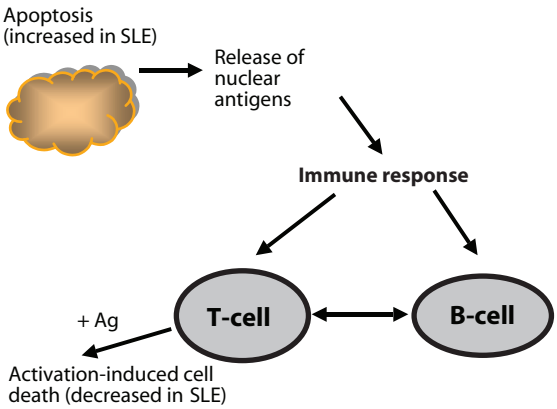


FIGURE 5 Aberrations of apoptosis in human lupus. The increased apoptotic rate of various cell types (monocytes, keratinocytes, etc.) leads to an increased production of nuclear antigens that stimulate the immune system and increase the number of activated lymphocytes. Decreased elimination of activated cells may further contribute to the size of the activated lymphocyte pool.

patients with SLE are relatively resistant to a TCR-mediated death stimulus, although they display intact anti-Fas mAb-mediated cell death. Defective antigen-mediated cell death can contribute to increased numbers of activated autoreactive cells in lupus patients (Fig. 5).

Because death of activated cells involves intracellular TNF-α [166] and because lupus cells produce decreased amounts of TNF-α [257] resulting from a TNF-α polymorphism that is different from that of normal cells [258], it was considered that a decreased expression of intracellular TNF-α may contribute to the decreased CD3-mediated lupus T-cell death. Indeed, permeabilized lupus T cells were found to display significantly lower amounts of TNF-α. Apparently, a functional Fas/Fas-ligand path and adequate amounts of intracellular TNF-α are needed for the CD3-mediated T-cell death. It was concluded that decreased TCR-mediated cell death in T cells from patients with SLE is not due to a dysfunction in Fas expression and function. The possible role of a defective Fas-ligand function needs to be determined. Furthermore, adequate levels of intracellular TNF-α may be necessary for proper execution of the activation-induced cell death cascade. Prolonged survival of autoreactive T cells can lead to increased autoantibody production. The fact that activation-induced cell death is decreased in SLE T cells can be important for the disease process *in vivo*, where engagement of the TCR on the surface of activated T cells, either by circulating or tissue-bound autoantigens, could fail to cause cell deletion, thus supporting the perpetuation of the autoimmune process.

Altered Mitochondrial Hyperpolarization, Reactive Oxygen Intermediates

Disruption of the mitochondrial transmembrane potential ($\Delta\psi_m$) has been proposed as the point of no return in apoptotic signaling [592]. Data indicate that elevation of $\Delta\psi_m$, i.e., mitochondrial hyperpolarization, occurs in the early phase of Fas-, p53-, and H_2O_2-induced apoptosis. Indeed, elevation of $\Delta\psi_m$ precedes caspase activation, phosphatidylserine externalization, and disruption of $\Delta\psi_m$ in Fas- and H_2O_2-induced apoptosis [592–594].

Deviations in key mitochondrial checkpoints of SLE T cells associated with abnormal apoptosis have been identified [595]. $\Delta\psi_m$ and mitochondrial reactive oxygen intermediate (ROI) production were elevated, but glutathione (GSH) levels were diminished compared to healthy or RA controls. A low GSH is consistent with increased ROI production due to the utilization of reducing equivalents. Ongoing oxidative stress *in vivo* may lead to a skewed expression of transcription factors AP-1 and NF-κB [207, 208] and, further downstream, expression of IL-2, TNF, and IL-10 [596]. Of note, increased spontaneous apoptosis of lymphocytes has been linked to increased IL-10 production, release of FasL, and overexpression of the FasR in SLE [597, 23]. Elevated nitric oxide (NO) production may also contribute to increased spontaneous apoptosis [598, 599]. Indeed, increased ROI levels confer sensitivity to H_2O_2, NO, TNF, and Fas-induced cell death [594]. Therefore, elevated baseline ROI and $\Delta\psi_m$ levels may have key roles in the enhanced spontaneous death of SLE PBL.

EFFECT OF TREATMENT ON THE FUNCTION OF LUPUS CELLS

General Considerations

Studies of the cellular immune dysfunction in patients with SLE are criticized by the fact that most, eventually all, patients take some form of immunomodulatory treatment [32]. Careful temporary withdrawal of the treatment prior to the study may not always guarantee the lack of effect of the treatment on the immune function under study. Drugs affect immune functions at differential doses. For example, corticosteroids suppress the autologous mixed lymphocyte reaction at physiological concentrations, whereas they have no effect on the mixed lymphocytes reaction unless they are used in pharmacologic doses [600]. Corticosteroids at nanomolar (subphysiologic) concentrations may enhance the immune system instead [601]. Such will be the situation in a patient from whom the low steroid dose is withheld

for 24 or 48 h. Steroids have pleiotropic effects on the expression of molecules that are involved in T-cell signaling, such as the TCR ζ chain. Specifically, culture of human lymphocytes with 10 n*M* dexamethasone caused increased transcription of the *TCR* ζ chain, whereas culture with 100 n*M* suppressed ζ chain transcription and expression [602]. Interestingly, high concentrations of dexamethasone led to increased TCR-mediated $[Ca^{2+}]_i$ responses [603]. In addition, steroids modulate the expression of IL-2 by suppressing its transcription and destabilizing its message [604], most probably affecting the expression of other cytokines. Unfortunately, there are not any controlled studies on the effect of various treatments on the cellular immune functions in patients with SLE. Patients receiving treatment enter invariably the inactive phase of the disease. Changes in the immune system between active and inactive phases of the disease may well mean drug effect. Treatment of patients with SLE leads inevitably to decreased immunoglobulin production, particularly of anti-dsDNA antibodies. By inference, treatment affects/normalizes B- and T-cell function [322].

Cyclophosphamide

McCune and colleagues [605] administered cyclophosphamide intravenously to patients with SLE in six monthly doses. Patients improved clinically, anti-DNA antibody titers decreased, and serum C3 levels improved along with a decrease in B and T lymphocytes and their CD4 and CD8 subsets. This study was preliminary, uncontrolled, and it would be eventually necessary to know whether the same cellular changes would have taken place in the absence of cyclophosphamide.

Plasmapheresis

Plasmapheresis was proven finally to have no effect on clinical manifestation and cellular immune functions in patients with SLE, despite the rational expectations that had led in its launching in the treatment of lupus [606].

Total Lymphoid Irradiation

Total lymphoid irradiation of patients with severe lupus nephritis seems to have more definitive effects by decreasing the number of peripheral lymphocytes and their major subsets and by their ability to mount proliferative responses *in vitro* [607, 608]. Similarly, *in vivo*, in these patients the levels of antibodies to tetanus toxoid and diphtheria toxin were decreased and rose only sluggishly following immunization [609]. Total lymphoid irradiation exerts its effects by acting on T cells rather than on B cells. Ig production by MNC from patients with SLE who were treated with total lymphoid irradiation was decreased following stimulation with pokeweed mitogen, which is a T-cell-dependent B-cell stimulus, and normal following stimulation with EBV and *S. aureus* Cowan 1, which are T-cell-independent B-cell stimuli [610]. Treatment of an autoimmune murine strain (New Zealand black X New Zealand white)F1 with total lymphoid irradiation resulted in a significant reduction of $CD5^+$ B cells and anti-DNA antibody production [611] and restoration of the thymic architecture [612].

DRUGS IN THE PATHOGENESIS OF SLE

A number of commonly used medications (hydralazine, procainamide, etc.) have been associated with the development of clinical and laboratory features of SLE. Manifestations are characterized by the rarity of vital organ involvement [613]. Peripheral B cells from these patients secrete increased amounts of immunoglobulin while their T cells seem to provide excessive help to B cells [125]. Study of the molecular mechanisms whereby drugs cause increased autoreactivity and autoimmunity is worthwhile, as these drugs represent distinct, identifiable extrinsic factors. $CD4^+$ tetanus toxoid-specific cloned T cells treated with 5-azacytidine (an inhibitor of DNA methylation) proliferate in response to autologous class II MHC gene products in the absence of tetanus toxoid [614]. 5-Azacytidine has also been shown to change the $CD8^+$ phenotype of T cells to $CD4^+$ [391]. Rendering $CD4^+$ cells autoreactive following treatment with DNA methylation inhibitors is an attractive phenomenon, as these cells can go on and induce autoimmunity. Because hydralazine and procainamide cause SLE and are known to bind to DNA [615, 616], they were tested and found to inhibit DNA methylation and induce self-reactivity to tetanus toxoid-specific human T-cell clones [617]. Ultraviolet light, another external factor frequently entertained in the pathogenesis of SLE, also causes extensive DNA demethylation, which is associated with gene (metallothionein-I) activation [618]. Because the methylation status of genes determines their transcription rate in general [619], one can speculate that various inciting factors may lead to autoimmunity by interfering with the methylation of crucial genes. It is difficult at the present time to speculate which are these genes. It was hypothesized that the *c-myc* oncogene and interleukin-2 gene might be involved, and their methylation status was tested using

an enzyme cut approach. Despite these negative results, the hypomethylated gene hypothesis might hold true for other genes that may be more central in the pathogenesis of SLE. Richardson *et al*. [390] found that DNA from T cells from patients with SLE is less methylated than DNA from normal T cells. This conclusion was achieved by measuring the deoxymethylcytosine content of lupus T lymphocyte DNA. DNA methyltransferase activity in lupus T lymphocyte nuclear proteins was decreased and was considered to be responsible for the hypomethylation of lupus T cell DNA. Interestingly, non-T cells from lupus patients expressed normal DNA methylation levels. The significance of these findings is undermined by the fact that T-cell DNA is hypomethylated only in one-half of the patients with lupus and that patients with rheumatoid arthritis share the same abnormality.

Inhibition of DNA methylation in various T-cell lines led to overexpression of LFA-1, which may contribute to T-cell autoreactivity and increased T-cell homing to target tissues as discussed earlier (costimulation) [389].

In mature T cells, DNA methylation is a postsynthetic event mediated by the enzyme DNA methyltransferase 1 (Dnmt1). Dnmt1 levels increase following T-cell stimulation [620]. It was possible that the signaling defects in the proximal and middle pathways might be associated with abnormal signaling in the distal pathways as well, contributing to decreased Dnmt1 levels. Moreover, Dnmt1 levels are also regulated by signals that traverse the JNK and ERK pathways, possibly acting through AP-1 sites in an intronic promoter [621].

The mechanisms underlying deficient Dnmt1 activity in T lymphocytes from subjects with SLE have been studied following stimulation with PMA/IO. The phosphorylation of ERK1/2 and JNK was analyzed. Compared to controls, MEK (MAPK kinase)-catalyzed ERK1/2 phosphorylation in SLE-unfractionated PBMC and CD4 T cells was significantly less, despite normal levels of the ERK proteins. This decrease in ERK phosphorylation was directly proportional to disease activity [622]. The functional significance of decreased signaling was tested by treating normal T cells as well as the leukemic Jurkat T-cell line with PD98059, a selective MEK inhibitor. The MEK inhibitor decreased Dnmt1 mRNA content and enzymatic activity to levels comparable to SLE T cells and caused globally hypomethylated DNA [622]. These observations support the hypothesis that decreased signaling through the ERK pathway contributes to the DNA hypomethylation characteristic of SLE T cells and possibly to the immunopathogenesis of SLE via mechanisms analogous to other DNA methylation inhibitors [623]. The mechanism causing the defect in SLE is presently unknown.

SEX HORMONES IN CELLULAR IMMUNE RESPONSES

The fact that SLE is preponderant among women during their reproductive life and is more frequent among male patients with Klinefelter's syndrome has implicated that hormones have a role in the pathogenesis of autoimmunity. Sex hormone manipulation experiments in lupus-prone murine strains and other *in vitro* experimental evidence have shown that female sex hormones facilitate the development of autoimmunity [624]. Specifically, testosterone enhances suppressor cell activity, whereas estrogens have a direct stimulatory effect on B cells and a suppressive effect on regulator cell activity [625, 626].

Experiments suggest a negative role for estrogens in B-cell proliferation [627]. Hypogonadal female mice display decreased B-cell lymphopoiesis that is restored with estrogen replacement [628]. Estrogens inhibit clonal expansion of B-cell precursors *in vitro* [629] and B-cell lymphopoiesis in pregnant mice [630]. These experiments show clearly that estrogens regulate B-cell biology through binding to the estrogen receptor and contrast previous concepts that estrogens promote autoimmunity. The opposite conclusions may represent the fact that the previous studies were performed in lupus-prone strains, whereas the latter were performed in normal mice.

Studies in human systems have provided indirect evidence on the role of sex hormones in the development of autoimmunity. Lymphocytes from both men and women respond more vigorously to pokeweed mitogen in the presence of estradiol, whereas they show diminished responses in the presence of testosterone [631]. This indicates that estrogen may play a permissive role in B-cell overactivity, provided that other factors are present. In another set of experiments, estrogens were shown to suppress apoptosis in normal and lupus peripheral blood MNC, thus increasing the chances for longer survival of autoreactive cells [632].

Human keratinocytes bind anti-Ro and anti-La antibodies if cultured in the presence of estradiol [633]. This relates to the clinical observation that there is a strong association between antibodies to Ro antigens and cutaneous lupus erythematosus [634]. In addition to estrogens, ultraviolet light induces binding of anti-Ro antibodies to skin cells [635]. Estrogens and ultraviolet light thus represent two permissive factors for the development of skin manifestations in patients with SLE. Pathogenically, both factors could precipitate skin pathology in patients who have anti-Ro antibodies or alternatively they could increase the antigenicity of skin autoantigens and elicit an autoimmune response.

Studies have begun addressing the molecular mechanisms that underlie regulation of the expression of genes that are involved in the pathogenesis of SLE by estrogens. For example, it was shown that estradiol upregulates the expression of the antiapoptotic gene *Bcl-2* in B cells of anti-DNA antibody transgenic mice [636]. In other experiments, it was shown that calcineurin steady-state mRNA levels and phosphatase activity rose in SLE T cells cultured in the presence of estrogen. The increase was dose dependent and hormone specific. T cells have both α and β types of estrogen receptors and, interestingly the exposure of normal T cells or T cells from male SLE patients to estrogen does not lead to the same calcineurin upregulation [637, 638].

FROM PATHOGENESIS TO THE DEVELOPMENT OF NOVEL TREATMENTS

Current therapeutic schemes are directed by individual disease manifestations rather than a general diagnosis of lupus. Involvement of vital organs (kidney, CNS, lungs, heart, severe hematologic disease) usually calls for intense treatment, including high doses of corticosteroids and cytotoxic drugs, whereas manifestations from nonvital organs can be treated with moderate or low doses of corticosteroids, antimalarials, and nonsteroidal anti-inflammatory drugs [639, 640]. Pulse cyclophosphamide has emerged as the treatment of choice for severe visceral involvement [641–644]. Corticosteroids, alkylating agents, or calcineurin inhibitors (such as cyclosporine and FK506) may be used in selected cases of patients with membranous lupus nephropathy [645, 646].

Understanding the processes involved in the pathogenesis of lupus has made possible the design of rational therapeutic approaches (Table 8). The enormous recent progress in the field of cytokines has raised expectations for their use in the management of autoimmune diseases. Indeed, a fusion molecule between the TNF-α receptor and the Fc portion of IgG was shown to significantly improve the clinical course of patients with rheumatoid arthritis [647]. It should be cautioned that lupus is a pathogenically different disease, and treatment with TNF-α-neutralizing biologics may exacerbate it. Patients treated with a humanized anti-TNF-α antibody developed autoantibodies, but not clinical disease [648, 649], an observation that suggests such reagents should not be used in lupus patients.

In an experimental model of antiphospholipid syndrome, an intraperitoneal injection of IL-3 prevented fetal loss [650]. IL-3 has been introduced in clinical trials for the treatment of neutropenias in patients with myelodysplastic and aplastic syndromes [651] and has been noted to have minimal toxicity. Although the justification for use in the prevention of fetal loss originated from the observation that these animals have low serum IL-3 levels, its mechanism of action is not clear.

TABLE 8 Rationalized Therapy for Lupus

Interruption of cognate cell interaction with antibodies or fusion molecules comprising a ligand and the Fc portion of human Ig
CTLA4-Ig
Anti-B7-1 or -2 antibodies
Anti-CD40 ligand antibody
Neutralization of cytokines with antibodies or fusion molecules (cytokine receptor human Ig)
Anti-IL-10 antibody
Anti-TNF-α antibody
TNF-α receptor–FcIgG fusion molecule
Lymphokines
Hormones (DHEA)
Inhibition of complement activation
Bone marrow transplantation
Reestablishment of tolerance (T and B cell)
Modulation of cell signaling
Gene therapy

Neutralization of IL-10 may present another therapeutic option as adjunct therapy for lupus and other systemic autoimmune diseases. Continuous infusion of the anti-IL-10 antibody of NZB/NZW mice from birth improved survival dramatically, indicating either that IL-10 is directly involved in promoting disease activity by acting on B lymphocytes and other cells or that elimination of IL-10 reversed the cytokine ratio in favor of the type 2 group of cytokines [251]. A major concern with all candidate lymphokines is the fact that they are pleiotropic. Patients with various forms of malignancies who have been treated with either IL-2 or IFN-α have developed a wide range of autoimmune diseases, including thyroiditis and SLE [652].

Complement activation is abundant in lupus patients as manifested by the fact that the circulating immune complexes have attached C3 split products [536], and the levels of various complement split products [653] and the membrane attack complex [654] are increased in the sera and urine of lupus patients. The use of biologics that would interfere with complement activation could be of therapeutic value; an anti-C5 antibody was shown to prolong life in NZB/NZW mice [655], and a humanized anti-C5 monoclonal antibody has been considered for use in lupus patients. The use of regulatory surface membrane molecules, such as decay accelerat-

ing factor (DAF), in the treatment of lupus should also be considered. Crry-Ig was shown to inhibit nephritis in a nephrotoxic serum animal model [656]. Crry is the murine analogue of human DAF and CD69 and inhibits both classical and alternative pathways of complement activation. Similarly, a transgenic mouse secreting Crry was resistant to the effects of nephrotoxic serum [657].

Disruption of the cognate interruption between T and B cells has been predicted and proven to be helpful in lupus. A B7-binding construct (CTLA4 molecule fused with the heavy chain of Ig to provide decreased plasma clearance rates) was given to mice that develop lupus spontaneously, and the rate of survival increased along with lower autoantibody titers and reduced kidney pathology [381]. This clinical effect is associated with a suppressive effect on the activated $CD4^+$ cells and the cells that produce anti-DNA antibodies [658].

Interruption of the CD40–gp39 (CD-40L) interaction in SNF1 autoimmune mice with the anti-gp39 antibody resulted in a decreased expansion of autoimmune memory B cells and long-term clinical improvement [395]. Experiments showing therapeutic effects in autoimmune mice following interruption of the B7–CD28 and CD40–gp39 paths indicate that they are involved serially in the production of antibody. These results have instigated ongoing human trials using humanized anti-CD40 ligand antibodies and combined treatments to achieve better and longer lasting effects [659, 660]. Indeed, when CTLA4Ig was combined with anti-gp39, there was sustained inhibition of autoantibody production and renal disease [661].

For patients with severe disease, immunosuppression may be intensified to the point of myelosuppression or hematopoietic ablation. Hematopoiesis and immunity may then be reconstituted rapidly by the reinfusion of $CD34^+$ progenitor cells. This approach has been tried in a limited number of patients with lupus and other autoimmune diseases who had failed standard treatment [662, 663]. Although the number of patients is small and the follow-up period is short, it seems that either the severe immunosuppression or the redevelopment of the immune system with possibly less autoimmune features may have beneficial effects on the clinical expression of the disease.

Hormonal treatment of lupus patients to reverse the effect of endogenous androgens with dehydroepiandrosterone has been tested in double blind controlled studies and found to have a minimal effect, which may justify its use as a steroid-sparing agent or treatment of mild manifestations in selected patients [664–666].

As discussed previously, lupus patients have T cells that respond to defined autoantigens and may display certain TCR selection. These studies helped us understand the pathogenesis of the disease and also provided rationale for specific immunologic interventions [189]. The identification of putative autoantigens may prove useful in reestablishing tolerance. Indeed, myelin basic protein (MBP) disease-inducing T cells have been deleted successfully by a high dose of MBP antigen in a murine model of autoimmune encephalomyelitis [166]. Additionally, oral administration of chicken collagen II in a double-blinded clinical study to patients with rheumatoid arthritis resulted in significant clinical improvement [167]. Histone-defined peptides have been identified in mice [667] and humans [668] that can stimulate T-cell-dependent anti-DNA antibody production and provide the basis for the development of peptide-based tolerogenic therapy for humans similar to that demonstrated in mice with lupus [669].

At the B-cell level, two approaches are currently entertained in clinical trials. In the first, a construct is used that consists of four short DNA fragments conjugated to a backbone. This construct modulates surface anti-DNA Ab of the surface membrane of B cells, which thereupon become unresponsive to further stimulation [670]. In the second one, humanized antibodies were directed against the B lymphocyte stimulator (BlyS). BlyS levels are increased in sera of patients with SLE [671, 672].

The described cellular biochemical abnormalities that characterize lupus cells may become the target of pharmaceutical intervention. The tyrosine kinase inhibitor tyrphostin AG490 was shown to prevent binding of freshly isolated mouse lymph node cells and of i*n vivo* activated lymphocytes to the brain vessel endothelium by inhibiting the expression of adhesion molecules, and systemic administration of the drug in a murine model of experimental allergic encephalomyelitis decreased disease activity [673, 674]. Therefore, drugs that interfere with cell signaling events may prove to be helpful in the treatment of lupus.

GENE THERAPY FOR SLE

The MRL-fas^{lpr} mouse has a mutation in the *Fas* gene that leads to defective lymphocyte apoptosis, lympho-proliferation, distinct immunoregulatory abnormalities, and systemic autoimmune manifestations similar to those of lupus, arthritis, and vasculitis. Reintroduction of *Fas* into these animals corrects the majority of the abnormalities [675]. Because such correction of a single missing gene is of no practical consideration in the treatment of human autoimmunity, attempts should be

made to design and deliver genes whose products can reverse autoimmune pathology. Lawson *et al.* [676] described a chimeric protein consisting of a soluble form of the interferon-γ receptor, which blocks the action of IFN-γ, fused to the Fc portion of IgG, which helps stabilize this bioactive protein in the circulation.

Gene therapy involves the insertion and expression of foreign DNA into the host cell. Viral vectors usually serve as effective carriers to insert DNA into cells by transduction, but each system has unique advantages and disadvantages. In most murine and human studies, modified retroviruses have been used that lack one or more viral structural proteins [677]. Gene therapy has been extensively considered in the treatment of arthritis in animal models [678]. Examples include the intra-articular delivery of IL-4 using a retroviral vector that improved inflammation in a rat model of adjuvant arthritis [679] and retroviral delivery of IL-13 that suppressed collagen-induced arthritis in mice [680]. In one attractive approach [681], human fibroblasts were transduced with a dominant negative form of IκB that blocks the translocation of nuclear factor κB to the nucleus. This transgene results in cell death only in the presence of TNF-α, so if the vector were injected into a human joint, fibroblasts and other cells would die only if TNF-α were present, i.e., if there were active inflammation.

Antigen-specific forms of immunotherapy employ either whole protein antigens or individual immunodominant peptide epitopes. The most important theoretical limitation to such therapies is whether epitope-specific forms of therapy will modulate an immune response directed to multiple epitopes or multiple target proteins. At least in mice, induction of transferable suppressor T cells can indeed regulate polyspecific autoimmune responses, apparently by promoting the release of regulatory cytokines that act on bystander-activated cells with different specificities. For example, nucleosome-defined peptides have been used successfully in the treatment of murine lupus, indicating that it is possible to reestablish tolerance in systemic autoimmune diseases when more than one antigen is involved [669]. The Scott laboratory, which pioneered the use of tolerizing peptides [682] and proteins [683] fused to the N terminus of the IgG heavy chain, found immune suppression to multiple epitopes expressed by the antigenic protein. Gene therapeutics for specific diseases may require this multiple epitope approach if bystander suppression mechanisms are not activated, as in the uveitis model described, in which immune regulation was not transferable by T cells.

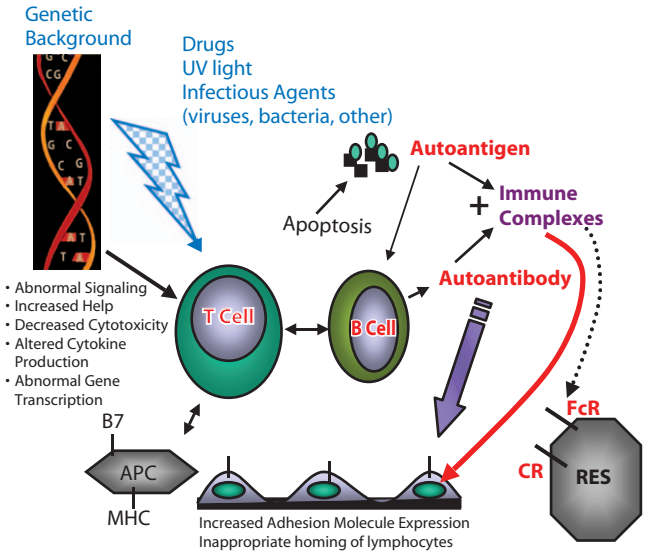


FIGURE 6 Overview of the pathogenetic processes in human SLE. Multiple genetic, environmental, and hormonal factors instigate a number of cellular and cytokine abnormalities. These abnormalities lead to an increased production of autoantibodies, which either directly or after forming complexes with autoantigens and activating complement deposit in tissues and initiate an inflammatory response. Immune complexes are formed in excessive amounts in lupus patients and are cleared at decreased rates because the number and/or the function of Fc and complement receptors is decreased (APC, antigen-presenting cells; RES, reticuloendothelial system).

PATHOGENESIS: AN OVERVIEW

The synthesis of the described cellular and lymphokine aberrations under a unified hypothesis is a difficult task. In the foregoing discussion, multiple recognitive, regulatory, and effector system abnormalities were mentioned (Fig. 6).

The fundamental event in the pathogenesis of SLE is B-cell overactivity, which is responsible for the overproduction of pathogenic autoantibodies, which either alone or in concert with immune complexes is responsible for tissue pathology and clinical manifestations. The relevant pathology and clinical manifestations of each of the produced autoantibodies are described in other chapters of this book. B-cell overactivity is the result of concomitant action of multiple factors. Although human B-cell overactivity has not been associated with a single gene, there is indication that overactive early B-cell receptor-initiated B-cell responses are independent of disease activity [499] and therefore possible inherent. A locus in murine chromosome 4 has been found to harbor a gene that is responsible for B-cell overactivity [684, 685]. Studies on the human

genome are bound to identify genes that contribute to the described cellular aberrations in human lupus. Hormonal factors obviously play a serious role in the expression of human lupus. Although *in vitro* studies suggest that estradiol enhances B-cell function, *in vivo* studies reported by the group of Kincade [627, 629, 630, 686] in normal mice clearly show that B-cell development in normal mice is suppressed by estrogens. Estrogens may "facilitate" the expression of autoimmunity in a more complex manner. Environmental factors have been strongly associated with the clinical expression of disease. Prominent among the environmental factors are drugs that induce lupus and infectious agents. Possible mechanisms for the action of these drugs were discussed earlier. In brief, lupus-inducing drugs may cause demethylation of DNA, possibly of the "lupus"-associated genes. Hypomethylated genes are transcribed at higher rates. Ultraviolet light is strongly associated with the clinical expression of the disease in certain patients. Ultraviolet light may be pathogenic by virtue of (a) causing increased binding of anti-SSA(Ro) antibodies to epidermal cells and (b) causing DNA demethylation. Hypomethylating drugs and environmental factors may directly affect the transcription of relevant genes or indirectly by causing the overexpression of adhesion molecules that are involved in B-cell activation [389]. Finally, immunoregulatory aberrations of the T-cell control or the lymphokine milieu are clearly responsible for increased B-cell function.

T cells play a crucial role in the regulation of B-cell function. Deficient suppressor cell function has a role in B-cell overactivity. Even if someone disclaims a generalized suppressor cell deficiency in the pathogenesis of lupus, antigen-specific suppressor cell deficiency contributes to the production of antibody excess. However, many T-cell subsets have been identified as providing excessive help to B cells to produce antibody in general and pathogenic antibodies specifically [183]. $CD4^+$, $CD8^+$, and abnormally expanded αβ and γδ $CD4^-CD4^-$ $CD3^+$ T cells provide increased amounts of help to B cells to produce antibody. Although not impressive, certain biases in the selection of the TCR repertoire (Table 3) speak for the role of autoantigen-mediated stimulation of T cells and for the potential of specific immunotherapy in lupus [189].

The importance of deficient cytotoxic responses in the pathogenesis of human lupus can not be underestimated. Specific cytotoxic cells able to eliminate EBV, and presumably other virus-infected cells, may be a cornerstone in the pathogenesis of the disease [128]. Similarly, antigen-nonspecific cytotoxic $CD56^+$ cells are vital in the control of the immune response [687]. The latter deficient cytotoxic responses extend to relatives of lupus patients and therefore introduce the possibility of another locus that may contribute independently to the expression of the disease.

The known increased levels of IL-6 and IL-10 in lupus patients may stimulate B cells, whereas decreased levels of IFN-γ and IL-2 to the deficient cytotoxic responses. However, it is difficult to interpret T-cell overactivity. In certain animal autoimmune models and possibly in human disease, the lymphokine response may be polarized in favor of type 1 or type 2 lymphokine production. Although evidence shows that human lupus is a disease with a type 2 lymphokine profile [267], data from a study of lupus-prone mice suggest that lupus may not be a polarized disease in terms of lymphokine production [20]. Study of the regulation of transcription of lymphokines in human lupus [688] has indicated that additional independent genetic defects may contribute to the pathogenesis of human lupus. Is it possible to treat lupus with lymphokines or lymphokine function-interfering agents such as soluble receptors and antibodies? Such possibilities deserve consideration and were discussed earlier in this chapter.

Aberrations in the expression of costimulatory and adhesion molecules have been identified and found to contribute to the pathogenesis of the disease. First, overexpression of adhesion molecules in target tissues may fuel the inflammatory response by causing an inappropriate homing of inflammatory cells [19] or contribute to the abnormal regulation of T- and B-cell function. The role of B7 and CD40, CD40 ligand has been elucidated (see earlier discussion). However, we do not understand what causes the abnormal regulation of their expression on the cell surface membrane.

The role of apoptosis in autoimmunity cannot be overestimated. With the exception of rare cases, structural defects of the apoptosis-involved molecules are not responsible for the aberrant apoptosis in human lupus as it is in certain murine lupus-prone strains. Lymphocytes and monocytes and possibly keratinocytes undergo spontaneous apoptosis at higher than normal rates and contribute to an increased pool of autoantigens, whereas the activation-induced death of lymphocytes is decreased in lupus and contributes to the increased survival of activated T cells.

Abnormalities in antigen receptor-initiated lymphocyte signaling have been identified and characterized. These abnormalities offer a dual purpose: (1) they explain some of the heterogeneous cellular abnormalities and (2) they may lead to the introduction of novel regiments that may help lupus patients by correcting abnormal signaling processes.

The just described synthesis has not taken into consideration all available information and furthermore cannot explain all the immunological abnormalities that have been described in patients with SLE. This does not

belittle their importance in the pathogenesis of the disease. A crucial supposition in understanding the pathogenesis of SLE is the heterogeneity of the disease. Clinical heterogeneity is forerun by etiologic heterogeneity. Each factor mentioned in this discussion may have relative weight in the pathogenesis of the disease in any given patient with SLE.

It is expected that a better understanding of the molecular events that underlie abnormal cellular functions will be found in the near future. Newly characterized molecular aberrations will unify the diverse cellular aberrations. In addition, genes that are associated with these defects will be identified. At this moment it is generally accepted that human lupus, as perceived clinically today, is the result of multiple factors that act in concert to exceed a "threshold" that permits the expression of clinical disease. Many of these factors have a clear genetic origin, and their effect is modified by other genetic factors and/or environmental factors.

Acknowledgments

Work in the author's laboratory was supported by PHS Grants RO1 AI 42269, RO1 AI 42782, RO1 AI 49954, and NO1 AI 95383.

References

1. Hiramatsu, M., Balow, J. E., and Tsokos, G. C. (1986). Production of nephritic factor of the alternative complement pathway by Epstein Barr virus-transformed B cell lines derived from a patient with membranoproliferative glomerulonephritis. *J. Immunol*. **136**, 4451–4455.
2. Ehrlich, P. (1990). On autoimmunity with special references to cell life. *Proc. R. Soc. Lond. Biol*. **66B**, 424.
3. Burnet, F. M. (1959). "The Clonal Selection Theory of Acquired Immunigy." Cambridge Univ. Press, Cambridge.
4. Howe, M. L., Goldstein, A. L., and Battisto, J. R. (1970). Isogeneic lymphocyte interaction: recognition of self antigens by cells of the neonatal thymus. *Proc. Natl. Acad. Sci. USA* **67**, 613–619.
5. Jerne, N. K. (1971). Towards a network theroy to the immune system. *Ann. Immunol*. **125C**, 373–389.
6. Cantor, H., and Boyse, E. A. (1976). Regulation of cellular and humoral immune responses by T cell subclasses. *Cold Spring Harb. Symp. Quant. Biol*. **41**, 23–36.
7. Horwitz, D. A., Gray, J. D., Ohtsuka, K., Hirokawa, M., and Takahashi, T. (1997). The immunoregulatory effects of NK cells: The role of TGF-beta and implications for autoimmunity. *Immunol. Today* **18**, 538–542.
8. Kumar, V., Kono, D. H., Urban, J. L., and Hood, L. (1989). The T-cell receptor repertoire and autoimmune diseases. *Annu. Rev. Immunol*. **7**, 657–682.
9. Theofilopoulos, A. N., Kofler, R., Singer, P. A., and Dixon, F. J. (1989). Molecular genetics of murine lupus models. *Adv. Immunol*. **46**, 61–109.
10. Datta, S. K. (1989). A search for the underlying mechanisms of systemic autoimmune disease in NZB × SWR model. *Clin. Immunol. Immunopathol*. **51**, 141–156.
11. Theofilopoulos, A. N. (1995). The basis of autoimmunity. I. Mechanisms of aberrant self-recognition. *Immunol. Today* **16**, 90–98.
12. Theofilopoulos, A. N. (1995). The basis of autoimmunity. II. Genetic predisposition. *Immunol. Today* **16**, 150–159.
13. Theofilopoulos, A. N. (1996). Genetics of systemic autoimmunity. *J. Autoimmun*. **9**, 207–210.
14. Mountz, J. D., Wu, J., Cheng, J., and Zhou, T. (1994). Autoimmune disease: A problem of defective apoptosis. *Arthritis Rheum*. **37**, 1415–1420.
15. Elkon, K. B. (1994). Apoptosis in SLE: Too little or too much? *Clin. Exp. Rheumatol*. **12**, 553–559.
16. June, C. H., Bluestone, J. A., Nadler, L. M., and Thompson, C. B. (1994). The B7 and CD28 receptor families. *Immunol. Today* **15**, 321–331.
17. Sfikakis, P. P., and Tsokos, G. C. (1995). Lymphocyte ahesion molecules in autoimmune rheumatic diseases: Basic issues and clinical expectations. *Clin. Exp. Rheum*. **13**, 763–777.
18. Guinan, E. C., Gribben, J. G., Boussiotis, V. A., Freeman, G. J., and Nadler, L. M. (1994). Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. *Blood* **84**, 3261–3282.
19. Sfikakis, P. P., and Via, C. S. (1997). Expression of CD28, CTLA4, CD80, and CD86 molecules in patients with autoimmune rheumatic diseases: Implications for immunotherapy. *Clin. Immunol. Immunopathol*. **83**, 195–198.
20. Handwerger, B. S., Rus, V., da Silva, L., and Via, C. S. (1994). The role of cytokines in the immunopathogenesis of lupus. *Springer Semin. Immunopathol*. **16**, 153–180.
21. Horwitz, D. A., and Jacob, C. (1994). The cytokine network in the pathogenesis of systemic lupus erythematosus and possible therapeutic implications. *Springer Semin. Immunopathol*. **181**, 200.
22. Gause, W. C., Halvorson, M. J., Lu, P., Greenwald, R., Linsley, P., Urgan, J. F., and Finkelman, F. D. (1997). The function of costimulatory molecules and the development of IL-4-producing T cells. *Immunol. Today* **18**, 115–120.
23. Kirou, K. A., and Crow, M. K. (1999). New pieces to the SLE cytokine puzzle. *Clin. Immunol*. **91**, 1–5.
24. Tsokos, G. C., and Kammer, G. M. (2000). Molecular aberrations in human systemic lupus erythematosus. *Mol. Med. Today* **6**, 418–424.
25. Tsokos, G. C., and Liossis, S. N. (1999). Immune cell signaling defects in lupus: Activation, anergy and death. *Immunol. Today* **20**, 123–128.
26. Tsokos, G. C., Wong, H. K., Enyedy, E. J., and Nambiar, M. P. (2000). Immune cell signaling in lupus. *Curr. Opin. Rheumatol*. **12**, 355–363.
27. Boswell, J., and Schur, P. H. (1989). Monocyte function in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **52**, 271–278.
28. Linker-Israeli, M., Bakke, A. C., Kitridou, R. C., Gendler, S., Gillis, S., and Horwitz, D. A. (1983). Defective pro-

duction of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus (SLE). *J. Immunol*. **130**, 2651–2655.
29. Andersen, L. S., Petersen, J., Svenson, M., and Bendtzen, K. (1999). Production of IL-1beta, IL-1 receptor antagonist and IL-10 by mononuclear cells from patients with SLE. *Autoimmunity* **30**, 235–242.
30. Oppenheim, J. J., Koopman, W. J., Wahl, L. M., and Dougherty, S. F. (1980). Prostaglandin E2 rather than lymphocyte-activating factor produced by activated human mononuclear cells stimulates increases in muring thymocyte cAMP. *Cell Immunol* **49**, 64–73.
31. Arrend, W. P., Joslin, E. G., and Mussoni, R. J. (1985). Effects of immune complexes on production by human monocytes of interleukin 1 or an interleukin 1 inhibitor. *J. Immunol*. **134**, 3868–3877.
32. Tsokos, G. C., and Balow, J. E. (1984). Cellular immune responses in systemic lupus erythematosus. *Prog. Allergy* **35**, 93–161.
33. Markenson, J. A., Morgan, J. W., Lockchin, M. D., Joachim, C., and Winfield, J. B. (1978). Responses of franctionated cells from patients with systemic lupus erythematosus normals to plant mitogen: Evidence for a suppressor population of monocytes. *Proc. Soc. Exp. Biol. Med*. **158**, 5–9.
34. Markenson, J. A., Lockshin, M. D., Joachim, C., and Morgan, J. (1980). Suppressor monocytes in patients with systemic lupus erythematosus: Evidence of suppressor activity associated with a cell-free soluble product of monocytes. *J. Lab. Clin. Med*. **95**, 40–48.
35. Weill, B. J., and Renoux, M. L. (1988). Mononuclear phagocytes from patients with active systemic lupus erythematosus down-regulate the specific in vitro reactivity of autologous lymphocytes to double-stranded DNA. *Clin. Exp. Immunol*. **72**, 43–49.
36. Laughter, A. H., Lidsky, M. D., and Twomey, J. J. (1979). Suppression of immunglobulin synthesis by monocytes in health and in patients with systemic lupus erythmatosus. *Clin. Immunol. Immunopathol*. **14**, 435–440.
37. Tsokos, G. C., and Balow, J. E. (1981). Cytotoxic responses to alloantigens in systemic lupus erythematosus. *J. Clin. Immunol*. **1**, 208.
38. Tsokos, G. C., and Balow, J. E. (1981). Spontaneous and pokeweed mitogen-induced plaque-forming cells in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **21**, 172–183.
39. Sasaki, T., Shibata, S., Hirabayashi, Y., Sekiguchi, Y., and Yoshinaga, K. (1989). Accessory cell activity of monocytes in anti-DNA antibody production in systemic lupus erythematosus. *Clin. Exp. Immunol*. **77**, 37–42.
40. Jandl, R. C., George, J. L., Silberstein, D. S., Eaton, R. B., and Schur, P. H. (1987). The effect of adherent cell-derived factors on immunoglobulin and anti-DNA synthesis in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **42**, 344–359.
41. Muryoi, T., Sasaki, T., Sekiguchi, Y., Tamate, E., Takai, O., and Yoshinaga, K. (1989). Impaired accessory cell function of monocytes in systemic lupus erythematosus. *J. Clin. Lab. Immunol*. **28**, 123–128.
42. Mene, P., Pecci, G., Cinotti, G. A., Pugliese, G., Pricci, F., and Pugliese, F. (1998). Eicosanoid synthesis in peripheral blood monocytes: A marker of disease activity in lupus nephritis. *Am. J. Kidney Dis*. **32**, 778–784.
43. Shirakawa, F., Vamashito, V., and Susuki, H. (1985). Decrease in HLA-DR-positive monocyte in patients with SLE. *J. Immunol*. **134**, 3560–3562.
44. Shirakawa, F., Vamashito, V., and Susuki, H. (1985). Reduced function of the HLA-DR-positive monocytes in patients with SLE. *J. Clin. Immunol*. **5**, 396–403.
45. Nagai, H., Sztein, M. B., Steeg, P. S., Hooks, J. J., Oppenheim, J. J., and Steinberg, A. D. (1984). Diminished peripheral blood monocyte DR antigen expression in systemic lupus erythematosus. *Clin. Exp. Rheumatol*. **2**, 131–137.
46. Steinbach, F., Henke, F., Krause, B., Thiele, B., Burmester, G. R., and Hiepe, F. (2000). Monocytes from systemic lupus erythematous patients are severely altered in phenotype and lineage flexibility. *Ann. Rheum. Dis*. **59**, 283–288.
47. Shirakawa, F., Yamashita, U., and Suzuki, H. (1987). Monocyte (macrophage)-specific antibodies in patients with systemic lupus erythematosus (SLE). *J. Clin. Immunol*. **7**, 121–129.
48. Blanco, P., Palucka, A. K., Gill, M., Pascual, V., and Banchereau, J. (2001). Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* **294**, 1540–1543.
49. Preble, O. T., Rothko, K., Klippel, J. H., Friedman, R. M., and Johnston, M. I. (1983). Interferon-induced 2′-5′ adenylate synthetase in vivo and interferon production in vitro by lymphocytes from systemic lupus erythematosus patients with and without circulating interferon. *J. Exp. Med*. **157**, 2140–2146.
50. Cederblad, B., Blomberg, S., Vallin, H., Perers, A., Alm, G. V., and Ronnblom, L. (1998). Patients with systemic lupus erythematosus have reduced numbers of circulating natural interferon-a. *J. Autoimmun*. **11**, 465–470.
51. Baxevanis, C. N., Reclos, G. J., Papamichail, M., and Tsokos, G. C. (1987). Prothymosin alpha restores the depressed autologous and allogeneic mixed lymphocyte responses in patients with systemic lupus erythematosus. *Immunopharmacol. Immunotoxicol*. **9**, 429–440.
52. Baxevanis, C. N., Reclos, G. J., and Papamichail, M. (1990). Decreased HLA-DR antigen expression on monocytes causes impaired suppressor cell activity in multiple sclerosis. *J. Immunol*. **144**, 4166–4171.
53. Reclos, G. J., Baxevanis, C. N., Sfagos, C., Papageorgiou, C., Tsokos, G. C., and Papamichail, M. (1987). Multiple sclerosis. II. Effects of prothymosin alpha on the autologous and allogeneic MLR in patients with multiple sclerosis. *Clin. Exp. Immunol*. **70**, 336–344.
54. Richardson, B. C., Yung, R. L., Johnson, K. J., Rowse, P. E., and Lalwani, N. D. (1996). Monocyte apoptosis in patients with acitive lupus. *Arthritis Rheum*. **39**, 1432–1434.
55. Iliopoulos, A. G., and Tsokos, G. C. (1996). Immunopathogenesis and spectrum of infections in systemic

lupus erythematosus. *Semin. Arthritis Rheum*. **25**, 318–336.
56. Cairns, A. P., Crockard, A. D., McConnell, J. R., Courtney, P. A., and Bell, A. L. (2001). Reduced expression of CD44 on monocytes and neutrophils in systemic lupus erythematosus: Relations with apoptotic neutrophils and disease activity. *Ann. Rheum. Dis*. **60**, 950–955.
57. Seres, T., Csipo, I., Kiss, E., Szegedi, G., and Kavai, M. (1998). Correlation of Fc gamma receptor expression of monocytes with clearance function by macrophages in systemic lupus erythematosus. *Scand. J. Immunol*. **48**, 307–311.
58. Scheinecker, C., Zwolfer, B., Koller, M., Manner, G., and Smolen, J. S. (2001). Alterations of dendritic cells in systemic lupus erythematosus: Phenotypic and functional deficiencies. *Arthritis Rheum*. **44**, 856–865.
59. DeHoratius, R. J., Tung, K. S., and Pincus, T. (1980). Reduced T-lymphocytes subsets in systemic lupus erythematosus: Effects of immune complexes and lymphocytotoxic antibodies. *Clin. Immunol. Immunopathol*. **17**, 245–256.
60. Glinski, W., Gershwin, M. E., Budman, D. R., and Steinberg, A. D. (1976). Study of lymphocyte subpopulations in normal humans and patients with SLE by fractionation of peripheral blood lymphocyte on a discontinuous Ficoll gradient. *J. Clin. Invest*. **57**, 604.
61. Hamilton, M. E., and Winfield, J. B. (1979). T cells in systemic lupus erythematosus. *Arthritis Rheum*. **22**, 1–6.
62. Steinberg, A. S., Klassen, L. W., Budman, D. R., and Williams, G. W. (1979). Immunofluorescence studies of anti-T cell antibodies and T cells in SLE: Selective loss of brightly staining T cells in active disease. *Arthritis Rheum*. **22**, 114–122.
63. Winfield, J. B., Winchester, R. J., and Kunkel, H. G. (1975). Association of cold reactive anti-lymphocyte antibodies with lymphopenia in systemic lupus erythematosus. *Arthritis Rheum*. **18**, 587–594.
64. Winfield, J. B. (1985). Anti-lymphocyte antibodies in systemic lupus erythematosus. *Clin. Rheum. Dis*. **11**, 523–549.
65. Wernet, P., and Kunkel, H. G. (1973). Antibodies to a specific surface antigen of T cells in human sera inhibiting mixed leukocyte culture reactions. *J. Exp. Med*. **138**, 1021.
66. Schocket, R. S., and Kohler, P. F. (1981). Lymphocytotoxic antibodies in systemic lupus erythematosus and clinically related diseases. *Arthritis Rheum*. **22**, 1060–1063.
67. Smolen, J. S., Chused, T. M., Leiserson, W. M., Reeves, J. P., Alling, D., and Steinberg, A. D. (1982). Heterogeneity of immunoregulatory T-cell subsets in systemic lupus erythematosus: Correlation with clinical features. *Am. J. Med*. **72**, 783–790.
68. Bakke, A. C., Kirkland, P. A., Kitridou, R. C., Quismorio, F. P., Rea, T., Ehresman, G. R., and Horwitz, D. A. (1983). T lymphocyte subsets in systemic lupus erythematosus. *Arthritis Rheum*. **26**, 745.
69. Lambris, J. D., and Tsokos, G. C. (1986). The biology and pathophysiology of complement receptors. *Anticancer Res*. **6**, 515–523.
70. Krych, M., Atkinson, J. P., and Holers, V. M. (1992). Complement receptors. *Curr. Opin. Immunol*. **4**, 8–13.
71. Bakke, A. C., Gray, J. D., Abo, W., Quismorio, F. P., Jr., Lash, A., Cooper, S. M., and Horwitz, D. A. (1986). Studies on human blood lymphocytes with iC3b (type 3) complement receptors. I. Granular, Fc-IgG receptor positive and negative subsets in healthy subjects and patients with systemic lupus erythematosus. *J. Immunol*. **136**, 1253–1259.
72. Gray, J. D., Lash, A., Bakke, A. C., Kitridou, R. C., and Horwitz, D. A. (1987). Studies on human blood lymphocytes with iC3b (type 3) complement receptors. III. Abnormalities in patients with active systemic lupus erythematosus. *Clin. Exp. Immunol*. **67**, 556–564.
73. Fuller, T. C., Trevithic, J. E., Fuller, A. A., Colvin, R. B., Cosimi, A. B., and Kung, P. C. (1984). Antigenic polymorphism of the T4 differentiation antigen expressed onhelper/inducer lymphocytes. *Hum. Immunol*. **9**, 89–102.
74. Stohl, W., and Kunkel, H. G. (1984). Heterogeneity in expression of the T4 epitope in black individuals. *Scand. J. Immunol*. **20**, 273–278.
75. Stohl, W., Crow, M. K., and Kunkel, H. G. (1985). Systemic lupus erythematosus with deficiency of the T4 epitope on T helper cells. *N. Engl. J. Med*. **312**, 1671–1678.
76. Levinson, A. L., Hoxie, J. A., Kornstein, M. J., Zembryski, D., Mathews, D. M., and Schreiber, A. D. (1985). Absence of the OKT4 epitope on blood T cells and thymus cells in a patient with thymoma, hypogammaglobulinemia and red blood cell aplasia. *J. Allergy Clin. Immunol*. **76**, 433–439.
77. Raziuddin, S., Nur, M. A., and Al-Janadi, M. A. (1990). CD4+ T lymphocyte epitope deficiency and function in systemic lupus erythematosus and rheumotoid arthritis. *Arthritis Rheum*. **33**, 1864–1865.
78. Messner, R. P., Kindstrom, F. D., and Williams, J. R., Jr. (1973). Peripheral blood lymphocyte cell surface markers during the course of systemic lupus erythematosus. *J. Clin. Invest*. **52**, 3046–3056.
79. Winfield, J. B., and Mimura, T. (1992). Pathogenetic significance of anti-lymphocyte autoantibodies in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **63**, 13–16.
80. Twomey, J. J., Laughter, A. H., and Steinberg, A. D. (1978). A serum inhibitor of immune regulation in patients with systemic lupus erythematosus. *J. Clin. Invest*. **62**, 713–715.
81. Sakane, T., Steinberg, A. D., Reeves, J. P., and Green, I. (1979). Studies of immune functions of patients with systemic lupus erythematosus: Complement-dependent immunoglobulin M anti-thymus-derived cell antibodies preferentially inactivate suppressor cells. *J. Clin. Invest*. **63**, 654–768.
82. Sakane, T., Steinberg, A. D., Reeves, J. P., and Green, I. (1979). Studies of immune functions of patients with systemic lupus erythematosus: T-cell subsets and antibodies to T-cell subsets. *J. Clin. Invest*. **64**, 1260–1269.
83. Sakane, T., Honda, M., Taniguchi, Y., and Kotani, H. (1981). Separation of concanavalin A-induced human

suppressor and helper T cells by the autologous erythrocyte rosette technique. *J. Clin. Invest*. **68**, 447–453.
84. Sakane, T., Kotani, H., Takada, S., Murakawa, Y., and Ueda, Y. (1983). A defect in the suppressor circuits among OKT4+ cell populations in patients with systemic lupus erythematosus occurs independently of a defect in the OKT8+ suppressor T cell function. *J. Immunol*. **131**, 753–761.
85. Yamada, A., Cohen, P. L., and Winfield, J. B. (1985). Subset specificity of antilymphocyte antibodies in systemic lupus erythematosus: Preferential reactivity with cells bearing the T4 and autologous erythrocyte receptor phenotypes. *Arthritis Rheum*. **28**, 262.
86. Yamada, A., Shaw, N., and Winfield, J. B. (1985). Surface antigen specificity of cold-reactive IgM antibophocyte antibodies in systemic lupus erythematosus. *Arthritis Rheum*. **28**, 44–51.
87. Honda, M., Sakane, T., Steinberg, A. D., Koran, H., Tsunematsu, T., Moriyama, K., and Fukase, M. (1982). Studies of immune functions of patients with systemic lupus erythematosus: Antibodies to desialized rather than intact T cells preferentially bind to an eliminate suppressor effector T cells. *J. Clin. Invest*. **69**, 940–949.
88. Mimura, T., Fernsten, P., Jarjour, W., and Winfield, J. B. (1990). Autoantibodies specific for different isoforms of CD45 in systemic lupus erythematosus. *J. Exp. Med*. **172**, 653–656.
89. Clark, E. A., and Ledbetter, J. A. (1989). Leukocyte cell surface enzymology: CD45(LCA, T200) is a protein tyrosine phosphatase. *Immunol. Today* **10**, 225.
90. Mustelin, T., Coggeshall, K. M., and Altman, A. (1989). Rapid activation of the T-cell tyrosine protein kinase pp56lck by the CD45 phosphotyrosine phosphatase. *Proc Natl. Acad. Sci. USA* **86**, 6302–6306.
91. Takeuchi, T., Pang, M., Amano, K., Koide, J., and Abe, T. (1997). Reduced protein tyrosine phosphatase (PTPase) activity of CD45 on peripheral blood lymphocytes in patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **109**, 20–26.
92. Yamada, A., and Winfield, J. B. (1984). Inhibition of soluble antigen-induced T cell proliferation by warm-reactive antibodies to activated T cells in systemic lupus erythematosus. *J. Clin. Invest*. **74**, 1948–1960.
93. Charpentier, B., Carnaud, C., and Bach, J. F. (1979). Selective depression of the xenogeneic cell-mediated lympholysis in systemic lupus erythematosus. *J. Clin. Invest*. **64**, 351–360.
94. Rook, A. H., Tsokos, G. C., Quinnan, G. V., Balow, J. E., Stocks, N., Phelan, M. A., and Djeu, J. Y. (1982). Antibodies to natural killer cells in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **24**, 179–185.
95. Edwards, B. S., Searles, R. P., Brozek, C. M., Richards, R., Savage, S. M., Nolla, H., and Hoffman, C. L. (1987). Isotype and cytotoxicity spectra of anti-lymphocyte antibodies in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **45**, 333–347.
96. Rekvig, O. P., and Hannestad, K. (1977). Properties of antinuclear antibodies that cross-react with plasma membranes. *Scand. J. Immunol*. **6**, 1041–1054.
97. Rekvig, O. P., and Hannestad, K. (1979). The specificity of human autoantibodies that react with bothe cell nuclei and plasma membranes: The nuclear antigen is present on core mononucleosomes. *J. Immunol*. **123**, 2673–2681.
98. Searles, R. P., Messner, R. P., and Bankhurst, A. D. (1979). Cross-reactivity of antilymphocyte and antinuclear antibodies in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **14**, 292–299.
99. Bluestein, H. G., and Zvafler, N. J. (1976). Brain reactive lymphocytotoxic antibodies inthe serum of patients with systemic lupus erythematosus. *J. Clin. Invest*. **57**, 509–516.
100. Bresnihan, B., Grigor, R. R., Oliver, M., Lewkonia, R. M., Hughes, G. R. V., Lovins, R. E., and Faulk, W. P. (1977). Immunologic mechanism for spontaneous abortion in systemic lupus erythematosus. *Lancet* **i**, 1205–1207.
101. Jacob, L., Tron, F., Bach, J.-F., and Louvard, D. (1984). A monoclonal anti-DNA antibody also bind to cell surface proteins. *Proc. Natl. Acad. Sci. USA* **81**, 3843–3845.
102. Jacob, L., Lety, M.-A., and Louvard, D. (1985). Dinding of a monoclonal anti-DNA autoantibodies to identical protein(s) present at the surface of several human cell types involved in lupus pathogenesis. *J. Clin. Invest*. **75**, 315.
103. Jacob, L., Lety, M.-A., Chogquette, D., Vaird, J.-P., Jacov, F., Louvard, D., and Bach, J.-F. (1987). Presence of antibodies against a cell surface protein, cross-reactive with DNA in systemic lupus erythematosus: A marker of the disease. *Proc. Natl. Acad. Sci. USA* **84**, 2956–2959.
104. Inghirami, G., Simon, J., Balow, J. E., and Tsokos, G. C. (1988). Activated T lymphocytes in the peripheral blood of patients with systemic lupus erythematosus induce B cells to produce immunoglobulin. *Clin. Exp. Rheumatol*. **6**, 269–276.
105. Tsuchiya, N., Mitamura, T., Goto, M., Moroi, Y., Kinoshita, M., Yokohari, R., and Miyamoto, T. (1988). Two-dimensional flow cytometric analysis of peripheral blood T lymphocytes from patients with systemic lupus erythematosus: Preferential expression of HLA-DR antigen on the surface of Leu 2a+ cells. *J. Rheumatol*. **15**, 946–951.
106. Viallard, J. F., Bloch-Michel, C., Neau-Cransac, M., Taupin, J. L., Garrigue, S., Miossec, V., Mercie, P., Pellegrin, J. L., and Moreau, J. F. (2001). HLA-DR expression on lymphocyte subsets as a marker of disease activity in patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **125**, 485–491.
107. Boumpas, D. T., Mark, G. E., and Tsokos, G. C. (1986). Oncogenes and autoimmunity. *Anticancer Res*. **6**, 491–497.
108. Eleftheriades, E. G., Boumpas, D. T., Balow, J. E., and Tsokos, G. C. (1989). Transcriptional and post-transcriptional mechanisms are responsible for the increased expression of c-myc protooncogene in lymphocytes from patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **52**, 507–515.

109. Suzuki, H., Nakanishi, K., Steinberg, A., and Green, I. (1986). Induction of c-myc expression early in the course of B-cell activation: Studies in normal humans and patients with systemic lupus erythematosus. *Int. Arch. Allergy Appl. Immunol*. **79**, 380–387.
110. Tanaka, T., Saiki, O., Negoros, S., Igarashi, T., Kuritani, T., Hara, H., Suemura, M., and Kishimoto, S. (1989). Decreased expression of interleukin-2 binding molecules (p 70/75) in T cells from patients with systemic lupus erythematosus. *Arthritis Rheum*. **32**, 552–559.
111. Ishida, H., Kumagai, S., Umehara, H., Sano, H., Tagaya, Y., Yodoi, J., and Imura, H. (1987). Impaired expression of high affinity interleukin 2 receptor on activated lymphocytes from patients with systemic lupus erythematosus. *J. Immunol*. **139**, 1070–1074.
112. Abdou, N. I., Sagawa, A., Passual, E., Herbert, G., and Sabeghee, S. (1976). Suppressor T cell abnormality in idiopathic SLE. *Clin. Immunol. Immunopathol*. **6**, 192–199.
113. Bresnihan, B., and Jasin, H. E. (1977). Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. Clin. Invest*. **59**, 106–116.
114. Fauci, A. S., Steinberg, A. D., Haynes, B. F., and Whalen, G. (1978). Immunoregulatory abberrations in systemic lupus erythematosus. *J. Immunol*. **121**, 1473–1479.
115. Miller, K. B., and Schwartz, R. S. (1979). Familial abnormalities of suppressor-cell function in systemic lupus erythematosus. *N. Engl. J. Med*. **301**, 803–809.
116. Ruiz-Arguelles, A., Alarcon-Segovia, D., Llorente, L. W., and DelGindice-Knipping, J. A. (1980). Heterogeneity of the spontaneously expanded an mitogen-induced generation of suppressor cell function of T cells on B cells systemic lupus erythematosus. *Arthritis Rheum*. **23**, 1004–1009.
117. Sakane, T., Steinberg, A. D., and Green, I. (1978). Studies of immune function of patients with systemic lupus erythematosus. I. Dysfunction of suppressor T cell activity related to impaired generation of, rather than response to, suppressor cell. *Arthritis Rheum*. **21**, 657–664.
118. Tsokos, G. C., and Balow, J. E. (1982). Suppressor T cells in SLE: Lack of defective in vitro suppressor cell generation in patients with active disease. *J. Clin. Lab. Immunol*. **8**, 83–90.
119. Nies, K., Boyer, R., Stevens, R., and Louie, J. (1980). Antitetanus toxoid antibody synthesis after booster immunization in systemic lupus erythematosus: Comparison of the in vitro and in vivo responses. *Arthritis Rheum*. **23**, 1343–1350.
120. Kaufman, D. V., and Bostwick, E. (1979). Defective suppressor T-cell activity in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **13**, 9–18.
121. Coovadia, H. M., Mackay, I. R., and d'Apice, A. J. F. (1981). Suppressor cells assayed by three different methods in patients with chronic active hepatitis and systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **18**, 268–275.
122. Morimoto, C. (1978). Loss of suppressor T-lymphocyte function in patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol*. **32**, 125–133.
123. Morimoto, C., Abe, T., and Homma, M. (1979). Altered function of suppressor T lymphocytes in patients with active systemic lupus erythematosus: in vitro immune response to autoantigen. *Clin. Immunol. Immunopathol*. **13**, 161–170.
124. Krakauer, R. S., Clough, J. D., Alexander, T., Sundeen, T., and Sauder, D. N. (1980). Suppressor cell defect in SLE: Relationship to native DNA binding. *Clin. Exp. Immunol*. **40**, 72–76.
125. Miller, K. B., and Salem, D. (1982). Immune regulatory abnormalities produced by procainamide. *Am. J. Med*. **73**, 487–492.
126. Alarcon-Segovia, D., and Palacios, R. (1981). Differences in immunoregulatory T cell circuits between diphenylhydantoin-related and spontaneously occuring systemic lupus erythematosus. *Arthritis Rheum*. **24**, 1086–1092.
127. Frajman, M., Diaz-Jouanen, E., Alcocer-Varela, J., Fishbein, E., Guevara, M., and Alarcon-Segovia, D. (1983). Effect of pregnancy on functions of circulating T cells from patients with systemic lupus erythematosus: Correction of T-cell suppression and autologous mixed-lymphocyte responses. *Clin. Immunol. Immunopathol*. **29**, 94–102.
128. Tsokos, G. C., Magrath, I. T., and Balow, J. E. (1983). EBV induces normal B cell responses but defective suppressor T cell responses in patients with SLE. *J. Immunol*. **131**, 1797–1781.
129. Golding, B., Tsokos, G. C., Fleisher, T., Muchmore, A. V., and Blaese, R. M. (1986). The role of nonactivated and interferon-gamma activated monocytes in regulating normal and SLE patient B cell responses to TNP-*Brucella abortus*. *J. Immunol*. **137**, 103–107.
130. Takada, S., Veda, Y., Suzuki, N., Murakawa, Y., Hosimo, T., Green, I., Steinberg, A. D., Horwitz, D. A., and Sakane, T. (1985). Abnormalities in autologous mixed lymphocyte reaction-activated immunologic processes in systemic lupus erythematosus and their possible correction by interleukin-2. *Eur. J. Immunol*. **15**, 262–267.
131. Crow, M. K. (1985). Enhancement of the impaired autologous mixed leukocyte reaction in patients with systemic lupus erythematosus. *J. Clin. Invest*. **76**, 807–815.
132. Schattner, A., Miller, K. B., Daburaki, Y., and Schwartz, R. S. (1986). Suppressor cell function and anti-DNA antibody idiotypes in the serum of SLE patients and their first degree relatives. *Clin. Immunol. Immunopathol*. **41**, 417–426.
133. Filaci, G., Bacilieri, S., Fravega, M., Monetti, M., Contini, P., Ghio, M., Setti, M., Puppo, F., and Indiveri, F. (2001). Impairment of CD8+ T suppressor cell function in patients with active systemic lupus erythematosus. *J. Immunol*. **166**, 6452–6457.
134. Prud'Homme, G. J., Park, C. L., Fieser, T. M., Kotler, R., Dixon, R. J., and Theofilopoulos, A. N. (1983). Identification of a B cell differentiation factor(s) spontaneously

produced by proliferating T cells in murine lupus strains of the 1pr/1pr genotype. *J. Exp. Med*. **157**, 730–742.
135. Shivakumar, S., Tsokos, G. C., and Datta, S. K. (1989). T cell receptor alpha/beta expressing double-negative (CD4-/CD8-) and CD4+ T helper cells in humans augment the production of pathogenic anti-DNA autoantibodies associated with lupus nephritis. *J. Immunol*. **143**, 103–112.
136. Linker-Israeli, M., Quismorio, F. P., Jr., and Horwitz, D. A. (1990). CD8+ lymphocytes from patients with systemic lupus erythematosus sustain, rather than suppress, spontaneous polyclonal IgG production and synergize with CD4+ cells to support autoantibody synthesis. *Arthritis Rheum*. **33**, 1216–1225.
137. Katz, P., Whalen, G., Mitchell, S. R., Cupps, T. R., and Evans, M. (1990). Modulation of expression of mitogen-induced T cell-dependent B cell responses by natural killer cells. *Clin. Immunol. Immunopathol*. **55**, 148–155.
138. Delfraissy, J. F., Segond, P., Galanaud, P., Wallon, C., Massias, P., and Dormont, J. (1980). Depressed primary in vitro antibody response in untreated systemic lupus erythematosus: T helper cell defect and lack of defective suppressor cell function. *J. Clin. Invest*. **66**, 141–148.
139. Kumagai, S., Sredni, B., House, S., Steinberg, A. D., and Green, I. (1982). Defective regulation of B lymphocyte colony formation in patients with systemic lupus erythematosus. *J. Immunol*. **128**, 258–262.
140. Beale, M. G., Nash, G. S., Bertovich, M. J., and MacDermott, R. P. (1982). Similar disturbances in B cell activity and regulatory T cell function in Henoch-Schonlein purpura and systemic lupus erythematosus. *J. Immunol*. **128**, 486–491.
141. Nies, K. M., Stevens, R., and Louie, J. S. (1980). Normal T cell regulation of IgG aynthesis in systemic lupus erythematosus. *J. Clin. Lab. Immunol*. **4**, 69–75.
142. Datta, S. K., Patel, H., and Berry, D. (1987). Induction of a cationic shift in IgG anti-DNA autoantibodies: Role of T helper cells with classical and novel phenotypes in three murine models of lupus nephritis. *J. Exp. Med*. **165**, 1252–1268.
143. Gallo, G. R. R., Caulin-Glaser, T., and Lamm, M. E. (1981). Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice. *J. Clin. Invest*. **67**, 1305–1313.
144. Mannik, M., Gautheir, V. J., Stapleton, S. A., and Agodoa, L. Y. C. (1987). Immune complexes with cationic antibodies deposit in glomeruli more effectively than cationic antibodies alone. *J. Immunol*. **138**, 4209–4219.
145. Madaio, M. P., Carlson, J., Cataldo, J., Ucci, A., Migliorini, P., and Pankewycz, O. (1987). Murine monoclonal anti-DNA antibodies bind directly to glomerular antigens and form immune deposits. *J. Immunol*. **138**, 2883–2889.
146. Sainis, K., and Datta, S. K. (1988). CD4+ T cell lines with selective patterns of autoreactivity as wall as CD4-CD8- T helper cell lines augment the production of idiotypes shared by pathogenic anti-DNA autoantibodies in the NZB×5WR model of lupus nephritis. *J. Immunol*. **140**, 2215–2224.
147. Morimoto, C., Steinberg, A. D., Letvin, N. L., Hagan, M., Takeuchi, T., Daley, J., Levine, H., and Schlossman, S. F. (1987). A defect of immunoregulatory T cell subsets in systemic lupus erythematosus patients demonstrated with anti-2H4 antibody. *J. Clin. Invest*. **79**, 762–768.
148. Rajagopalan, S., Zordan, T., Tsokos, G. C., and Datta, S. K. (1990). Pathogenic anti-DNA autoantibody-inducing T helper cell lines from patients with active lupus nephritis: Isolation of CD4–8- T helper cell lines that express the gamma delta T-cell antigen receptor. *Proc. Natl. Acad. Sci. USA* **87**, 7020–7024.
149. Sieling, P. A., Porcelli, S. A., Duong, B. T., Spada, F., Bloom, B. R., Diamond, B., and Hahn, B. H. (2000). Human double-negative T cells in systemic lupus erythematosus provide help for IgG and are restricted by CD1c. *J. Immunol*. **165**, 5338–5344.
150. Minota, S., Cameron, B., Welch, W. J., and Winfield, J. B. (1988). Autoantibodies to the constitutive 73-KD member of the hsp70 family of heat shock proteins in systemic lupus erythematosus. *J. Exp. Med*. **168**, 1475–1480.
151. Winfield, J. B., and Jarjour, W. N. (1990). Stress proteins, autoimmunity, and autoimmune disease. *Curr. Top. Microbiol. Immunol*. **167**, 161–189.
152. Kiang, J. G., and Tsokos, G. C. (1996). Cell signaling and heat shock protein expression. *J. Biomed. Sci*. **3**, 379–388.
153. Winfield, J. B. (1989). Stress proteins, arthritis, and autoimmunity. *Arthritis Rheum*. **32**, 1497–1504.
154. Lydyard, P. M., and van Eden, W. (1990). Heat shock proteins: Immunity and immunopathology. *Immunol. Today* **11**, 228–229.
155. Born, W., Hall, L., Dallas, A., Boymel, J., Shinnick, T., Young, D., Brennan, P., and O'Brien, R. (1991). Recognition of a peptide antigen by heat shock-reactive gamma-delta T lymphocytes. *Science* **249**, 67–69.
156. van Eden, W., Thole, J. E. R., van der Zee, R., Noordzij, A., van Embden, J. D. A, Hensen, E. J., and Choen, I. R. (1988). Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. *Nature* **331**, 171–173.
157. Holoshitz, J., Koning, F., Coligan, J. E., de Bruyn, J., and Strober, S. (1989). Isolation of CD4-CD8- mycobacteria-reactive T lymphocytes clones from rheumatoid arthritis synoval fluid. *Nature* **339**, 226.
158. Gaston, J. S. H., Life, P. F., Jenner, P. J., Colston, M. J., and Bacon, P. A. (1990). Recognition of a mycobacteria-specific epitope in the 65-kd heat-shock protein by synovial fluid-derived T cell clones. *J. Exp. Med*. **171**, 831–841.
159. Liossis, S. N. C., Ding, D. Z., Kiang, J. G., and Tsokos, G. C. (1997). Overexpression of the heat shock protein 70 enhances the TCR/CD3- and Fas/Apo-1/CD95-mediated apoptotic cell death in Jurkat T cels. *J. Immunol*. **158**, 5668–5675.
160. Gabai, V. L., Merlin, A. B., Mosser, D. D., Caron, A. W., Rits, S., Shifrin, V. I., and Sherman, M. Y. (1997). Hsp70 prevents activation of stress kinases: A novel pathway of

cellular thermotolerance. *J. Biol. Chem*. **272**, 18033–18037.
161. Hoffman, R. W. (2001). T cells in the pathogenesis of systemic lupus erythematosus. *Front. Biosci*. **6**, D1369–D1378.
162. Hoffman, R. W., Takeda, Y., Sharp, G. C., Lee, D. R., Hill, D. L., Kaneoka, H., and Caldwell, C. W. (1993). Human T cell clones reactive against U-small nuclear ribonucleoprotein autoantigens from connective tissue disease patients and healthy individuals. *J. Immunol*. **151**, 6460–6469.
163. Talken, B. L., Schafermeyer, K. R., Bailey, C. W., Lee, D. R., and Hoffman, R. W. (2001). T cell epitope mapping of the Smith antigen reveals that highly conserved Smith antigen motifs are the dominant target of T cell immunity in systemic lupus erythematosus. *J. Immunol*. **167**, 562–568.
164. Talken, B. L., Holyst, M. M., Lee, D. R., and Hoffman, R. W. (1999). T cell receptor beta-chain third complementarity-determining region gene usage is highly restricted among Sm-B autoantigen-specific human T cell clones derived from patients with connective tissue disease. *Arthritis Rheum*. **42**, 703–709.
165. Crow, M. K., DelGiudice-Asch, G., Zehetbauer, J. B., Lawson, J. L., Brot, N., Weissbach, H., and Elkon, K. B. (1994). Autoantigen-specific T cell proliferation induced by the ribosomal P2 protein in patients with systemic lupus erythematosus. *J. Clin. Invest*. **94**, 345–352.
166. Critchfield, J. M., Racke, M. K., Zuniga-Pfluecker, J. C., Cannella, B., Raine, C. S., Goverman, J., and Lenardo, M. J. (1994). T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis. *Science* **263**, 1139–1143.
167. Trentham, D. E., Dynesius-Trentham, R. A., Orav, E. J., Combitchi, D., Lorenzo, C., Sewell, K. L., Hafler, D. A., and Weiner, H. L. (1993). Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* **261**, 1669–1670.
168. Albertini, R. J., O'Neil, J. P., Nicklas, J. A., Heintz, N., and Kelleher, P. C. (1985). Alterations of the hprt gene in human in vivo-derived 6-thioguanine resistant T lymphocytes. *Nature* **316**, 369–371.
169. O'Neill, J. P., McGinniss, M. J., Berman, J. K., Sullivan, L. M., Nicklas, J. A., and Albertini, R. J. (1987). Refinement of a T-lymphocyte cloning assay to quantify the in vivo thioguanine-resistant mutant frequency in humans. *Mutagenesis* **2**, 87–94.
170. Henderson, L., Cole, H., Cole, J., James, S. E., and Green, M. (1986). Detection of somatic mutations in man: Evaluation of the microtitre cloning assay for T-lymphocytes. *Mutagenesis* **1**, 195–200.
171. Allegretta, M., Nicklas, J. A., Sriram, S., and Albertini, R. J. (1990). T cells responsive to myelin basic protein in patients with multiple sclerosis. *Science* **247**, 718–721.
172. Gmelig-Meyling, F., Dawisha, S., and Steinberg, A. D. (1992). Assessment of in vivo frequency of mutated T cells in patients with systemic lupus erythematosus. *J. Exp. Med*. **175**, 297–300.
173. Dawisha, S. M., Gmelig-Meylig, F., and Steinberg, A. D. (1994). Assessment of clinical parameters associated with increased frequency of mutant T cells in patients with systemic lupus erythematosus. *Arthrititis Rheum*. **37**, 270–277.
174. Theocharis, S., Sfikakis, P. P., Lipnick, R. N., Klipple, G. L., Steinberg, A. D., and Tsokos, G. C. (1995). Characterization of in vivo mutated T cell clones from patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **74**, 135–142.
175. Sfikakis, P. P., Tesar, J., Theocharis, S. E., Klipple, G. L., and Tsokos, G. C. (1994). Increased frequency of in vivo hprt gene mutated T cells in the peripheral blood of patients with systemic sclerosis. *Ann. Rheum. Dis*. **53**, 122–127.
176. Holyst, M. M., Hill, D. L., Sharp, G. C., and Hoffman, R. W. (1994). Increased frequency of mutations in the hprt gene of T cells isolated from patients with anti-U1-70 kD-autoantibody-positive connective tissue disease. *Int. Arch. Allergy Immunol*. **105**, 234–237.
177. Dunckley, H., Gatenby, P. A., and Serjeantson, S. W. (1988). T-cell receptor and HLA class II RFLPs in systemic lupus erythematosus. *Immunogenetics* **27**, 393–395.
178. Posnett, D. N., Gottlieb, A., Bussel, J. B., Friedman, S. M., Chiorazzi, N., Li, Y., Svabo, P., Farid, N. R., and Robinson, M. A. (1988). T cell antigen receptors in autoimmunity. *J. Immunol*. **141**, 1963–1969.
179. Frank, M. B., McArthur, R., Harley, J. B., and Fujisaku, A. (1990). Anti-Ro(SSA) autoantibodies are associated with T cell receptor b genes in systemic lupus erythematosus. *J. Clin. Invest*. **85**, 33–39.
180. Tebib, J. G., Alcocer-Virela, J., Alarcon-Segovia, A., and Schur, P. H. (1990). Association between a T cell receptor restriction fragment length polymorphism and systemic lupus erythematosus. *J. Clin. Invest*. **86**, 1961–1967.
181. Oishi, Y., Sumida, T., Sakamoto, A., Kita, Y., Kurasawa, K., Nawata, Y., Takabayashi, K., Takahashi, H., Yoshida, S., Taniguchi, M., Saito, Y., and Iwamoto, I. (2001). Selective reduction and recovery of invariant Valpha24JalphaQ T cell receptor T cells in correlation with disease activity in patients with systemic lupus erythematosus. *J. Rheumatol*. **28**, 275–283.
182. Desai-Mehta, A., Mao, C., Rajagopalan, S., Robinson, T., and Datta, S. K. (1995). Structure and specificity of T cell receptors expressed by potentially pathogenic anti-DNA autoantibody-inducing T cells in human lupus. *J. Clin. Invest*. **95**, 531–541.
183. Mohan, C., and Datta, S. K. (1995). Lupus: Key pathogenic mechanisms and contributing factors. *Clin. Immunol. Immunopathol*. **77**, 209–220.
184. Olive, C., Gatenby, P. A., and Serjeantson, S. W. (1994). Restricted junctional diversity of T cell recepotr delta gene rearrangements expressed in systemic lupus erythematosus (SLE) patients. *Clin. Exp. Immunol*. **97**, 430–438.
185. Rajagopalan, S., Mao, C., and Datta, S. K. (1992). Pathogenic autoantibody-inducing gamma/delta T helper cells from patients with lupus nephritis express unusual T cell receptors. *Clin. Immunol. Immunopathol*. **62**, 344–350.
186. Okubo, M., Kurokawa, M., Ohto, H., Nishimaki, T., Nishioka, K., Kasukawa, R., and Yamamoto, K. (1996). Clonotype analysis of peripheral blood T cells and

autoantigen-reactive T cells from patients with mixed connective tissue disease. *J. Immunol*. **153**, 3784–3790.
187. Talken, B. L., Lee, D. R., Caldwell, C. W., Quinn, T. P., Schafermeyer, K. R., and Hoffman, R. W. (1999). Analysis of T cell receptors specific for U1-70 kD small nuclear ribonucleoprotein autoantigen: The alpha chain complementarity determining region three is highly conserved among connective tissue disease patients. *Hum. Immunol*. **60**, 200–208.
188. Kato, T., Kurokawa, M., Sasakawa, H., Masuko-Hongo, K., Matsui, T., Sekine, T., Tanaka, C., Yamamoto, K., and Nishioka, K. (2000). Analysis of accumulated T cell clonotypes in patients with systemic lupus erythematosus. *Arthritis Rheum*. **43**, 2712–2721.
189. Datta, S. K., Kaliyaperumal, A., and Desai-Mehta, A. (1997). T cells of lupus and molecular targets for immunotherapy. *J. Clin. Immunol*. **17**, 11–20.
190. Steinman, L. (1996). A few autoreactive cells in an autoimmune infiltrate control a vast populaiton of nonspecifc cells: A tale of smart bombs and the infantry. *Proc. Natl. Acad. Sci. USA* **93**, 2253–2256.
191. Linker-Israeli, M., and Deans, R. (1991). Dysregulated lymphokine production in systemic lupus erythematosus (SLE). *Ann. N.Y. Acad. Sci*. **687**, 567–569.
192. Alcocer-Varela, J., and Alarcon-Segovia, D. (1982). Decreased production of and response to interleukin-2 by cultured lymphocytes from patients with systemic lupus erythematosus. *J. Clin. Invest*. **69**, 1388–1392.
193. Murakawa, Y., Takada, S., Ueda, Y., Suzuki, N., Hoshino, T., and Sakane, T. (1985). Characterization of T lymphocyte subpopulations responsible for deficient interleukin 2 activity in patients with systemic lupus erythematosus. *J. Immunol*. **134**, 187–195.
194. Huang, Y. P., Perrin, L. H., Miescher, P. A., and Zubler, R. H. (1988). Correlation of T and B cell activities in vitro and serum IL-2 levels in systemic lupus erythematosus. *J. Immunol*. **141**, 827–833.
195. Linker-Israeli, M., Bakke, A. C., Quismorio, F. P., Jr., and Horwitz, D. A. (1985). Correction of interleukin-2 production in patients with systemic lupus erythematosus by removal of spontaneously activated suppressor cells. *J. Clin. Invest*. **75**, 762–768.
196. Horwitz, D. A., Linker-Israeli, M., Gray, J. D., and Lemoine, C. (1987). Functional properties of CD8 positive lymphocyte subsets in systemic lupus erythematosus. *J. Rheumatol*. **14**, 49–52.
197. Linker-Israeli, M., Quismorio, F. P., Jr., and Horwitz, D. A. (1988). Further characterization of interleukin-2 production by lymphocytes of patients with systemic lupus erythematosus. *J. Rheumatol*. **15**, 1216–1222.
198. Linker-Israeli, M., Gray, J. D., Quismorio, F. P., Jr., and Horwitz, D. A. (1988). Characterization of lymphocytes that suppress IL-2 production in systemic lupus erythematosus. *Clin. Exp. Immunol*. **73**, 236–241.
199. Sierakowski, S., and Kucharz, E. J. (1988). Phorbol myristate acetate (PMA) reverses inhibition of interleukin-2 production by T lymphocytes of patients with systemic lupus erythematosus. *Med. Interne*. **26**, 67–73.
200. Murakawa, Y., and Sakane, T. (1988). Deficient phytohemagglutinin-induced interleukin-2 activity in patients with inactive systemic lupus erythematosus is correctable by the addition of phorbol myristate acetate. *Arthritis Rheum*. **31**, 826–833.
201. Hishikawa, T., Tokano, Y., Sekigawa, I., Ando, S., Takasaki, Y., Hashimoto, H., Hirose, S., Okumura, K., Abe, M., and Shirai, T. (1990). HLA-DP+ T cells and deficient interleukin-2 production in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **55**, 285–296.
202. Zubler, R. H., and Miescher, P. A. (1990). Role of T lymphocytes in systemic lupus erythematosus. *Ann. Med. Interne. (Paris)* **141**, 208–212.
203. Sakane, T., Takada, S., Suzuki, N., Tsuchida, T., Murakawa, Y., and Ueda, Y. (1986). Deficiencies in suppressor T cell activity seen in patients with active systemic lupus erythematosus are due to the dilution of normally functioning suppressor T cells by nonsuppressor T cells. *J. Immunol*. **137**, 3809–3813.
204. Warrington, R. J., Sauder, P. J., Homik, J., and Ofosu-Appiah, W. (1989). Reversible interleukin-2 response defects in systemic lupus erythematosus. *Clin. Exp. Immunol*. **77**, 163–167.
205. Warrington, R. J. (1988). Interleukin-2 abnormalities in systemic lupus erythematosus and rheumatoid arthritis: A role for overproduction of interleukin-2 in human autoimmunity. *J. Rheumatol*. **15**, 616–620.
206. Miyagi, J., Minato, N., Sumiya, M., Kasahara, T., and Kano, S. (1989). Two types of antibodies inhibiting interleukin-2 production by normal lymphocytes in patients with systemic lupus erythematosus. *Arthritis Rheum*. **32**, 1356–1364.
207. Wong, H. K., Kammer, G. M., Dennis, G., and Tsokos, G. C. (1999). Abnormal NF-kappaB activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-relA protein expression. *J. Immunol*. **163**, 1682–1689.
208. Wong, H. K., Kammer, G. M., Mishra, N., Dennis, G., and Tsokos, G. C. (1999). Activator protein-1 (AP-1) regulation in lymphocytes from patients with systemic lupus erythematosus. *Arthritis Rheum*. **42**, S1446.
209. Solomou, E. E., Juang, Y. T., Gourley, M. F., Kammer, G. M., and Tsokos, G. C. (2001). Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus. *J. Immunol*. **166**, 4216–4222.
210. Djeu, J. Y., Kasahara, T., Balow, J. E., and Tsokos, G. C. (1986). Decreased interleukin 2 inhibitor in sera of patients with autoimmune disorders. *Clin. Exp. Immunol*. **65**, 279–285.
211. Kucharz, E. J., Sierakowski, S., and Goodwin, J. S. (1988). Decreased activity of interleukin-2 inhibitor in plasma of patients with systemic lupus erythematosus. *Clin. Rheumatol*. **7**, 87–90.
212. Wolf, R. E., and Brelsford, W. G. (1988). Soluble interleukin-2 receptors in systemic lupus erythematosus. *Arthritis Rheum*. **31**, 729–735.
213. Campen, D. H., Horwitz, D. A., Quismorio, F. P., Jr., Ehresmann, G. R., and Martin, W. J. (1988). Serum levels

of interleukin-2 receptor and activity of rheumatic diseases characterizied by immune sytem activation. *Arthritis Rheum.* **31**, 1358–1364.
214. Wood, N. C., Symons, J. A., and Duff, G. W. (1988). Serum interleukin-2 receptor in rheumatoid arthritis: A prognostic indicator of disease activity? *J. Autoimmun.* **1**, 353–360.
215. Tokano, Y., Murashima, A., Takasaki, Y., Hashimoto, H., Okomura, K., and Mirose, S. (1989). Relation between soluble IL-2 receptor and clinical findings in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**, 803–809.
216. Keystone, E. C., Snow, K. M., Bombardier, C., Chang, C.-H., Nelson, D. L., and Rubin, L. A. (1988). Elevated soluble interleukin-2 receptor levels in the sera and synovial fluids of patients with rheumatoid arthritis. *Arthritis Rheum.* **31**, 844–849.
217. Balderas, R. S., Josemovic-Alasevic, O., Diamanstein, T., Dixon, F. J., and Theofilopoulos, A. N. (1986). Elevated titers of cell-free interleukin 2 receptor in serum of lupus mice. *J. Immunol.* **139**, 1496–1500.
218. Rubin, L. A. (1990). The soluble interleukin-2 receptor in rheumatic disease. *Arthritis Rheum.* **33**, 1145–1148.
219. Wigfall, D. R., Sakai, R. S., Wallace, D. J., and Jordan, S. C. (1988). Interleukin-2 receptor expression in peripheral blood lymphocytes from systemic lupus erythematosus patients: Relationship to clinical activity. *Clin. Immunol. Immunopathol.* **47**, 354–362.
220. Tsokos, G. C., Smith, P. L., Christian, C. B., Lipnick, R. L., Balow, J. E., and Djeu, J. Y. (1985). Interleukin-2 resotres the depressed allogeneic cell mediated lympholysis and natural killer cell activity in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **34**, 379–386.
221. Tsokos, G. C., Boumpas, D. T., Smith, P. L., Djeu, J. Y., and Balow, J. E. (1988). Deficient gamma-IFN production in patients with SLE. *Arthritis Rheum.* **29**, 1210–1215.
222. Neighbour, P. A., and Grayzel, A. I. (1981). Interferon production in vitro by leuocytes from patients with systemic lupus erythematosus and rheumatoid arthritis. *Clin. Exp. Immunol.* **45**, 576–582.
223. Strannegard, O., Hermodsson, S., and Westberg, G. (1982). Interferon and natural killer cells in systemic lupus erythematosus. *Clin. Exp. Immunol.* **50**, 246–252.
224. McKenna, R. M., Wilkins, J. A., and Warrington, R. J. (1988). Lymphokine production in rheumatoid arthritis and systemic lupus erythematosus. *J. Rheumatol.* **15**, 1639–1642.
225. Takeuchi, T., Tsuzaka, K., Pang, M., Amano, K., Koide, J., and Abe, T. (1998). TCR zeta chain lacking exon 7 in two patients with systemic lupus erythematosus. *Int. Immunol.* **10**, 911–921.
226. Tsuzaka, K., Takeuchi, T., Onoda, N., Pang, M., and Abe, T. (1998). Mutations in T cell receptor zeta chain mRNA of peripheral T cells from systemic lupus erythematosus patients. *J. Autoimmun.* **11**, 381–385.
227. Prud'Homme, G. J., Kono, D. H., and Theofilopoulos, A. N. (1995). Quantitative polymerase chain reaction analysis reveals marked overexpression of interleukin-1 beta, interleukin-1 and interferon-gamma mRNA in the lymph nodes of lupus-prone mice. *Mol. Immunol.* **32**, 495–503.
228. Seelig, H. P., Ehrfeld, H., and Renz, M. (1994). Interferon-gamma-inducible protein p16: A new target of antinuclear antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 1672–1683.
229. Jacob, C. O., van der Meide, P. H., and McDevitt, H. O. (1987). In vivo treatment of (NZB × NZW)F1 lupus-like nephritis with monoclonal antibody to gamma interferon. J. *Exp. Med.* **166**, 798–803.
230. Balomenos, D., Rumold, R., and Theofilopoulos, A. N. (1998). Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J. Clin. Invest* **101**, 364–371.
231. Peng, S. L., Moslehi, J., and Craft, J. (1997). Roles of interferon-gamma and interleukin-4 in murine lupus. *J. Clin. Invest* **99**, 1936–1946.
232. Haas, C., Ryffel, B., and Le Hir, M. (1997). IFN-gamma is essential for the development of autoimmune glomerulonephritis in MRL/Ipr mice. *J. Immunol.* **158**, 5484–5491.
233. Schwarting, A., Wada, T., Kinoshita, K., Tesch, G., and Kelley, V. R. (1998). IFN-gamma receptor signaling is essential for the initiation, acceleration, and destruction of autoimmune kidney disease in MRL-Fas(lpr) mice. *J. Immunol.* **161**, 494–503.
234. Haas, C., Ryffel, B., and Le Hir, M. (1998). IFN-gamma receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB × NZW)F1 mice. *J. Immunol.* **160**, 3713–3718.
235. Kishimoto, T., and Hirano, T. (1988). Molecular regulation of B lymphocyte response. *Annu. Rev. Immunol.* **6**, 485–512.
236. Linker-Israeli, M., Honda, M., Nand, R., Mandyam, R., Mengesha, E., Wallace, D. J., Metzger, A., Beharier, B., and Klinenberg, J. R. (1999). Exogenous IL-10 and IL-4 down-regulate IL-6 production by SLE-derived PBMC. *Clin. Immunol.* **91**, 6–16.
237. Linker-Israeli, M., Wallace, D. J., Prehn, J., Michael, D., Honda, M., Taylor, K. D., Paul-Labrador, M., Fischel-Ghodsian, N., Fraser, P. A., and Klinenberg, J. R. (1999). Association of IL-6 gene alleles with systemic lupus erythematosus (SLE) and with elevated IL-6 expression. *Genes Immun.* **1**, 45–52.
238. Hirohata, S., and Miyamoto, T. (1990). Elevated levels of interleukin-6 in cerebrospinal fluid from patients with systemic lupus erythematosus and central nervous system. *Arthritis Rheum.* **33**, 644–649.
239. Horii, Y., Muraguchi, A., Iwano, M., Matsuda, T., Hirayama, T., Yamada, H., Fujii, Y., Dohi, K., Ishikawa, H., and Ohmoto, Y. (1989). Involvement of IL-6 in mesangial proliferative glomerulonephritis. *J. Immunol.* **143**, 3949–3955.
240. Nagafuchi, H., Suzuki, N., Mizushima, Y., and Sakane, T. (1993). Constitutive expression of IL-6 receptors and their role in the excessive B cell function in patients with systemic lupus erythematosus. *J. Immunol.* **151**, 6525–6534.

241. Finck, B. K., Chan, B., and Wofsy, D. (1994). Interleukin 6 promotes murine lupus in NZB/NZW F1 mice. *J. Clin. Invest.* **94**, 585–591.
242. Briere, F., Servet-Delprat, C., Bridon, J. M., Saint-Remy, J. M., and Banchereau, J. (1994). Human interleukin 10 induces naive surface immunoglobulin D+ (sIgD+) B cells to secrete IgG1 and IgG3. *J. Exp. Med.* **179**, 757–762.
243. Bacchetta, R., Bigler, M., Touraine, J. L., Parkman, R., Tovo, P. A., Abrams, J., de Waal Malefyt, R., de Vries, J. E., and Roncarolo, M. G. (1994). High levels of interleukin 10 production in vivo are associated with tolerance in SCID patients transplanted with HLA mismatched hematopoietic stem cells. *J. Exp. Med.* **179**, 493–502.
244. Llorente, L., Zou, W., Levy, Y., Richaud-Patin, Y., Wijdenes, J., Alcocer-Varela, J., Morel-Fourrier, B., Brouet, J. C., Alarcon-Segovia, D., and Galanaud, P. (1995). Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med.* **181**, 839–844.
245. Linker-Israeli, M. (1992). Cytokine abnormalities in human lupus. *Clin. Immunol. Immunopathol.* **63**, 10–12.
246. Linker-Israeli, M., Deans, R. J., Wallace, D. J., Prehn, J., Ozeri-Chen, T., and Klinenberg, J. R. (1991). Elevated levels of endogenous IL-6 in systemic lupus erythematosus: A putative role in pathogenesis. *J. Immunol.* **147**, 117–123.
247. Gibson, A. W., Edberg, J. C., Wu, J., Westendorp, R. G., Huizinga, T. W., and Kimberly, R. P. (2001). Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. *J. Immunol.* **166**, 3915–3922.
248. Nakashima, H., Akahoshi, M., Tanaka, Y., Yamaoka, K., Ogami, E., Nagano, S., Arinobu, Y., Niiro, H., Otsuka, T., and Niho, Y. (1999). Polymorphisms within the interleukin-10 receptor cDNA gene (IL10R) in Japanese patients with systemic lupus erythematosus. *Rheumatology* (*Oxford*) **38**, 1142–1144.
249. Llorente, L., Richaud-Patin, Y., Fior, R., Alcocer-Varela, J., Wijdenes, J., Fourrier, B. M., Galanaud, P., and Emilie, D. (1994). In vivo production of interleukin-10 by non-T cells in rheumatoid arthritis, Sjogren's syndrome, and systemic lupus erythematosus: A potential mechanism of B lymphocyte hyperactivity and autoimmunity. *Arthritis Rheum.* **37**, 1647–1655.
250. Cush, J. J., Splawski, J. B., Thomas, R., McFarlin, J. E., Schulze-Koops, H., Davis, L. S., Fujita, K., and Lipsky, P. E. (1995). Elevated interleukin-10 levels in patients with rheumatoid arthritis. *Arthritis Rheum.* **38**, 96–104.
251. Ishida, H., Muchamuel, T., Sakaguchi, S., Andrade, S., Menon, S., and Howard, M. (1994). Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* **179**, 305–310.
252. Tokano, Y., Morimoto, S., Kaneko, H., Amano, H., Nozawa, K., Takasaki, Y., and Hashimoto, H. (1999). Levels of IL-12 in the sera of patients with systemic lupus erythematosus (SLE): Relation to Th1- and Th2-derived cytokines. *Clin. Exp. Immunol.* **116**, 169–173.
253. Horwitz, D. A., Gray, J. D., Behrendsen, S. C., Kubin, M., Rengaraju, M., Ohtsuka, K., and Trinchieri, G. (1998). Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupus erythematosus. *Arthritis Rheum.* **41**, 838–844.
254. Alleva, D. G., Kaser, S. B., and Beller, D. I. (1998). Intrinsic defects in macrophage IL-12 production associated with immune dysfunction in the MRL/++ and New Zealand Black/White F1 lupus-prone mice and the Leishmania major-susceptible BALB/c strain. *J. Immunol.* **161**, 6878–6884.
255. Liu, T. F., and Jones, B. M. (1998). Impaired production of IL-12 in system lupus erythematosus. II: IL-12 production in vitro is correlated negatively with serum IL-10, positively with serum IFN-gamma and negatively with disease activity in SLE. *Cytokine* **10**, 148–153.
256. Okubo, T., Hagiwara, E., Ohno, S., Tsuji, T., Ihata, A., Ueda, A., Shirai, A., Aoki, I., Okuda, K., Miyazaki, J., and Ishigatsubo, Y. (1999). Administration of an IL-12-encoding DNA plasmid prevents the development of chronic graft-versus-host disease (GVHD). *J. Immunol.* **162**, 4013–4017.
257. Jacob, C. O., and McDevitt, H. O. (1988). Tumour necrosis factor-a in murine autoimmune "lupus" nephritis. *Nature* **331**, 356–358.
258. Jacob, C. O., Fronek, Z., Lewis, G. D., Koo, M., Hansen, J. A., and McDevitt, H. O. (1990). Heritable major histocompatibility complex class II-associated differences in production of tumor necrosis factor alpha: Relevance to genetic predisposition to systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **87**, 1233–1237.
259. Yu, C. L., Chang, K. L., Chiu, C. C., Chiang, B. N., Han, S. H., and Wang, S. R. (1989). Defective phagocytosis, decreased tumour necrosis factor-alpha production, and lymphocyte hyporesponsiveness predispose patients with systemic lupus erythematosus to infections. *Scand. J. Rheumatol.* **18**, 97–105.
260. Maury, C. P. J., and Teppo, A. M. (1989). Tumor crecosis factor in the serum of patients with systemic lupus erythematosus. *Arthritis Rheum.* **32**, 146–150.
261. Gray, J. D., Liu, T., Huynh, N., and Horwitz, D. A. (2001). Transforming growth factor beta enhances the expression of CD154 (CD40L) and production of tumor necrosis factor alpha by human T lymphocytes. *Immunol. Lett.* **78**, 83–88.
262. Yamagiwa, S., Gray, J. D., Hashimoto, S., and Horwitz, D. A. (2001). A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. *J. Immunol.* **166**, 7282–7289.
263. Ohtsuka, K., Gray, J. D., Stimmler, M. M., Toro, B., and Horwitz, D. A. (1998). Decreased production of TGF-beta by lymphocytes from patients with systemic lupus erythematosus. *J. Immunol.* **160**, 2539–2545.
264. Funauchi, M., Yu, H., Sugiyama, M., Ikoma, S., Ohno, M., Kinoshita, K., Hamada, K., and Kanamaru, A. (1999). Increased interleukin-4 production by NK T cells in systemic lupus erythematosus. *Clin. Immunol.* **92**, 197–202.

265. Lee, S., Kaneko, H., Sekigawa, I., Tokano, Y., Takasaki, Y., and Hashimoto, H. (1998). Circulating interleukin-16 in systemic lupus erythematosus. *Br. J. Rheumatol.* **37**, 1334–1337.
266. Aringer, M., Stummvoll, G. H., Steiner, G., Koller, M., Steiner, C. W., Hofler, E., Hiesberger, H., Smolen, J. S., and Graninger, W. B. (2001). Serum interleukin-15 is elevated in systemic lupus erythematosus. *Rheumatology* (*Oxford*) **40**, 876–881.
267. Hagiwara, E., Gourley, M. F., Lee, S., and Klinman, D. K. (1996). Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10: interferon-gamma-secreting cells in the peripheral blood. *Arthritis Rheum.* **39**, 379–385.
268. Masutani, K., Akahoshi, M., Tsuruya, K., Tokumoto, M., Ninomiya, T., Kohsaka, T., Fukuda, K., Kanai, H., Nakashima, H., Otsuka, T., and Hirakata, H. (2001). Predominance of Th1 immune response in diffuse proliferative lupus nephritis. *Arthritis Rheum.* **44**, 2097–2106.
269. Akahoshi, M., Nakashima, H., Tanaka, Y., Kohsaka, T., Nagano, S., Ohgami, E., Arinobu, Y., Yamaoka, K., Niiro, H., Shinozaki, M., Hirakata, H., Horiuchi, T., Otsuka, T., and Niho, Y. (1999). Th1/Th2 balance of peripheral T helper cells in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 1644–1648.
270. Kanik, K. S., Hagiwara, E., Shumacher, R., Wilder, R. L., and Klinman, D. K. (1997). Distinct patterns of cytokine secretion characterize new onset synovitis versus chronic rheumatoid arthritis. *J. Rheumatol.* **25**, 16–22.
271. Bell, D. A. (1978). Cell-mediated immunity in systemic lupus erythematosus: Observations on in vitro cell-mediated immune responses in relationship to number of potentially reactive T cells, disease activity in treatment. *Clin. Immunol. Immunopathol.* **9**, 301–317.
272. Gottlieb, A. B., Lahita, R. G., Chiorazzi, N., and Kunkel, H. G. (1979). Immune function in systemic lupus erythematosus: Impairment of in virto T-cell proliferation and in vivo antibody response to exogenous antigen. *J. Clin. Invest.* **63**, 885.
273. Horwitz, D. A., and Garrett, M. A. (1977). Lymphocyte reactivity to mitogens in subjects with systemic lupus erythematosus, rheumatoid arthritis and scleroderma. *Clin. Exp. Immunol.* **27**, 92–99.
274. Horwitz, D. A., Garrett, M. A., and Craig, A. H. (1977). Serum effects on mitogenic reactivity in sybjects with systemic lupus erythematosus, rheumatoid arthritis and scleroderma: Technical considerations and lack of correlation with anti-lymphocyte antibodies. *Clin. Exp. Immunol.* **27**, 100–110.
275. Offner, H., Konat, G., Legg, N. J., Raun, N. E., Winterberg, H., and Clausen, J. (1980). Antigenic stimulation of active E-rosette-forming lymphocytes in multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis. *Clin. Immunol. Immunopathol.* **16**, 367–373.
276. Utsinger, P. D., and Yount, W. J. (1977). Phytohemagglutinin response in systemic lupus erythematosus: Reconstitution experiments using highly purified lymphocyte subpopulations and monocytes. *J. Clin. Invest.* **60**, 626–638.
277. Goldman, J. A., Kitwin, L. E., Adams, R. C., Kruegar, R. C., and Hess, E. V. (1972). Cellular immunity to nuclear antigens in systemic lupus erythematosus. *J. Clin. Invest.* **51**, 2669–2677.
278. Horwitz, D. A., and Cusar, J. B. (1975). A relationship between impaired cellular immunity, humoral suppression of lymphocyte function and severity of systemic lupus erythematosus. *Am. J. Med.* **58**, 829–835.
279. Landry, M. (1977). Phagocyte function and cell-mediated immunity in systemic lupus erythematosus. *Arch. Derm.* **113**, 147–154.
280. Tsokos, G. C., Balow, J. E., Huston, D. P., Wei, N., and Decker, J. L. (1982). Effect of plasmapheresis on T and B lymphocyte functions in patients with systemic lupus erythematosus: A double-blind study. *Clin. Exp. Immunol.* **48**, 449.
281. Malave, I., Layrisse, Z., and Layrisse, M. (1975). Dose-dependent hypo-reactivity to phytohemagglutinin in systemic lupus erythematosus. *Cell. Immunol.* **15**, 231.
282. Rosenthal, C. J., and Franklin, E. C. (1975). Depression of cellular-mediated immunity in systemic lupus erythematosus: Related to disease activity. *Arthritis Rheum.* **18**, 207–217.
283. Via, C. S., Tsokos, G. C., Stocks, N. I., Clerici, M., and Shearer, G. M. (1990). Human in vitro allogeneic responses: Demonstration of three pathways of T helper cell activation. *J. Immunol.* **144**, 2524–2528.
284. Suciu-Foca, N., Buda, J. A., Theim, T., and Reemtsma, K. (1974). Impaired responsiveness of lymphocytes in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **18**, 295.
285. Sakane, T., Steinberg, A. D., and Green, I. (1978). Failure of autologous mixed lymphocyte reactions between T and non-T cells in patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **75**, 3464–3468.
286. Kuntz, M., Innes, J., and Weksler, M. (1976). Lymphocyte transformation induced by autologous or allogeneic non-T-lymphocytes. IV. Human T-lymphocyte proliferation induced by autologous or allergenis non-T-lymphocytes. *J. Exp. Med.* **143**, 1042–1054.
287. Smith, J. B., and Knowlton, R. P. (1979). Activation of suppressor T-cells in human autologous mixed lymphocyte culture. *J. Immunol.* **123**, 419–422.
288. Weksler, M. E., and Kosak, R. (1978). Lymphocyte transformation induced by autologous cells. V. Generation of immunologic memory and specificity during the autologous mixed lymphocyte reaction. *J. Exp. Med.* **146**, 1833.
289. Sakane, T., Steinberg, A. D., Arnett, F. C., Reinertsen, J. L., and Green, I. (1979). Studies of immune functions of patients with systemic lupus erythematosus. *Arthritis Rheum.* **22**, 770–776.
290. Kuntz, M., Innes, J. B., and Weksler, M. (1979). The cellular basis of the imparied autologous mixed lymphocyte reaction in patients with systemic erythematosus. *J. Clin. Invest.* **63**, 151–153.
291. Takeuchi, T., Tanaka, S., Steinberg, A. D., Matsuyama, T., Daley, J., Schlossman, S. F., and Morimoto, C. (1988). Defective expression of the 2H4 molecule after autol-

ogous mixed lymphocyte reaction activation in systemic lupus erythematosus patients. *J. Clin. Invest.* **82**, 1288–1294.
292. Andrianakos, A. A., Tsichlis, P. N., Merikas, E. C., Marketos, S. G., Sharp, J. T., and Merikas, G. E. (1977). Cell-mediated immunity in systemic lupus erythematosus. *Clin. Exp. Immunol.* **30**, 89–93.
293. Hahn, B. H., Bagby, M. K., and Osterland, C. K. (1973). Abnormalities of delayed hypersensitivity in systemic lupus erythematosus. *Am. J. Med.* **55**, 25–31.
294. Horwitz, D. A. (1972). Impaired delayed hypersensitivity in systemic lupus erythematosus. *Arthritis Rheum.* **15**, 353.
295. Tanaka, Y., Watanabe, K., Suzuki, M., Saito, K., Oda, S., Suzuki, H., Eto, S., and Yamashita, U. (1989). Spontaneous production of bone-resorbing lymphokines by B cells in patients with systemic lupus erythematosus. *J. Clin. Immunol.* **9**, 415–420.
296. Trejaut, J., Dunckley, H., Sullivan, J. S., Doran, T., and Chapman, J. (1990). Comparison of serological class II typing with DNA-DR and DNA-DQ typing of kidney donors and recipients. *Transplant. Proc.* **22**, 2133–2133.
297. Foad, B. S. I., Khullar, S., Freimer, E. H., Kirsner, A. B., and Sheon, R. P. (1975). Cell-mediated immunity in systemic lupus erythematosus: Aalterations with advancing age. *J. Lab. Clin. Med.* **85**, 132.
298. Abe, T., Toguchi, T., Takeuchi, T., Kiyotaki, M., and Homma, M. (1981). Mitogenic responses to lipopolysaccharide by B lymphocytes from patients with systemic lupus erythematosus. *Scand. J. Immunol.* **15**, 475–482.
299. Blasini, A. M., Stekman, I. L., Gonzalez, F., Tositti, M. L., and Rodriguez, M. A. (1994). T lymphocytes from patients with systemic lupus erythematosus show increased response to interleukin-2 after costimulation with OKT3 monoclonal antibody and phorbol esters. *Clin. Immunol. Immunopathol.* **70**, 66–72.
300. Blasini, A. M., Stekman, I. L., Leon-Ponte, M., Caldera, D., and Rodriguez, M. A. (1993). Increased proportion of responders to a murine anti-CD3 monoclonal antibody of the IgG1 class in patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **94**, 423–428.
301. Stekman, I. L., Blasini, A. M., Leon-Ponte, M., Baroja, M. L., Abadi, I., and Rodriguez, M. A. (1991). Enhanced CD3-mediated T lymphocyte proliferation in patients with systemic lupus erythematosus. *Arthritis Rheum.* **34**, 459–467.
302. Kammer, G. M. (1983). Impaired T cell capping and receptor regeneration in active systemic lupus erythematosus: Evidence for a disorder intrinsic to the T lymphocyte. *J. Clin. Invest.* **72**, 1686.
303. Kammer, G. M., Smith, J. A., and Mitchell, R. (1983). Capping of human T cell specific determinants: Kinetics of capping and receptor reexpression and regulation by the cytoskeleton. *J. Immunol.* **130**, 38–44.
304. Kammer, G. M., and Rundolph, S. A. (1984). Regulation of human T lymphocyte surface antigen mobility by a purinergic receptor. *J. Immunol.* **133**, 3298–3302.
305. Kammer, G. M., Boehem, C. A., and Rundolph, S. A. (1986). Role of adenylate cyclase on human T-lymphocyte surface antigen capping. *Cell Immunol.* **101**, 251–258.
306. Hasler, P., Schultz, L. A., and Kammer, G. M. (1990). Defective cAMP-dependent phosphorylation of intact T lymphocytes in active systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **87**, 1978–1982.
307. Phi, N. C., Takats, A., Binh, V. H., Vien, C. V., Gonzalez-Cabello, R., and Gergely, P. (1989). Cyclic AMP level of lymphocytes in patients with systemic lupus erythematosus and its relation to disease activity. *Immunol. Lett.* **23**, 61–64.
308. Kammer, G. M., Khan, I. U., and Malemud, C. J. (1994). Deficient type I protein kinase A isozyme activity in systemic lupus erythematosus T lymphocytes. *J. Clin. Invest.* **94**, 422–430.
309. Travali, S., Koniecki, J., Petralia, S., and Basegra, R. (1990). Oncogenes in growth and development. *FASEB J.* **4**, 3209–3214.
310. Tsokos, G. C. (1987). Immunological aspects in humans. **106**, 82–84.
311. Boumpas, D. T., Popovic, M., Mann, D. L., Balow, J. E., and Tsokos, G. C. (1986). Type C retroviruses of the human T cell leukemia family are not evident in patients with systemic lupus erythematosus. *Arthritis Rheum.* **29**, 185–188.
312. Green, J. A., Dawson, A. A., and Walker, W. (1978). Systemic lupus erythematosus and lymphoma. *Lancet* **2**, 753–756.
313. Agudelo, C. A., Schumacher, H. R., Glick, J. H., and Molina, J. (1981). Non-Hodgkin's lymphoma in systemic lupus erythematosus: Report of 4 cases with ultrastructural studies in 2. *J. Rheumatol.* **8**, 69–78.
314. Stevanovic, G., Cramer, A. D., Taylor, C. R., and Lukes, R. J. (1983). Immunoblastic sarcoma in patients with sytemic lupus erythematosus-like disorders. *Arch. Pathol. Lab. Med.* **107**, 589–592.
315. Milligan, D. W., and Chang, J. G. (1980). Systemic lupus erythematosus and lymphoma. *Acta. Heamatol.* **64**, 109–110.
316. Kassan, S. S., Thomas, T. L., Moutsopoulos, H. M., Hoover, R., Kimberly, R. P., Budman, D. R., Costa, J., Decker, J. L., and Chused, T. M. (1978). Increased risk of lymphoma in sicca syndrome. *Ann. Intern. Med.* **89**, 888–892.
317. Talal, N., and Bunim, J. J. (1964). The development of malignant lymphoma in the course of Sjoren's syndrome. *Am. J. Med.* **36**, 529–540.
318. Lewis, R. B., Castor, C. W., Knisley, R. E., and Bole, G. C. (1976). Frequency of neoplasia in systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* **19**, 1256–1260.
319. Louie, S., and Schwartz, R. S. (1978). Immunodeficiency and the pathogenesis of lymphoma and leukemia. *Semin. Hematol.* **15**, 117–138.
320. Wange, R. L., and Samelson, L. E. (1996). Complex complexes: Signaling at the TCR. *Immunity* **5**, 197–205.
321. Weiss, A., and Littman, D. R. (1994). Signal transduction by lymphocyte antigen receptors. *Cell* **76**, 263–274.

322. Fox, D. A., and McCune, W. J. (1989). Immunologic and clinical effects of cytotoxic drugs used in the treatment of rheumatoid arthritis and systemic lupus erythematosus. *Concepts Immunopathol.* **7**, 20–78.
323. Liossis, S. N., Ding, D. Z., Dennis, G. J., and Tsokos, G. C. (1998). Altered pattern of TCR/CD3-mediated protein-tyrosyl phosphorylation in T cells from patients with systemic lupus erythematosus: Deficient expression of the T-cell receptor zeta chain. *J. Clin. Invest.* **101**, 1448–1457.
324. Vassilopoulos, D., Kovacs, B., and Tsokos, G. C. (1995). TCR/CD3 complex-mediated signal transduction pathway in T cells and cell lines from patients with systemic lupus erythematosus. *J. Immunol.* **155**, 2269–2281.
325. Liossis, S. N., Hoffman, R. W., and Tsokos, G. C. (1998). Abnormal early TCR/CD3-mediated signaling events of a snRNP- autoreactive lupus T cell clone. *Clin. Immunol. Immunopathol.* **88**, 305–310.
326. Tsokos, G. C., and Liossis, S. N. (1998). Lymphocytes, cytokines, inflammation, and immune trafficking. *Curr. Opin. Rheumatol.* **10**, 417–425.
327. Brundula, V., Rivas, L. J., Blasini, A. M., Paris, M., Salazar, S., Stekman, I. L., and Rodriguez, M. A. (1999). Diminished levels of T cell receptor zeta chains in peripheral blood T lymphocytes from patients with systemic lupus erythematosus. *Arthritis Rheum.* **42**, 1908–1916.
328. Kovacs, B., Vassilopoulos, D., Vogelgesang, S. A., and Tsokos, G. C. (1996). Defective CD3-mediated cell death in activated T cells from patients with systemic lupus erythematosus: Role of decreased intracellular TNF-alpha. *Clin. Immunol. Immunopathol.* **81**, 293–302.
329. Jensen, J. P., Hou, D., Ramsburg, M., Taylor, A., Dean, M., and Weissman, A. M. (1992). Organization of the human T cell receptor zeta/eta gene and its genetic linkage to the Fc gamma RII-Fc gamma RIII gene cluster. *J. Immunol.* **148**, 2563–2571.
330. Stacey, M., Barlow, A., and Hulten, M. (1997). Human T-cell receptor zeta chain gene Map position 1q23. 1. *Chromosome Res.* **5**, 279.
331. Weissman, A. M., Hou, D., Orloff, D. G., Modi, W. S., Seuanez, H., O'Brien, S. J., and Klausner, R. D. (1988). Molecular cloning and chromosomal localization of the human T-cell receptor zeta chain: Distinction from the molecular CD3 complex. *Proc. Natl. Acad. Sci. USA* **85**, 9709–9713.
332. Moser, K. L., Gray-McGuire, C., Kelly, J., Asundi, N., Yu, H., Bruner, G. R., Mange, M., Hogue, R., Neas, B. R., and Harley, J. B. (1999). Confirmation of genetic linkage between human systemic lupus erythematosus and chromosome 1q41. *Arthritis Rheum.* **42**, 1902–1907.
333. Gaffney, P. M., Kearns, G. M., Shark, K. B., Ortmann, W. A., Selby, S. A., Malmgren, M. L., Rohlf, K. E., Ockenden, T. C., Messner, R. P., King, R. A., Rich, S. S., and Behrens, T. W. (1998). A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc. Natl. Acad. Sci. USA* **95**, 14875–14879.
334. Shai, R., Quismorio, F. P., Jr., Li, L., Kwon, O. J., Morrison, J., Wallace, D. J., Neuwelt, C. M., Brautbar, C., Gauderman, W. J., and Jacob, C. O. (1999). Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum. Mol. Genet.* **8**, 639–644.
335. Nambiar, M. P., Enyedy, E. J., Warke, V. G., Krishnan, S., Dennis, G., Wong, H. K., Kammer, G. M., and Tsokos, G. C. (2001). T cell signaling abnormalities in systemic lupus erythematosus are associated with increased mutations/polymorphisms and splice variants of T cell receptor zeta chain messenger RNA. *Arthritis Rheum.* **44**, 1336–1350.
336. Caplan, S., and Baniyash, M. (2000). Searching for significance in TCR-cytoskeleton interactions. *Immunol. Today* **21**, 223–228.
337. Nambiar, M. P., Enyedy, E. J., Warke, V. G., Krishnan, S., Dennis, G., Kammer, G. M., and Tsokos, G. C. (2001). Polymorphisms/mutations of TCR-zeta-chain promoter and 3′ untranslated region and selective expression of TCR zeta-chain with an alternatively spliced 3′ untranslated region in patients with systemic lupus erythematosus. *J. Autoimmun.* **16**, 133–142.
338. Reichlin, M. (1999). Autoantibodies to intracellular antigens in systemic lupus erythematosus patients that bind and penetrate cells. In "Lupus: Molecular and Cellular Pathogenesis." (G. M. Kammer and G. C. Tsokos, eds.), pp. 389–398. Humana Press, Clifton, NJ.
339. Juang, Y.-T., Solomou, E. E., Rellahan, B., and Tsokos, G. C. (2002). Phosphorylation and O-linked glycosylation of Elf-1 leads to its translocation to the nucleus and binding to the promoter of the T cell receptor z chain. *J. Immunol.* **168**.
340. Nambiar, M. P., Enyedy, E. J., Fisher, C. U., Krishnan, S., Warke, V. G., Gilliland, W., Oglesby, R., and Tsokos, G. C. (2002). Abnormal expression of various molecular forms, fractions and splice variants of TCR z chain in T cells from patients with systemic lupus erythematosus. *Arthritis Rheum.* **45**.
341. Enyedy, E. J., Nambiar, M. P., Liossis, S. N., Dennis, G., Kammer, G. M., and Tsokos, G. C. (2001). Fc epsilon receptor type I gamma chain replaces the deficient T cell receptor zeta chain in T cells of patients with systemic lupus erythematosus. *Arthritis Rheum.* **44**, 1114–1121.
342. Russell, J. H., Rush, B. J., Abrams, S. I., and Wang, R. (1992). Sensitivity of T cells to anti-CD3-stimulated suicide is independent of functional phenotype. *Eur. J. Immunol.* **22**, 1655–1658.
343. Reichert, T. E., Day, R., Wagner, E. M., and Whiteside, T. L. (1998). Absent or low expression of the zeta chain in T cells at the tumor site correlates with poor survival in patients with oral carcinoma. *Cancer Res.* **58**, 5344–5347.
344. Cardi, G., Heaney, J. A., Schned, A. R., Phillips, D. M., Branda, M. T., and Ernstoff, M. S. (1997). T-cell receptor zeta-chain expression on tumor-infiltrating lymphocytes from renal cell carcinoma [published erratum appears in *Cancer Res.* **57**(21), 4973 (1997)]. *Cancer Res.* **57**, 3517–3519.
345. Shores, E. W., and Love, P. E. (1997). TCR zeta chain in T cell development and selection. *Curr. Opin. Immunol.* **9**, 380–389.
346. Shores, E. W., Tran, T., Grinberg, A., Sommers, C. L., Shen, H., and Love, P. E. (1997). Role of the multiple T cell

receptor (TCR)-zeta chain signaling motifs in selection of the T cell repertoire. *J. Exp. Med.* **185**, 893–900.
347. Noraz, N., Schwarz, K., Steinberg, M., Dardalhon, V., Rebouissou, C., Hipskind, R., Friedrich, W., Yssel, H., Bacon, K., and Taylor, N. (2000). Alternative antigen receptor (TCR) signaling in T cells derived from ZAP-70-deficient patients expressing high levels of Syk. *J. Biol. Chem.* **275**, 15832–15838.
348. Glinski, W., Gershwin, M. E., and Steinberg, A. D. (1976). Fractionation of cells on a discomtinuous Ficoll gradient: Study of subpopulations of human T cells using anti-T-cell antibodies from patients with systemic lupus erythematosus. *J. Clin. Invest.* **57**, 604–614.
349. Simons, K., and Ikonen, E. (1997). Functional rafts in cell membranes. *Nature* **387**, 569–572.
350. Ilangumaran, S., He, H. T., and Hoessli, D. C. (2000). Microdomains in lymphocyte signalling: beyond GPI-anchored proteins. *Immunol. Today* **21**, 2–7.
351. Montixi, C., Langlet, C., Bernard, A. M., Thimonier, J., Dubois, C., Wurbel, M. A., Chauvin, J. P., Pierres, M., and He, H. T. (1998). Engagement of T cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. *EMBO J.* **17**, 5334–5348.
352. Ilangumaran, S., Arni, S., Echten-Deckert, G., Borisch, B., and Hoessli, D. C. (1999). Microdomain-dependent regulation of Lck and Fyn protein-tyrosine kinases in T lymphocyte plasma membranes. *Mol. Biol. Cell* **10**, 891–905.
353. Kosugi, A., Saitoh, S., Noda, S., Yasuda, K., Hayashi, F., Ogata, M., and Hamaoka, T. (1999). Translocation of tyrosine-phosphorylated TCRzeta chain to glycolipid-enriched membrane domains upon T cell activation. *Int. Immunol.* **11**, 1395–1401.
354. Xavier, R. M., Nakamura, M., and Tsunematsu, T. (1994). Isolation and characterization of a human nonspecific suppressor factor from ascitic fluid of systemic lupus erythematosus: Evidence for a human counterpart of the monoclonal nonspecific suppressor factor and relationship to the T cell receptor alpha-chain. *J. Immunol.* **152**, 2624–2632.
355. Demetriou, M., Granovsky, M., Quaggin, S., and Dennis, J. W. (2001). Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation. *Nature* **409**, 733–739.
356. Chui, D., Sellakumar, G., Green, R., Sutton-Smith, M., McQuistan, T., Marek, K., Morris, H., Dell, A., and Marth, J. (2001). Genetic remodeling of protein glycosylation in vivo induces autoimmune disease. *Proc. Natl. Acad. Sci. USA* **98**, 1142–1147.
357. Mandler, R., Birch, R. E., Polmar, S. H., Kammer, G. M., and Rudolph, S. A. (1982). Abnormal adenosine-induced immunosuppression and cAMP metabolism in T lymphocytes of patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **79**, 7542–7546.
358. Kammer, G. M., and Mitchell, E. (1988). Impaired mobility of human T lymphocyte surface molecules during inactive systemic lupus erythematosus: Relationship to a defective cAMP pathway. *Arthritis Rheum.* **31**, 88–98.
359. Laxminarayana, D., Khan, I. U., Mishra, N., Olorenshaw, I., Tasken, K., and Kammer, G. M. (1999). Diminished levels of protein kinase A RI alpha and RI beta transcripts and proteins in systemic lupus erythematosus T lymphocytes. *J. Immunol.* **162**, 5639–5648.
360. Laxminarayana, D., and Kammer, G. M. (2000). Messenger RNA mutations on type I protein kinase A regulatory subunit alpha in T lymphocytes of a subject with systemic lupus erythematosus. *Int. Immunol.* **12**, 1521–1529.
361. Tada, Y., Nagasawa, K., Yamauchi, Y., Tsukamoto, H., and Niho, Y. (1991). A defect in the protein kinase C system in T cells from patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **60**, 220–231.
362. Matache, C., Stefanescu, M., Onu, A., Tanaseanu, S., Matei, I., Frade, R., and Szegli, G. (1999). p56lck activity and expression in peripheral blood lymphocytes from patients with systemic lupus erythematosus. *Autoimmunity* **29**, 111–120.
363. Grolleau, A., Kaplan, M. J., Hanash, S. M., Beretta, L., and Richardson, B. (2000). Impaired translational response and increased protein kinase PKR expression in T cells from lupus patients. *J. Clin. Invest.* **106**, 1561–1568.
364. Park, D. J., Min, H. K., and Rhee, S. G. (1992). Inhibition of CD3-linked phospholipase C by phorbol ester and by cAMP is associated with decreased phosphotyrosine and increased phosphoserine contents of PLC-gamma 1. *J. Biol. Chem.* **267**, 1496–1501.
365. Ghosh, S., May, M. J., and Kopp, E. B. (1998). NF-kappa B and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* **16**, 225–260.
366. Solomou, E. E., Juang, Y. T., and Tsokos, G. C. (2001). Protein kinase c-theta participates in the activation of cyclic amp-responsive element-binding protein and its subsequent binding to the—180 site of the il-2 promoter in normal human t lymphocytes. *J. Immunol.* **166**, 5665–5674.
367. Mishra, N., Khan, I. U., Tsokos, G. C., and Kammer, G. M. (2000). Association of deficient type II protein kinase A activity with aberrant nuclear translocation of the RIIbeta subunit in systemic lupus erythematosus T lymphocytes. *J. Immunol.* **165**, 2830–2840.
368. Williams, B. R. (1999). PKR; a sentinel kinase for cellular stress. *Oncogene* **18**, 6112–6120.
369. Jagus, R., Joshi, B., and Barber, G. N. (1999). PKR, apoptosis and cancer. *Int. J. Biochem. Cell Biol.* **31**, 123–138.
370. Feuerstein, N., Chen, F., Madaio, M., Maldonado, M., and Eisenberg, R. A. (1999). Induction of autoimmunity in a transgenic model of B cell receptor peripheral tolerance: Changes in coreceptors and B cell receptor-induced tyrosine-phosphoproteins. *J. Immunol.* **163**, 5287–5297.
371. Vratsanos, G. S., Jung, S., Park, Y. M., and Craft, J. (2001). CD4(+) T cells from lupus-prone mice are hyperresponsive to T cell receptor engagement with low and high affinity peptide antigens: A model to explain spontaneous T cell activation in lupus. *J. Exp. Med.* **193**, 329–337.
372. Via, C. S., Tsokos, G. C., Bermas, B., Clerici, M., and Shearer, G. M. (1993). T cell-antigen-presenting cell interactions in human systemic lupus erythematosus:

Evidence for heterogeneous expression of multiple defects. *J. Immunol.* **151**, 3914–3922.
373. Tsokos, G. C., Kovacs, B., Sfikakis, P. P., Theocharis, S., Vogelgesang, S., and Via, C. S. (1996). Defective antigen presenting cell function in patients with systemic lupus erythematosus: Role of the B7–1 (CD80) costimulatory molecule. *Arthritis Rheum.* **39**, 600–609.
374. Sfikakis, P. P., Oglesby, R., Sfikakis, P., and Tsokos, G. C. (1996). B7/BB1 provides an important costimulatory signal for CD3-mediated T lymphocyte proliferation in patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **96**, 8–14.
375. Plana, M., Font, J., Vinas, O., Martorell, J., Ingelmo, M., and Vives, J. (1994). Responsiveness of T lymphocytes from systemic lupus erythematosus to signals provided through CD26 antigen. *Clin. Immunol. Immunopathol.* **72**, 227–232.
376. Thomas, R., and Quinn, C. (1996). Functional differentiation of dendritic cells in rheumatoid arthritis: Role of Cd86 in the synovium. *J. Immunol.* **156**, 3074–3086.
377. Balsa, A., Dixey, J., Sansom, D. M., Maddison, P. J., and Hall, N. D. (1996). Differential expression of the costimulatory molecules B7. 1 (CD80) and B7. 2 (CD86) in rheumatoid synovial tissue. *Br. J. Rheumatol.* **35**, 33–37.
378. Folzenlogen, D., Hofer, M. F., Leung, D. Y., Freed, J. H., and Newell, M. K. (1997). Analysis of CD80 and CD86 expression on peripheral blood B lymphocytes reveals increased expression of CD86 in lupus patients. *Clin. Immunol. Immunopathol.* **83**, 199–204.
379. Bijl, M., Horst, G., Limburg, P. C., and Kallenberg, C. G. (2001). Expression of costimulatory molecules on peripheral blood lymphocytes of patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **60**, 523–526.
380. Zhou, T., Weaver, C., Linsley, P. S., and Mountz, J. D. (1994). T cells of staphylococcal enterotoxin B-tolerized autoimmune MRL-lpr/lpr mice require co-stimulation through the B7-CD28/CTL-4 pathway for activation and can be reanergized in vivo by stimulation of the T cell receptor in the absence of this co-stimulatory signal. *Eur. J. Immunol.* **24**, 1019–1025.
381. Singh, R. R., Kumar, V., Ebling, F. M., Southwood, S., Sette, A., Sercarz, E. E., and Hahn, B. H. (1995). T cell determinants from autoantibodies to DNA can upregulate autoimmunity in murine systemic lupus erythematosus. *J. Exp. Med.* **181**, 2017–2027.
382. Nasir, A., Ferbel, B., Salminen, W., Barth, R. K., and Gaspari, A. A. (1994). Exaggerated and persistent cutaneous delayed-type hypersensitivity in transgenic mice whose epidermal keratinocytes constitutively express B7–1 antigen. *J. Clin. Invest.* **94**, 892–898.
383. Williams, I. R., Ort, R. J., and Kuffer, T. S. (1994). Keratinocyte expression of B7–1 in transgenic mice amlifies the primary immune response to cutaneous antigens. *Proc. Natl. Acad. Sci. USA* **91**, 12780–12784.
384. Nickoloff, B. J., Mitra, R. S., Lee, K., Turka, L. A., Green, J., Thompson, C., and Shimizu, Y. (1993). Discordant expression of CD28 ligands, BB-1, and B7 on keratinocytes in vitro and psoriatic cells in vivo. *Am. J. Pathol.* **142**, 1029–1040.
385. Harlan, D. M., Hengartner, H., Huang, M. L., Kang, Y. H., Abe, R., Moreadith, R. W., Pircher, H., Gray, G. S., Ohashi, P. S., and Freeman, G. J. (1994). Mice expressing both B7–1 and viral glycoprotein on pancreatic beta cells along with glycoprotein-specific transgenic T cells develop diabetes due to a breakdown of T-lymphocyte unresponsiveness. *Proc. Natl. Acad. Sci. USA* **91**, 3137–3141.
386. von Herath, M. G., Guerder, S., Lewicki, H., Flavell, R. A., and Oldstone, M. B. A. (1995). Coexpression of B7–1 and viral ("self") transgenes in pancreatic beta cells can break peripheral ignorance and lead to spontaneous autoimmune diabetes. *Immunity* **3**, 727–738.
387. Belmont, H. M., Buyon, J., Giorno, R., and Abramson, S. (1994). Up-regulation of endothelial cell adhesion molecules characterizes disease activity in systemic lupus erythematosus: The Shwartzman phenomenon revisited. *Arthritis Rheum.* **37**, 376–383.
388. Tsokos, G. C. (1995). Lymphocytes, cytokines, inflammation, and immune trafficking. *Curr. Opin. Rheum.* **7**, 376–383.
389. Yung, R., Powers, D., Johnson, K., Amento, E., Carr, D., Laing, T., Yang, J., Chang, S., Hemati, N., and Richardson, B. (1996). Mechanisms of drug-induced lupus. II. T cells overexpressing LFA-1 cause a lupus-like disease in syngeneic mice. *J. Clin. Invest.*
390. Richardson, B., Scheinbart, L., Strahler, J., Gross, L., Hanash, S., and Johnson, M. (1990). Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* **33**, 1665–1673.
391. Richardson, B., Kahn, L., Lovett, E. J., and Hudson, J. (1986). Effect of an inhibitor of DNA methylation of T cells. I 5-azacytidine induces T4 expression of T8+ cells. *J. Immunol.* **137**, 35–39.
392. Richardson, B., Powers, D., Hooper, F., Yung, R. L., and O'Rourke, K. (1994). Lymphocyte function-associated antigen 1 overexpression and T cell autoreactivity. *Arthritis Rheum.* **37**, 1363–1372.
393. Grewal, I. S., Xu, J., and Flavell, R. A. (1996). Impairment of antigen-specific T-cell priming in mice lacking CD40 ligand. *Nature* **378**, 617–620.
394. van Essen, D., Kikutani, H., and Gray, D. (1996). CD40 ligand-tranduced co-stimulation of T cells in the development of helper function. *Nature* **378**, 620–623.
395. Mohan, C., Shi, Y., Laman, J. D., and Datta, S. K. (1995). Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J. Immunol.* **154**, 1470–1480.
396. Koshy, M., Berger, D., and Crow, M. K. (1996). Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J. Clin. Invest.* **98**, 826–837.
397. Desai-Mehta, A., Lu, L., Ramsey-Goldman, R., and Datta, S. K. (1996). Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J. Clin. Invest.* **97**, 2063–2073.
398. Kato, K., Santana-Sahagun, E., Rassenti, L. Z., Weisman, M. H., Tamura, N., Kobayashi, S., Hashimoto, H., and

Kipps, T. J. (1999). The soluble CD40 ligand sCD154 in systemic lupus erythematosus. *J. Clin. Invest* **104**, 947–955.
399. Vakkalanka, R. K., Woo, C., Kirou, K. A., Koshy, M., Berger, D., and Crow, M. K. (1999). Elevated levels and functional capacity of soluble CD40 ligand in systemic lupus erythematosus sera. *Arthritis Rheum.* **42**, 871–881.
400. Hynes, R. O. (1992). Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11–25.
401. Cybulsky, M. I. and Gimbrone, M. A. (1990). Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* **251**, 788–791.
402. Argenbright, L. W., and Barton, R. W. (1992). Interactions of leukocyte integrins with intercellular adhesion molecule 1 in the production of inflammatory vascular injury in vivo. *J. Clin. Invest.* **89**, 259–272.
403. Laffon, A., Garcia, V. R., Humbria, A., Postigo, A. A., Corbi, A. L., and Sanchez, M. F. (1991). Upregulated expression and function of VLA4 fibronectin receptors on human activated T cells in rheumatoid arthritis. *J. Clin. Invest.* **88**, 546–552.
404. Molad, Y., Buyon, J., Anderson, D. C., Abramson, S. B., and Cronstein, B. N. (1994). Intravascular neutrophil activation in systemic lupus erythematosus (SLE): Dissociation between increased expression of CD11b/CD18 and diminished expression of L-selectin on neutrophils from patients with active SLE. *Clin. Immunol. Immunopathol.* **71**, 281–286.
405. Belmont, H. M., Abramson, S. B., and Lie, J. T. (1996). Pathology and pathogenesis of vescular injury in systemic lupus erythematosus: Interactions of inflammatory cells and activated endothelium. *Arthritis Rheum.* **39**, 9–22.
406. Chiorazzi, N. (1987). An overview of cellular immune function in systemic lupus erythematosus. **1**, 23–63.
407. Goto, M., Tanimoto, K., and Horiuchi, Y. (1980). Natural cell mediated cytotoxicity in systemic lupus erythematosus: Suppression by antilymphocyte antibody. *Arthritis Rheum.* **23**, 1274–1281.
408. Hoffman, T. (1980). Natural killer funciton in systemic lupus erythematosus. *Arthritis Rheum.* **23**, 30–35.
409. Oshimi, K., Gonda, N., Sumiya, M., and Kano, S. (1980). Effects of corticosteroids on natural killer cell activity in systemic lupus erythematosus. *Clin. Exp. Immunol.* **40**, 83–88.
410. Jasin, H. E., and Ziff, M. (1975). Immunoglobulin synthesis by peripheral blood cells in systemic lupus erythematosus. *Arthritis Rheum.* **19**, 219–228.
411. Silverman, S. L., and Cathcart, E. S. (1980). Natural killing in systemic lupus erythematosus: Inhibitory effects of serum. *Clin. Immunol. Immunopathol.* **24**, 263.
412. Tsokos, G. C., Rook, A. H., Djeu, J. Y., and Balow, J. E. (1982). Natural killer cells in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **50**, 239–245.
413. Gaspar, M. L., Alvarez-Mon, M., and Gutierrez, C. (1988). Role of interleukin 2 in inducing normalization of natural killer activity in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **49**, 204–214.
414. Struyf, N. J., Snoeck, H. W., Bridts, C. H., DeClerck, L. S., and Stevens, W. J. (1990). Natural killer cell activity in Sjogren's syndrome and systemic lupus erythematosus: Stimulation with interferons and interleukin-2 and correlation with immune complexes. *Ann. Rheum. Dis.* **49**, 690–693.
415. Herberman, R. B., and Ortaldo, J. R. (1981). Natural killer cells: Their role in defense against disease. *Science* **214**, 24–30.
416. Kiessling, R., Petranyi, G., Karre, K., Jondal, M., Tracey, D., and Wigzel, H. (1976). Killer cells: A functional comparison between natural, immune T-cell and antibody-dependent in vitro systems. *J. Exp. Med.* **143**, 772–780.
417. Petersen, J., Rhodes, G., Roudier, J., and Vaughan, J. H. (1990). Altered immune response to glycine-rich sequences of Epstein-Barr nuclear antigen-1 in patients with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum.* **33**, 993–1000.
418. Tosato, G., Steinberg, A. D., Yarchoan, R., Heilman, C. A., Pike, S. E., De Seau, V., and Blaese, R. M. (1984). Abnormally elevated frequency of Epstein-Barr virus-infected B cells in the blood of patients with rheumatoid arthritis. *J. Clin. Invest.* **73**, 1789–1795.
419. Tosato, G., Steinberg, A. D., and Blaese, R. M. (1981). Defective EBV-specific suppressor T-cell function in rheumatoid arthritis. *N. Engl. J. Med.* **305**, 1238–1243.
420. Birx, D. L., Redfield, R. R., and Tosato, G. (1986). Defective regulation of Epstein-Barr virus infection in patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related disorders. *N. Engl. J. Med.* **314**, 874–879.
421. Mellemkjaer, L., Andersen, V., Linet, M. S., Gridley, G., Hoover, R., and Olsen, J. H. (1997). Non-Hodgkin's lymphoma and other cancers among a cohort of patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 761–768.
422. Abu-Shakra, M., Gladman, D. D., and Urowitz, M. B. (1996). Malignancy in systemic lupus erythematosus. *Arthritis Rheum.* **39**, 1050–1054.
423. Maca, R. D., Bonnard, G. D., and Herberman, R. B. (1979). The suppression of mitogen and alloantigen stimulated peripheral blood lymphocytes by cultured huma T lymphocytes. *J. Immunol.* **123**, 246–251.
424. Wright, J. K., Hughes, P., Gelsthorpe, K., Ward, A. M., and Rowell, N. R. (1981). Antibody-dependent and phytohaemagglutinin-induced lymphocyte cytotoxicity in systemic lupus erythematosus. *Ann. Rheum. Dis.* **40**, 11–17.
425. Froelich, C. J., Guiffaut, S., Sosenko, M., and Muth, K. (1989). Deficient interleukin-2-activated killer cell cytotoxicity in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **50**, 132–145.
426. Perlmann, P., and Perlmann, H. (1970). Contactual lysis of antibody-coated chicken erythrocytes by purified lymphocytes. *Cell. Immunol.* **1**, 300–315.
427. Van Boxel, J. A., Stobo, J. D., Paul, W. E., *et al.* (1972). Antibody-dependent lymphoid cell-mediated cytotoxicity: No requirements for thymus derived lymphocytes. *Science* **175**, 194.

428. Fakhri, O., and Hobbs, J. R. (1972). Target cell death eithout added complement after cooperation of 7S antibodies with non-immune lymphocytes. *Nature New Biol.* **235**, 177–178.
429. Moller, G., and Svehag S.-E. (1972). Specificity of lymphocyte-mediated cytotoxicity induced by in vitro antibody-coated target cells. *Cell. Immunol.* **4**, 1–19.
430. Walker, W. S. (1977). Mediation of macrophage cytolytic and phagocytic activities by antibodies of different classes and class-specific Fc-receptors. *J. Immunol.* **119**, 367–373.
431. Gershwin, M. E., Glinski, W., Chused, T., and Steinberg, A. D. (1977). Lymphocyte-dependent antibody-mediated cytolysis of DNA-anti-DNA-coated target cells using human and murine SLE effector populations. *Clin. Immunol. Immunopathol.* **8**, 280–291.
432. Feldman, J. L., Becker, M. J., Moutsopoulos, H., Fye, K., Blackman, M., Epstein, W. V., and Talal, N. (1976). Antibody-dependent cell-mediated cytotoxicity in selected autoimmune diseases. *J. Clin. Invest.* **58**, 173–179.
433. Diaz-Jouanen, E., Bankhurst, A. D., and Williams, R. C., Jr. (1976). Antibody-mediated lymphocytotoxicity in rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum.* **19**, 133–141.
434. Cooper, S. M., Harding, B., Mirick, G. R., Schneider, J., Quismorio, F. P., and Friou, G. J. (1978). Selective decrease in antibody-dependent cell-mediated cytotoxicity in systemic lupus erythematosus and progressive systemic sclerosis. *Clin. Exp. Immunol.* **34**, 235–240.
435. Scheinberg, M. A., and Cathcart, E. S. (1976). Antibody-dependent direct cytotoxicity of human lymphocytes. I. Studies on peripheral blood lymphocytes and sera of patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **24**, 323–327.
436. Schneider, J., Chin, W., Friou, G. J., Cooper, S. M., Harding, B., Hill, R. L., and Quismorio, F. P. (1975). Reduced antibody-dependent cell-mediated cytotoxicity in systemic lupus erythematosus. *Clin. Exp. Immunol.* **20**, 187–192.
437. Stohl, W. (1992). Impaired generation of polyclonal T cell-mediated cytolytic activity despite normal polyclonal T cell proliferation in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **63**, 163–172.
438. Stohl, W. (1995). Impaired polyclonal T cell cytolytic activity: A possible risk factor for systemic lupus erythematosus. *Arthritis Rheum.* **38**, 506–516.
439. Stohl, W., Elliott, J. E., Hamilton, A. S., Deapen, D. M., Mack, T. M., and Horwitz, D. A. (1996). Impaired recovery and cytolytic function of CD56+ T and non-T cells in systemic lupus erythematosus following in vitro polyclonal T cell stimulation: Studies in unselected patients with monozygotic disease-discordant twins. *Arthritis Rheum* **39**, 1840–1851.
440. Stohl, W., Elliott, J. E., Li, L., Podack, E. R., Lynch, D. H., and Jacob, C. O. (1997). Impaired nonrestricted cytolytic activity in systemic lupus erythematosus: Involvement of a pathway independent of Fas, tumor necrosis factor, and extracellular ATP that is associated with little detectable perforin. *Arthritis Rheum.* **40**, 1130–1137.
441. Theofilopoulos, A. N., Prud'Homme, G. J., and Dixon, F. J. (1985). Autoimmune aspects of systemic lupus erythematosus. *Concepts Immunopathol.* **1**, 190–218.
442. Theofilopoulos, A. N., and Dixon, F. J. (1981). Etiopathogenesis of murine SLE. *Immunol. Rev.* **55**, 179–216.
443. Klinman, D. M., Shirai, A., Ishigatsubo, Y., Conover, J., and Steinberg, A. D. (1991). Quantitation of IgM- and IgG-secreting B cells in the peripheral blood of patients with systemic lupus erythematosus. *Arthritis Rheum.* **34**, 1404–1410.
444. Klinman, D. M. (1992). Analysis of B lymphocyte cross-reactivity at the single cell level. *J. Immunol Methods* **152**, 217–225.
445. Klinman, D. M., Higgins, K. W., and Conover, J. (1991). Sequential immunizations with rgp120s from independent isolates of human immunodeficiency virus type 1 induce the preferential expansion of broadly crossreactive B cells. *J. Exp. Med.* **173**, 881–887.
446. Klinman, D. M. (1992). Similarities in B cell repertoire development between autoimmune and aging normal mice. *J. Immunol.* **148**, 1353–1358.
447. Klinman, D. M., Shirai, A., Conover, J., and Steinberg, A. D. (1994). Cross-reactivity of IgG anti-DNA secreting B cells in patients with systemic lupus erythematosus. *Eur. J. Immunol.* **24**, 53–58.
448. Paul, E., Iliev, A. A., Livneh, A., and Diamond, B. (1992). The anti-DNA-associated idiotype 8. 12 is encoded by the V lambda II gene family and maps to the vicinity of L chain CDR1. *J. Immunol.* **149**, 3588–3595.
449. Livneh, A., Or, G., Many, A., Gazit, E., and Diamond, B. (1993). Anti-DNA antibodies secreted by peripheral B cells of lupus patients have both normal and lupus-specific features. *Clin. Immunol. Immunopathol.* **68**, 68–73.
450. Hertzenberg, L. A., Stall, A., Lalor, P. A., Sidman, C., Moore, W., Parks, D., and Herzenberg, L. (1986). The Ly-B1 cell lineage. *Immunol. Rev.* **93**, 81–102.
451. Sidman, C. L., Shultz, L. D., Hardy, R. R., Hayakawa, K., and Herzenberg, L. A. (1986). Production of immunoglobulin isotypes by Ly1 + B cells in viable motheaten and normal mice. *Science* **232**, 1423–1425.
452. Talal, N. (1987). Overview of Sjogren's syndrome. *J. Dent. Res.* **66**, 672–674.
453. Plater-Zyberk, C., Maini, R. N., Lam, K., Kennedy, T. D., and Janossy, G. (1985). A rheumatoid arthritis B cell subset expresses a phenotype similaar to that in chronic leukemia. *Arthritis Rheum.* **28**, 971–976.
454. Youinou, P., Mackenzie, L., Jouquan, J., LeGoff, P., and Lydyard, P. (1987). CD5 positive B cells in patients with RA: PMA mediated ehnancement of detection. *Ann. Rheum. Dis.* **46**, 17–22.
455. Lydyard, P. M., Youinou, R., and Cooke, A. (1987). CD5 positive B cells in rheumadoid arthritis and CLL. *Immunol. Today* **8**, 37–38.
456. Boumsell, K., Bernard, A., Lepage, V., Degos, L., Lemerle, J., and Dausset, J. (1978). Some chronic lymphocytic leukemia cells being surface immunoglobulin share determinants with T cells. *Eur. J. Immunol.* **8**, 900–904.

457. Hayakawa, K., Hardy, R., Parks, D. R., Herzenberg, L. A., and Steinberg, A. D. (1984). Ly1 B cells: Functionally distinct lymphocytes that secreta IgM autoantibodies. *Proc Nat. Acad. Sci. USA* **81**, 2494–2498.
458. Raveche, E. S. (1990). Possible immunoregulatory role for CD5+ B cells. *Clin. Immunol. Immunopathol* **56**, 135–150.
459. Gavalchin, J., and Datta, S. K. (1987). The NZB X SWR model of lupus nephritis. II Autoantibodies deposited in renal lesions show a restricted idiotypic diversity. *J. Immunol.* **138**, 138–148.
460. Casali, P., and Notkins, A. L. (1989). CD5+ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. *Immunol. Today* **10**, 364–368.
461. Smith, H. R., and Olson, R. R. (1990). CD5+ B lymphocytes in systemic lupus erythematosus and rheumatoid arthritis. *J. Rheumatol.* **17**, 833–835.
462. Kazbay, K., and Osterland, C. K. (1990). The frequency of Leu 1+ B cells in autoantibody positive and negative autoimmune diseases and in renatal cord blood. *Clin. Exp. Rheum.* **8**, 231–235.
463. Casali, P., Burastero, S. E., Balow, J. E., and Notkins, A. L. (1989). High-affinity antibodies to ssDNA are produced by CD5- B cells in systemic lupus erythematosus. *J. Immunol.* **143**, 3476–3483.
464. Suzuki, N., Sakane, T., and Engleman, E. G. (1990). Anti-DNA antibodies production by CD5+ and CD5– B cells of patients with systemic lupus erythematosus. *J. Clin. Invest.* **85**, 238–247.
465. Datta, S. K., and Kalled, S. L. (1997). CD40-CD40 ligand interaction in autoimmune disease. *Arthritis Rheum.* **40**, 1735–1745.
466. Grammer, A. C., Bergman, M. C., Miura, Y., Fujita, K., Davis, L. S., and Lipsky, P. E. (1995). The CD40 ligand expressed by human B cells costimulates B cell responses. *J. Immunol.* **154**, 4996–5010.
467. Roth, R., Nakamura, T., and Mamula, M. J. (1996). B7 costimulation and autoantigen specificity enable B cells to activate autoreactive T cells. *J. Immunol.* **157**, 2924–2931.
468. Nies, K. M., and Louie, J. S. (1978). Impaired immunoglobulin synthesis by peripheral blood lymphocytes in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 51–57.
469. Russel, I. J., Conn, D. L., McKenna, C. H., and Stobo, J. D. (1980). Augmentation of immunoglobulin production inconnective tissur disorder. *Clin. Immunol. Immunopathol.* **16**, 221–232.
470. Blaese, R. M., Grayson, J., and Steinberg, A. D. (1980). Increased immunoglobulin-secreting cells in the blood of patients with active systemic lupus erythematosus. *Am. J. Med.* **69**, 345–350.
471. Budman, D. R., Merchant, E. B., Steinberg, A. D., Doft, B., Gershwin, M. E., Lizzo, E., and Reeves, J. P. (1977). Increased spontaneous activity of antibody-forming cells in the peripheral blood in patients with active SLE. *Arthritis Rheum.* **20**, 829–833.
472. Benenson, E. V., and Tsai, E. G. (1990). The rapid cytofluorometric indices of lymphocyte functional activity in rheumatoid arthritis and systemic lupus erythematosus. *Ter. Arkh.* **62**, 22–27.
473. Ginsburg, W. W., Finkelman, F. D., and Lipsky, P. E. (1979). Circulating and pokeweed-mitogen-induced immunoglobulin-secreting cells in systemic lupus erythematosus. *Clin. Exp. Immunol.* **35**, 76–88.
474. Cairns, E., St. Germain, J., and D = Bell, D. A. (1985). The in vitro production of anti-DNA antibody by cultured peripheral blood or tonsillar lymphoid cells from normal donors with SLE. *J. Immunol.* **135**, 3839–3844.
475. Ward, M. M., Dawson, D. V., Kredich, D. W., and Pisetsky, D. S. (1990). Expression of IgM and IgG autoantibodies in pediatric and adult systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **55**, 273–284.
476. Fauci, A. S., and Moutsopoulos, H. M. (1981). Polyclonally triggered B cells in the peripheral blood and bone marrow of normal individuals and in patients with systemic lupus erythematosus and primary Sjogren's syndrome. *Arthritis Rheum*. **24**, 577–583.
477. Bobrove, A. M., and Miller, P. (1977). Depressed *in vitro* B lymphocytesdiffenentiation in systemic lupus erythematosus. *Arthritis Rheum*. **20**, 1326–1333.
478. Clough, J. D., Frank, S. A., and Calabrese, L. H. (1980). Deficiency of T cell-mediated regulation of anti-DNA production in systemic lupus erythematosus. *Arthritis Rheum*. **23**, 24–29.
479. Okudaira, K., Tanimoto, K., Nakarmura, T., and Horiuchi, Y. (1980). Spontaneously enhanced *in vitro* immunoglobulin synthesis by B cells systemic lupus erythematosus. *Clin*. *Immunol*. *Immunopathol*. **16**, 267–278.
480. Tan, P., Pang, G., and Wilson, J. D. (1981). Immunoglobulin production *in vitro* by peripheral blood lymphocytes in systemic lupus erythematosus: Helper T cell defect and B cell hyperreactivity. *Clin*. *Exp*. *Immunol*. **44**, 548–554.
481. Prud'Homme, G. J., Fieser, T. M., Dixon, F. J., and Theofilopoulos, A. N. (1984). B-cell-tropic interleukins in murine systemic lupus erythematosus (SLE). *Immunol*. *Rev*. **78**, 159–183.
482. Humbert, M., and Galanaud, P. (1990). B-lymphocyte hyperreactivity and differentiation factors of T-lymphocytes in systemic lupus erythematosus. *Ann*. *Med*. *Interne*. (*Paris*) **141**, 213–216.
483. Suzuki, N., and Sakane, T. (1989). Induction of excessive B cell proliferation and differentiation by an *in vitro* stimulus in culture in human systemic lupus erythematosus. *J*. *Clin*. *Invest*. **83**, 937–944.
484. Martinez-Cordero, E., Alcocer-Varela, J., and Alarcon-Segovia, D. (1986). Stimulating and differentiation factors for human B lymphocytes in systemic lupus erythematosus. *Clin*. *Exp*. *Immunol*. **65**, 598–604.
485. Veda, Y., Sakane, T., and Tsunematsu, T. (1989). Hyperactivity of activated B cells to B cell growth factor in patients with systemic lupus erythematosus. *J*. *Immunol*. **143**, 3988–3993.
486. Delfraissy, J. F., Wallon, C., Vazquez, A., Dugas, B., Dormont, J., and Galanaud, P. (1986). B cell hyperactivity in systemic lupus erythematosus: Selectively enhanced

responsiveness to a high molecular weight B cell growth factor. *Eur. J. Immunol.* **16**, 1251–1256.
487. Flescher, E., Fossum, D., Ballester, A., Maizel, A., Sharma, S., and Talarl, N. (1990). Characterization of B cell growth in systemic lupus erythematosus: Effects of incombinant 12-KDa B cell growth factor, interleukin 4 and transforming growth Factor-b. *Eur. J. Immunol.* **20**, 2425–2430.
488. Golbus, J., Salata, M., Greenwood, J., Hudson, J., and Richardson, B. C. (1988). Increased immunoglobulin response to gamma-interferon by lymphocytes from patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **46**, 129–140.
489. Pelton, B. K., and Denman, A. M. (1987). Spontaneous production of B cell growth factors by SLE lymphocytes. *Clin. Exp. Immunol.* **67**, 159–166.
490. Warrington, R. J. (1988). B cell differentiation factor production in systemic lupus erythematosus. *J. Rheumatol.* **15**, 54–58.
491. Gaspar, M. L., Alvarez-Mon, M., and Gutierrez, C. (1988). The B-cell activation pathway in human systemic lupus erythematosus: Imbalanced *in vitro* production of lymphokines and association with serum analytical findings. *J. Clin. Immunol.* **8**, 266–274.
492. Kallenberg, C. G., van Dissel-Emiliani, F., Huitema, M. G., Limburg, P. C., and The, T. H. (1988). B-cell proliferation and differentiation in systemic lupus erythematosus and mixed connective tissue disease. *J. Clin. Lab. Immunol.* **26**, 55–61.
493. Schultz, L. A., Kammer, G. M., and Rudolph, S. A. (1988). Characterization of the human T lymphocyte adenosine receptor: Comparison of normal and systemic lupus erythematosus cells. *FASEB. J.* **2**, 244–250.
494. Tanaka, V., Saito, K., Shirakawa, F., Ota, T., *et al.* (1988). Production of B cell-stimulating factors by B cells in patients with systemic lupus erythematosus. *J. Immunol.* **141**, 3043–3049.
495. Uher, F., and Dickler, H. B. (1986). Cooperativity between B lymphocytes membrane molecules: Independent ligand occupancy and cross-linking of antigen receptors and Fc gamma receptors down-regulate B lymphocyte function. *J. Immunol.* **137**, 3124–3129.
496. Uher, F., and Dickler, H. B. (1986). Independent ligandoccupancy and cross-linking of surface Ig and Fc gamma receptors downregulates B-lymphocytes function: Evaluation in various B-lymphocyte populations. *Mol. Immunol.* **23**, 1177–1181.
497. Salata, M., Golbus, J., and Richardson, B. C. (1988). Diminished response to an inhibitory signal in lymphocytes from patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **71**, 439–444.
498. Ramirez, F., Searles, R. P., and Williams, R. C., Jr. (1987). Effects of immunoglobulin G from patients with systemic lupus erythematosus on human B cell function. *Clin. Immunol. Immunopathol.* **44**, 219–234.
499. Liossis, S. N., Kovacs, B., Dennis, G., Kammer, G. M., and Tsokos, G. C. (1996). B cells from patients with systemic lupus erythematosus display abnormal antigen receptor-mediated early signal transduction events. *J. Clin. Invest.* **98**, 2549–2557.
500. Cambier, J. C., Pleiman, C. M., and Clark, M. R. (1994). Signal transduction by the B cell antigen receptor and its coreceptors. *Annu. Rev. Immunol.* **12**, 457–486.
501. O'Keefe, T. L., Williams, G. T., Davies, S. L., and Neuberger, M. S. (1996). Hyperresponsive B cells in CD22-deficient mice. *Science* **274**, 798–801.
502. O'Keefe, T. L., Williams, G. T., Batista, F. D., and Neuberger, M. S. (1999). Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. *J. Exp. Med.* **189**, 1307–1313.
503. Liossis, S. N. C., Dimopoulos, M. A., and Sfikakis, P. P. (1999). Expression of the B cell inhibitory molecule CD22 in patients with systemic lupus erythematosus. **42**, S55.
504. Frank, M. M., Hamburger, M. I., Lawley, T. J., *et al.* (1979). Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. *N. Engl. J. Med.* **300**, 518.
505. Takai, T., Ono, M., Hikida, M., Ohmori, H., and Ravetch, J. V. (1996). Augmented humoral and anaphylactic responses in Fc gamma RII-deficient mice. *Nature* **379**, 346–349.
506. Chan, V. W., Meng, F., Soriano, P., DeFranco, A. L., and Lowell, C. A. (1997). Characterization of the B lymphocyte populations in Lyn-deficient mice and the role of Lyn in signal initiation and down-regulation. *Immunity* **7**, 69–81.
507. Liossis, S. N., Solomou, E. E., Dimopoulos, M. A., Panayiotidis, P., Mavrikakis, M. M., and Sfikakis, P. P. (2001). B-cell kinase lyn deficiency in patients with systemic lupus erythematosus. *J. Invest. Med.* **49**, 157–165.
508. Huck, S., Le Corre, R., Youinou, P., and Zouali, M. (2001). Expression of B cell receptor-associated signaling molecules in human lupus. *Autoimmunity* **33**, 213–224.
509. Ashman, R. E., White, R. H., Wiesenhutter, C., Cantor, Y., Lasarow, E., Liebling, M., and Talal, N. (1982). Panhypogammaglobulinemia in systemic lupus erythematosus *in vivo* demonstration of multiple cellular defects. *J. Allergy Clin. Immunol.* **70**, 465–473.
510. Sussman, G. L., Rivera, V. J., and Kohler, P. F. (1983). Transition from systemic lupus erythematosus to common variable hypogammaglobulinemia. *Ann. Intern. Med.* **99**, 32–35.
511. Epstein, R. J., Ogler, R. F., and Gatenby, P. A. (1984). Lupus erythematosus and panhypogammaglobulinemia. *Ann. Intern. Med.* **100**, 162–163.
512. Slepian, I. K., Schwarts, S. A., Weiss, J. J., Roth, S. L., and Mathews, K. P. (1984). Immunodeficiency with hyper IgM after systemic lupus erythematosus. *J. Allergy. Clin. Immunol.* **73**, 846–857.
513. Woo, P., Pereira, R. S., and Lever, A. M. L. (1984). Persistent immunoglobulin defieiency after prednisolone and antiepileptic therapy in a C2 deficiency patient with lupus-loke syndrome. *J. Rheumatol.* **11**, 828–831.
514. Goldstein, R., Izaguirre, C., Smith, C. D., Mierins, E., and Karch, J. (1985). Systemic lupus erythematosus

and common variable panhypogammaglobulinemia: A patient with absence of circulating B cells. *Arthritis Rheum*. **28**, 100–103.
515. Stein, A., Winkelstein, A., and Agarwal, A. (1985). Concurrent systemic lupus erythematosus and common varibale hypogammaglobulinemia. *Arthritis Rheum*. **28**, 462–465.
516. Tsokos, G. C., Smith, P. L., and Balow, J. E. (1986). Development of hypogammaglobulinemia in a patient with systemic lupus erythematosus. *Am. J. Med*. **81**, 1081–1084.
517. Cronin, M. E., Balow, J. E., and Tsokos, G. C. (1989). Immunoglobulin deficiency in patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol*. **7**, 359–364.
518. Birmingham, D. J. (1995). Erythrocyte complement receptors. *Crit. Rev. Immunol*. **15**, 133–154.
519. Kazatchkine, M. D., and Fearon, D. T. (1990). Deficiencies of human C3 complement receptors type 1 (CR1, CD35) and type 2 (CR2, CD21). *Immunodefic. Rev*. **2**, 17–41.
520. Bartholomew, W. R., and Shanahan, T. C. (1990). Complement components and receptors: Deficiencies and disease associations. *Immunol. Ser*. **52**, 33–51.
521. Iida, K., Mornaghi, R., and Nussenzweig, V. (1982). Complement receptor (CR1) deficiency in erythrocytes from patients with systemic lupus erythematosus. *J. Exp. Med*. **155**, 1427–1438.
522. Wilson, J. F., Wong, W. W., Schur, P. H., and Fearon, D. T. (1982). Mode of inheritance of decreased C3b receptors on erythrocytes of patients with systemic lupus erythematosus. *N. Engl. J. Med*. **307**, 981–986.
523. Cornillet, P., Gredy, P., Pennaforte, J. L., Meyer, O., Kazatchkine, M. D., and Cohen, J. H. (1992). Increased frequency of the long (S) allotype of CR1 (the C3b/C4b receptor, CD35) in patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **89**, 22–25.
524. Cohen, J. H., Lutz, H. U., Pennaforte, J. L., Bouchard, A., and Kazatchkine, M. D. (1992). Peripheral catabolism of CR1 (the C3b receptor, CD35) on erythrocytes from healthy individuals and patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol*. **87**, 422–428.
525. Moulds, J. M., Reveille, J. D., and Arnett, F. C. (1996). Structural polymorphisms of complement receptor 1 (CR1) in systemic lupus erythematosus (SLE) patients and normal controls of three ethnic groups. *Clin. Exp. Immunol*. **105**, 302–305.
526. Kumar, A., Sinha, S., Khandekar, P. S., Banerjee, K., and Srivastava, L. M. (1995). Hind III genomic polymorphism of the C3b receptor (CR1) in patients with SLE: Low erythrocyte CR1 expression is an acquired phenomenon. *Immunol. Cell Biol*. **73**, 457–462.
527. Satoh, H., Yokota, E., Tokiyama, K., Kawaguchi, T., and Niho, Y. (1991). Distribution of the HindIII restriction fragment length polymorphism among patients with systemic lupus erythematosus with different concentrations of CR1. *Ann. Rheum. Dis*. **50**, 765–768.
528. Tebib, J. G., Martinez, C., Granados, J., Alarcon-Segovia, D., and Schur, P. H. (1989). The frequency of complement receptor type 1 (CR1) gene polymorphisms in nine families with multiple cases of systemic lupus erythematosus. *Arthritis Rheum*. **32**, 1465–1469.
529. Cohen, J. H., Caudwell, V., Levi-Strauss, M., Bourgeois, P., and Kazatchkine, M. D. (1989). Genetic analysis of CR1 expression on erythrocytes of patients with systemic lupus erythematosus. *Arthritis Rheum*. **32**, 393–397.
530. Mitchell, J. A., and Sim, E. (1989). Size polymorphism of the erythrocyte complement receptor type 1 (CR1) in systemic lupus erythematosus induced by hydralazine. *Complement Inflamm*. **6**, 88–93.
531. Wilson, J. G., Wong, W. W., Murphy, E. E. 3d, Schur, P. H., and Fearon, D. T. (1987). Deficiency of the C3b/C4b receptor (CR1) of erythrocytes in systemic lupus erythematosus: Analysis of the stability of the defect and of a restriction fragment length polymorphism of the CR1 gene. *J. Immunol*. **138**, 2708–2710.
532. Walport, M. J., Ross, G. D., Mackworth-Young, C., Watson, J. V., Hogg, N., and Lachmann, P. J. (1985). Family studies of erythrocyte complement receptor type 1 levels: Reduced levels in patients with SLE are acquired, not inherited. *Clin. Exp. Immunol*. **59**, 547–554.
533. Wilson, J. G., Jack, R. M., Wong, W. W., Schur, P. H., and Fearon, D. T. (1985). Autoantibody to the C3b/C4b receptor and absence of this receptor from erythrocytes of a patient with systemic lupus erythematosus. *J. Clin. Invest*. **76**, 182–190.
534. Cook, J. M., Kazatchkine, M. D., Bourgeois, P., Mignon, F., Mery, J. P., and Kahn, M. F. (1986). Anti-C3b-receptor (CR1) antibodies in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **38**, 135–138.
535. Corvetta, A., Pomponio, G., Bencivenga, R., Luchetti, M. M., Spycher, M., Spaeth, P. J., and Danieli, G. (1991). Low number of complement C3b/C4b receptors (CR1) on erythrocytes from patients with essential mixed cryoglobulinemia, systemic lupus erythematosus and rheumatoid arthritis: Relationship with disease activity, anticardiolipin antibodies, complement activation and therapy. *J. Rheumatol*. **18**, 1021–1025.
536. Aguado, M. T., Lambris, J. D., Tsokos, G. C., Burger, R. Z., Bitter-Suermann, D., Tamerius, J. D., Dixon, F. J., and Theofilopoulos, A. N. (1985). Monoclonal antibodies against complement 3 neoantigens for detection of immune complexes and complement activation: Relationship between immune complex levels, state of C3 and numbers of receptors for C3b. *J. Clin. Invest*. **76**, 1418–1426.
537. Ross, G. D., Yount, W. J., Walport, M. J., Winfield, J. B., Parker, C. J., Fuller, C. R., Taylor, R. P., Myones, B. L., and Lachmann, P. J. (1985). Disease-associated loss of erythrocyte complement receptors (CR1, C3b receptors) in patients with systemic lupus erythematosus and other diseases involving autoantibodies and/or complement activation. *J. Immunol*. **135**, 2005–2014.
538. Thomsen, B. S., Nielsen, H., and Andersen, V. (1987). Erythrocyte CR1 (C3b/C4b receptor) levels and disease activity in patients with SLE. *Scand. J. Rheumatol*. **16**, 339–346.
539. Moldenhauer, F., David, J., Fielder, A. H., Lachmann, P. J., and Walport, M. J. (1987). Inherited deficiency of

erythrocyte complement receptor type 1 does not cause susceptibility to systemic lupus erythematosus. *Arthritis Rheum*. **30**, 961–966.
540. Fyfe, A., Holme, E. R., Zoma, A., and Whaley, K. (1987). C3b receptor (CR1) expression on the polymorphonuclear leukocytes from patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **67**, 300–308.
541. Tausk, F., Harpster, E., and Gigli, I. (1990). The expression of C3b receptor in the differentiation of discoid lupus erythematosus and systemic lupus erythematosus. *Arthritis Rheum*. **33**, 888–892.
542. Yoon, S. H., and Fearon, D. T. (1985). Characterization of a soluble form of the C3b/C4b receptor (CR1) in human plasma. *J. Immunol*. **134**, 3332–3338.
543. Pascual, M., Steiger, G., Sadallah, S., Paccaud, J. P., Carpentier, J. L., James, R., and Schifferli, J. A. (1994). Identification of membrane-bound CR1 (CD35) in human urine: Evidence for its release by glomerular podocytes. *J. Exp. Med*. **179**, 889–899.
544. Wilson, J. G., Ratnoff, W. D., Schur, P. H., and Fearon, D. T. (1986). Decreased expression of the C3b/C4b receptor (CR1) and the C3d receptor (CR2) on B lymphocytes and of CR1 on neutrophils of patients with systemic lupus erythematosus. *Arthritis Rheum*. **29**, 739–747.
545. Levy, E., Ambrus, J., Kahl, L., Molina, H., Tung, K., and Holers, V. M. (1992). T lymphocyte expression of complement receptor 2 (CR2/CD21): A role in adhesive cell-cell interactions and dysregulation in a patient with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol*. **90**, 235–244.
546. Marquart, H. V., Svendsen, A., Rasmussen, J. M., Nielsen, C. H., Junker, P., Svehag, S. E., and Leslie, R. G. (1995). Complement receptor expression and activation of the complement cascade on B lymphocytes from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol*. **101**, 60–65.
547. Takahashi, K., Kozono, Y., Waldschmidt, T. J., Berthiaume, D., Quigg, R. J., Barron, A., and Holers, V. M. (1997). Mouse complement receptors type 1 (CR1; CD35) and type 2 (CR2; CD21): Expression on normal B cells subpopulations and decreased levels during the development of autoimmunity in MRL/lpr mice. *J. Immuol*. **159**, 1557–1569.
548. Schur, P. H. (1983). Complement and lupus erythematosus. *Arthritis Rheum*. **25**, 793–798.
549. Schur, P. H. (1983). Complement studies of sera and other biologic fluids. *Hum. Pathol*. **14**, 338–342.
550. Perrin, L. H., Lambert, P. H., and Miescher, P. A. (1975). Complement breakdown products in plasma from patients with systemic lupus erythematosus and patients with membranoproliferative or other glomerulonephritis. *J. Clin. Invest*. **54**, 165–176.
551. Charlesworth, J. A., Gwyn-Williams, D., Sherington, E. Z., Lachmann, P., and Peters, D. K. (1974). Metabolic studies of the third component of complement and glycin-rich beta-gluco-protein in patients with hypocomplementemia. *J. Clin. Invest*. **53**, 1578–1587.
552. Hiramatsu, M., and Tsokos, G. C. (1988). Epstein-Barr virus transformed B cell lines derived from patients with systemic lupus erythematosus produce a nephritic factor of the classical complement pathway. *Clin. Immunol. Immunopathol*. **46**, 91–99.
553. Hiramatsu, M., and Tsokos, G. C. (1988). A factor activating complement via the alternative pathway in the supernatants of B cell lines transformed by Epstein-Barr virus and in sera obtained from patients with systemic lupus erythematosus. *Int. Arch. Allergy Appl. Immunol*. **86**, 209–214.
554. Faried, H. F., Tachibana, T., and Okuda, T. (1993). The secretion of the third component of complement (C3) by human polymorphonuclear leucocytes from both normal and systemic lupus erythematosus cases. *Scand. J. Immunol*. **37**, 19–28.
555. Tsokos, G. C., Berger, M., and Balow, J. E. (1984). Modulation of human B-cell immunoglobulin secretion by the C3b component of the complement. *J. Immunol*. **132**, 622–626.
556. Melchers, F., Erdei, A., Schulz, T., and Derich, M. (1985). Growth control of activated synchronized murine B cells by the C3b fragment of human complement. *Nature* **318**, 264–••.
557. Tsokos, G. C., Lambris, J. D., Finkelman, F. D., Anastassiou, E. D., and June, C. H. (1990). Monovalent ligands of complement receptor 2 inhibit whereas polyvalent ligands enhance anti-Ig-induced human B cell intracytoplasmic free calcium concentration. *J. Immunol*. **144**, 1640–1645.
558. Dempsey, P. W., Allison, M. E., Akkaraju, S., Goodnow, C. C., and Fearon, D. T. (1996). C3d of complement as a molecular adjuvant: Bridging innate and acquired immunity. *Science* **271**, 348–350.
559. Fearon, D. T., and Locksley, R. M. (1996). The instructive role of innate immunity in the acquired immune response. *Science* **272**, 50–53.
560. Yamane, K., Kono, I., Kabashima, T., Sakurai, T., and Kashiwagi, H. (1986). Monocyte-mediated suppression of T lymphocyte blastogenesis and its reversal by deoxyguanosine: Defects in patients with systemic lupus erythematosus. *Int. Arch. Allergy Appl. Immunol*. **80**, 132–138.
561. Vaux, D. L. (1993). Toward an understanding of the molecular mechanisms of physiological cell death. *Proc. Natl. Acad. Sci. USA* **90**, 786–789.
562. Orrenius, S., McCabe, M. J. J., and Nicotera, P. (1992). Ca(2+)-dependent mechanisms of cytotoxicity and programmed cell death. *Toxicol. Lett* **64–65**, 357–364.
563. Orrenius, S., McConkey, D. J., and Nicotera, P. (1991). Role of calcium in toxic and programmed cell death. *Adv. Exp. Med. Biol*. **283**, 419–425.
564. Zhivotovsky, B., Nicotera, P., Bellomo, G., Hanson, K., and Orrenius, S. (1993). Ca2+ and endonuclease activation in radiation-induced lymphoid cell death. *Exp. Cell Res*. **207**, 163–170.
565. Bissonnette, R. P., Echeverri, F., Mahboubi, A., and Green, D. R. (1992). Apoptotic cell death induced by c-myc is inhibited by bcl-2. *Nature* **359**, 552–554.
566. Itoh, N., and Nagata, S. (1993). A novel protein domain required for apoptosis. Mutational analysis of human fas antigen. *J. Biol. Chem*. **268**, 10932–10937.

567. Watanabe-Fukunaga, R., Brannan, C. I., Itoh, N., Yonehara, S., Copeland, N. G., Jenkins, N. A., and Nagata, S. (1992). The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. *J. Immunol.* **148**, 1274–1279.
568. Cheng, J., Liu, C., Koopman, W. J., and Mountz, J. D. (1995). Characterization of human Fas gene: Exon/intron organization and promoter region. *J. Immunol.* **154**, 1239–1245.
569. Latinis, k. M., Carr, L. L., Peterson, E. J., Norian, L. A., Eliason, S. L., and Koretzky, G. A. (1997). Regulation of CD95 (Fas) ligand expression by TCR-mediated signaling events. *J. Immunol.* **158**, 4602–4611.
570. Kovacs, B., and Tsokos, G. C. (1995). Cross-linking of the Fas/APO-1 antigen suppresses the CD3-mediated signal transduction events in human T lymphocytes. *J. Immunol.* 5543–5549.
571. Adachi, M., Watanabe-Fukunaga, R., and Nagata, S. (1993). Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. *Proc. Natl. Acad. Sci. USA* **90**, 1756–1760.
572. Watanabe-Fukunaga, R., Brannan, C. I., Copeland, N. G., Jenkins, N. A., and Nagata, S. (1992). Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* **356**, 314–317.
573. Wu, J., Zhou, T., He, J., and Mountz, J. D. (1993). Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene. *J. Exp. Med.* **178**, 461–468.
574. Wu, J., Zhou, T., Zhang, J., He, J., Gause, W. C., and Mountz, J. D. (1994). Correction of accelerated autoimmune disease by early replacement of the mutated lpr gene with the normal Fas apoptosis gene in the T cells of transgenic MRL-lpr/lpr mice. *Proc. Natl. Acad. Sci. USA* **91**, 2344–2348.
575. Takahashi, T., Tanaka, M., Brannan, C. I., Jenkins, N. A., Copeland, N. G., Suda, T., and Nagata, S. (1994). Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* **76**, 969–976.
576. Mysler, E., Bini, P., Ramos, P., Friedman, S. M., Krammer, P. H., and Elkon, K. B. (1994). The apoptosis-1/Fas protein in human systemic lupus erythematosus. *J. Clin. Invest.* **93**, 1029–1034.
577. Ohsako, S., Hara, M., Harigai, M., Fukasawa, C., and Kashiwazaki, S. (1994). Expression and function of Fas antigen and bcl-2 in human systemic lupus erythematosus lymphocytes. *Clin. Immunol. Immunopathol.* **73**, 109–114.
578. Emlen, W., Niebur, J., and Kadera, R. (1994). Accelerated *in vitro* apoptosis of lymphocytes from patients with systemic lupus erythematosus. *J. Immunol.* **152**, 3685–3692.
579. Aringer, M., Wintersberger, W., Steiner, C. W., Kiener, H., Presterl, E., Jaeger, U., Smolen, J. S., and Graninger, W. B. (1994). High levels of bcl-2 protein in circulating T lymphocytes, but not B lymphocytes, of patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 1423–1430.
580. Lorenz, H. M., Grunke, M., Hieronymus, T., Herrmann, M., Kuhnel, A., Manger, B., and Kalden, J. R. (1997). *In vitro* apoptosis and expression of apoptosis-related molecules in lymphocytes from patients with systemic lupus erythematosus and other autoimmune diseases. *Arthritis Rheum.* **40**, 306–317.
581. Kovacs, B., Liossis, S. N. C., Dennis, G. J., and Tsokos, G. C. (1997). Increased expression of functional Fas-ligand in activated T cells from patients with systemic lupus erythematosus. *Autoimmunity*.
582. Mohan, C., Adams, S., Stanik, V., and Datta, S. K. (1993). Nucleosome: A major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J. Exp. Med.* **177**, 1367–1381.
583. Casciola-Rosen, L. A., Anhalt, G., and Rosen, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317–1330.
584. Cheng, J., Zhou, T., Liu, C., Shapiro, J. P., Brauer, M. J., Kiefer, M. C., Barr, P. J., and Mountz, J. D. (1994). Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. *Science* **263**, 1759–1762.
585. Knipping, E., Krammer, P. H., Onel, K. B., Lehman, T. J., Myster, E., and Elkon, K. B. (1995). Levels of soluble Fas/APO-1/CD95 in systemic lupus erythematosus and juvenile rhematoid arthritis. *Arthritis Rheum.* **38**, 1735–1737.
586. Goel, N., Ulrich, D. T., StClair, E. W., Fleming, J. A., Lynch, D. H., and Seldin, M. F. (1995). Lack of correlation between serum soluble Fas/APO-1 levels and autoimmune disease. *Arthritis Rheum.* **38**, 1738–1743.
587. Nozawa, K., Kayagaki, N., TokanoY., Yagita, H., Okumura, K., and Hasimoto, H. (1997). Soluble Fas (APO-1, CD95) and soluble Fas lignad in rheumatic diseases. *Arthritis Rheum.* **40**, 1126–1129.
588. Mysler, E., Bini, P., Drappa, J., Ramos, P., Friedman, S. M., Krammer, P. H., and Elkon, K. B. (1994). The apoptosis-1/Fas protein in human systemic lupus erythematosus. *J. Clin. Invest.* **93**, 1029–1034.
589. Le Deist, F., Emile, J. F., Rieux-Laucat, F., Benkerrou, M., Roberts, I., Brousse, N., and Fischer, A. (1996). Clinical, immunological, and pathological cosequences of Fas-deficient conditions. *Lancet* **348**, 719–723.
590. Wu, J., Wilson, J., He, J., Xiang, L., Schur, P. H., and Mountz, J. D. (1996). Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. *J. Clin. Invest.* **98**, 1107–1113.
591. Kovacs, B., Szentendrei, T., Bednarek, J. M., Pierson, M. C., Mountz, J. D., Vogelgesang, S. A., and Tsokos, G. C. (1997). Persistent expression of a soluble form of Fas/APO1 in continuously activated T cells from a patient with SLE. *Clin. Exp. Rheumatol.* **15**, 19–23.
592. Banki, K., Hutter, E., Gonchoroff, N. J., and Perl, A. (1999). Elevation of mitochondrial transmembrane potential and reactive oxygen intermediate levels are early events and occur independently from activation of caspases in Fas signaling. *J. Immunol.* **162**, 1466–1479.

593. Puskas, F., Gergely, P., Jr., Banki, K., and Perl, A. (2000). Stimulation of the pentose phosphate pathway and glutathione levels by dehydroascorbate, the oxidized form of vitamin C. *FASEB J*. **14**, 1352–1361.
594. Banki, K., Hutter, E., Colombo, E., Gonchoroff, N. J., and Perl, A. (1996). Glutathione levels and sensitivity to apoptosis are regulated by changes in transaldolase expression. *J. Biol. Chem*. **271**, 32994–33001.
595. Gergely, Jr. P., Grossman, C., Niland, B., Puskas, F., Neupane, H., Allam, F., Banki, K., and Perl, A. (2001). Mitochondrial hyperpolarization and ATP depletion in patients with systemic lupus erythematosus. *Arthritis Rheum*. **47**.
596. Le Moine, O., Louis, H., Stordeur, P., Collet, J. M., Goldman, M., and Deviere, J. (1997). Role of reactive oxygen intermediates in interleukin 10 release after cold liver ischemia and reperfusion in mice. *Gastroenterology* **113**, 1701–1706.
597. Georgescu, L., Vakkalanka, R. K., Elkon, K. B., and Crow, M. K. (1997). Interleukin-10 promotes activation-induced cell death of SLE lymphocytes mediated by Fas ligand. *J. Clin. Invest*. **100**, 2622–2633.
598. Oates, J. C., Christensen, E. F., Reilly, C. M., Self, S. E., and Gilkeson, G. S. (1999). Prospective measure of serum 3-nitrotyrosine levels in systemic lupus erythematosus: Correlation with disease activity. *Proc. Assoc. Am. Phys.* **111**, 611–621.
599. Oates, J. C., Ruiz, P., Alexander, A., Pippen, A. M., and Gilkeson, G. S. (1997). Effect of late modulation of nitric oxide production on murine lupus. *Clin. Immunol. Immunopathol*. **83**, 86–92.
600. Ting, J. P. Y., and Ranney, D. F. (1980). Selective suppression of the murine autologous mixed lymphocyte reaction by physiolofy concentrations of hydrocortisone. Effects on cell surface Ia antigens. *Cell. Immunol*. **53**, 138.
601. Orson, F. M., Grayson, J., Pike, S., and Blasese, R. M. (1983). T cell-replacing factor for glucocorticosteroid-induced immunglobulin production: A unique steroid-dependent cytokine. *J. Exp. Med*. **158**, 1473.
602. Nambiar, M. P., Enyedy, E. J., Fisher, C. U., Warke, V. G., Juang, Y. T., and Tsokos, G. C. (2001). Dexamethasone modulates TCR zeta chain expression and antigen receptor-mediated early signaling events in human T lymphocytes. *Cell. Immunol*. **208**, 62–71.
603. Nambiar, M. P., Enyedy, E. J., Fisher, C. U., Warke, V. G., and Tsokos, G. C. (2001). High dose of dexamethasone upregulates TCR/CD3-induced calcium response independent of TCR zeta chain expression in human T lymphocytes. *J. Cell Biochem*. **83**, 401–413.
604. Boumpas, D. T., Anastassiou, E. D., Older, S. A., Tsokos, G. C., Nelson, D. L., and Balow, J. E. (1991). Dexamethasone inhibits human interleukin 2 but not interleukin 2 receptor gene expression *in vitro* at the level of nuclear transcription. *J. Clin. Invest*. **87**, 1739–1747.
605. McCune, W. J., Golbus, J., Zeldes, W., Bohlke, P., Dunne, R., and Fox, D. A. (1988). Clinical and immunologic effects of monthly administration of intravenous cyclophosphamide in severe systemic lupus erythematosus. *N. Engl. J. Med*. **318**, 1423–1431.
606. Balow, J. E., Austin, H. A., and Tsokos, G. C. (1984). Plasmapheresis: Therapy in immunologically mediated rheumatic and renal diseases. *Clin. Immunol. Rev*. **3**, 235–272.
607. Tanay, A., Schiffman, G., and Strober, S. (1981). Effect of total lymphoid irradiation on levels of serum autoantibodies in systemic lupus erythematosus and in rheumatoid arthritis. *Arthritis Rheum*. **29**, 26–31.
608. Chagnac, A., Kinberd, B. A., Farinas, M. C., Strober, S., Sibley, R. K., Hoppe, R., and Meyers, B. D. (1989). Outcome of the acute glomerular injury in proliferative lupus nephritis. *J. Clin. Invest*. **84**, 922–930.
609. Strober, S., Field, E., Hoppe, R. T., Kotzin, B. L., Shemesh, O., Engleman, E. G., Ross, J. C., and Myers, B. D. (1985). Treatment of intractable lupus nephritis with total lymphoid irradiation. *Ann. Intern. Med*. **102**, 450–458.
610. Solovera, J. J., Farinas, M. C., and Strober, S. (1988). Changes in B lymphocute function in rheumatoid arthritis and lupus nephritis after total lymphoid irradiation. *Arthritis Rheum*. **31**, 1481–1491.
611. Farinas, M., Stall, A. M., Solovera, J. J., Tarlinton, D. M., Herzenberg, L. A., and Strobers, S. (1990). Ly-1 B cells and disease activity in (New Zealand Black X New Zealand White) F1 mice: Effect of total irradiation. *Arthritis Rheum*. **33**, 553–562.
612. Farinas, M. C., Adkins, B., Stall, A. M., Weissman, I., and Strober, S. (1990). B cell infiltration of the thymic medulla in New Zealand Black, New Zealand white, and (New Zealand Black x New Zealand White) F1 mice: Effect of total lymphoid irradiation. *Arthritis* and *Rheum*. **33**, 702–110.
613. Weinstein, A. (1980). Drug-induced systemic lupus erythematosus. *Prog. Clin. Immunol*. **4**, 1–21.
614. Richardson, B. (1986). Effect of an inhibitor of DNA methylation on T cells II, 5-azacytidine induces self-reactivity in antigen-specific T4+ cells. *Hum. Immunol*. **17**, 456–470.
615. Stuart, R. K., and Hamilton, J. A. (1980). Hydralazine-pyrimidine interactions may explain hydralazine-induced lupus erythematosus. *Science* **208**, 402–404.
616. Thomas, T. J., and Messner, R. P. (1986). Effect of lupus-inducing drugs on the B to Z transition of synthetic DNA. *Arthritis Rheum*. **29**, 638–645.
617. Cornacchia, E., Golbus, J., Maybaum, J., Strahler, J., Hanash, S., and Richardson, B. (1988). Hydroclazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. *J. Immunol*. **140**, 2197–2200.
618. Lieberman, M. W., Beach, L. R., and Palmiter, R. D. (1983). Ultraviolet radiation-induced metallothionein-I gene activation is associated with extensive DNA demethylation. *Cell* **35**, 207–214.
619. Doerfler, W. (1983). DNA methylation and gene activity. *Annu. Rev. Biochem*. **52**, 93–124.
620. Yang, J., Deng, C., Hemati, N., Hanash, S. M., and Richardson, B. C. (1997). Effect of mitogenic stimulation and DNA methylation on human T cell DNA methyltransferase expression and activity. *J. Immunol*. **159**, 1303–1309.

621. Deng, C., Yang, J., Scott, J., Hanash, S., and Richardson, B. C. (1998). Role of the ras-MAPK signaling pathway in the DNA methyltransferase response to DNA hypomethylation. *Biol. Chem.* **379**, 1113–1120.
622. Deng, C., Kaplan, M. J., Yang, J., Ray, D., Zhang, Z., McCune, W. J., Hanash, S. M., and Richardson, B. C. (2001). Decreased Ras-mitogen-activated protein kinase signaling may cause DNA hypomethylation in T lymphocytes from lupus patients. *Arthritis Rheum.* **44**, 397–407.
623. Quddus, J., Johnson, K. J., Gavalchin, J., Amento, E. P., Chrisp, C. E., Yung, R. L., and Rchardson, B. C. (1993). Treating activated CD4+ T cells with either of two distinct DNA methyltransferase inhibitors, 5-azacytidine or procainamide, is sufficient to cause a lupus-like disease in syngeneic mice. *J. Clin. Invest.* **92**, 38–53.
624. Lahita, R. G. (1985). Sex steroids and the rheumatic disease. *Arthritis Rheum.* **28**, 121–126.
625. Weinstein, Y., and Berkovich, Z. (1981). Testosterone effect on bone marrow thymuc and expression T cells in the (NZBxNZW) F1 mice: Its relevance to autoimmunity. *J. Immunol.* **126**, 998–1002.
626. Brick, J. E., Wilson, D. A., and Walker, S. E. (1985). Hormonal modulation of reponses to thymuc-independent of thymuc-dependent antigen in autoimmune mice. *J. Immunol.* **134**, 3693.
627. Kincade, P. W., Medina, K. L., and Smithson, G. (1994). Sex hormones as neagative regulators of lymphopoiesis. *Immunol. Rev.* **137**, 119–134.
628. Smithson, G., Beamer, W. G., Shultz, K. L., Christianson, S. W., Shultz, L. D., and Kincade, P. W. (1994). Increased B lymphopoiesis in gentically sex steroid-deficient hyupogonadal (hpg) mice. *J. Exp. Med.* **180**, 717–720.
629. Smithson, G., Medina, K., Ponting, I., and Kincade, P. W. (1995). Estogen suppresses stromal cell-dependent lymphopoiesis in culture. *J. Immunol.* **155**, 3409–3417.
630. Medina, K. L. and Kincade, P. W. (1994). Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. *Proc. Natl. Acad. Sci. USA* **91**, 5382–5386.
631. Sthoeger, Z. M., Chiorazzi, N., and Lahita, R. G. (1988). Regulation of the immune response by sex hormone. I. *In vitro* effects of estradiol and testosterone on pokeweek mitogen-induced human B cell differentiation. *J. Immunol.* **141**, 91–98.
632. Evans, M. J., MacLaughlin, S., Marvin, R. D., and Abdou, N. I. (1997). Estogen decreases *in vitro* apoptosis of peripheral blood monouclear cells from women with normal menstrual cycles and decreases TNF-alpha production in SLE but not in normal cultures. *Clin. Immuno. Immunopathol.* **82**, 258–262.
633. Furukawa, F., Lyons, M. B., Lee, L. A., Coulter, S. N., and Norris, D. A. (1988). Estradiol enhances binding to cultured human keratinocytes of antibodies specific for SS-A/Ro and SS-B/La: Another possible mechanism for estradiol influence of lupus erythematosus. *J. Immunol.* **141**, 1480.
634. Sontheimer, R. D., Maddison, P. J., Reichlin, M., Jordon, R. E., Stastny, P., and Gillman, J. N. (1982). Serological and HLA association in subacute cutaneous lupus erythematosus, a clinical subset of lupus erythematosus. *Ann. Intern. Med.* **97**, 664–671.
635. LeFever, W. P., Norris, D. A., Ryan, S. R., Hiff, J. C., Lee, L. A., Kubo, M., Boyce, S. T., Kotzin, B. L., and Weston, W. L. (1984). Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultures human keratinocytes. *J. Clin. Invest.* **74**, 1545–1551.
636. Bynoe, M. S., Grimaldi, C. M., and Diamond, B. (2000). Estrogen up-regulates Bcl-2 and blocks tolerance induction of naive B cells. *Proc. Natl. Acad. Sci. USA* **97**, 2703–2708.
637. Rider, V., Foster, R. T., Evans, M., Suenaga, R., and Abdou, N. I. (1998). Gender differences in autoimmune diseases: Estrogen increases calcineurin expression in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **89**, 171–180.
638. Rider, V., and Abdou, N. I. (2001). Gender differences in autoimmunity: Molecular basis for estrogen effects in systemic lupus erythematosus. *Int. Immunopharmacol.* **1**, 1009–1024.
639. Boumpas, D. T., Austin, H. A., III, Fessler, B. J., Balow, J. E., Klippel, J. H., and Lockshin, M. D. (1995). Systemic lupus erythematosus: emerging concepts. 1. Renal, neuropsychiatric, cardiovascular, pulmonary, and hematologic disease. *Ann. Intern. Med.* **122**, 940–950.
640. Boumpas, D. T., Fessler, B. J., Austin, H. A., III, Balow, J. E., Klippel, J. H., and Lockshin, M. D. (1995). Systemic lupus erythematosus: Emerging concepts. 2. Dermatologic and joint disease, the antiphospholipid antibody syndrome, pregnancy and hormonal therapy, morbidity and mortality, and pathogenesis. *Ann. Intern. Med.* **123**, 42–53.
641. Austin, H. A., III, Klippel, J. H., Balow, J. E., le Riche, N. G., Steinberg, A. D., Plotz, P. H., and Decker, J. L. (1986). Therapy of lupus nephritis: Controlled trial of prednisone and cytotoxic drugs. *N. Engl. J. Med.* **314**, 614–619.
642. Gourley, M. F., Austin, H. A., III, Scott, D., Yarboro, C. H., Vaughan, E. M., Muir, J., Boumpas, D. T., Klippel, J. H., Balow, J. E., and Steinberg, A. D. (1996). Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis: A randomized, controlled trial. *Ann. Intern. Med.* **125**, 549–557.
643. Boumpas, D. T., Barez, S., Klippel, J. H., and Balow, J. E. (1990). Intermittent cyclophosphamide for the treatment of autoimmune thrombocytopenia in systemic lupus erythematosus. *Ann. Intern. Med.* **112**, 674–677.
644. Boumpas, D. T., Austin, H. A., Vaughn, E. M., Klippel, J. H., Steinberg, A. D., Yarboro, C. H., and Balow, J. E. (1992). Controlled trial of pulse methylprednisolone versus two regimens of pulse cyclophosphamide in severe lupus nephritis. *Lancet* **340**, 741–745.
645. Moroni, G., Maccario, M., Banfi, G., Quaglini, S., and Ponticelli, C. (1998). Treatment of membranous lupus nephritis. *Am. J. Kidney Dis.* **31**, 681–686.
646. Austin, H. A., III, Antonovych, T. T., MacKay, K., Boumpas, D. T., and Balow, J. E. (1992). NIH conference: Membranous nephropathy. *Ann. Intern. Med.* **116**, 672–682.

647. Moreland, L. W., Baumgartner, S. W., Schiff, M. H., Tindall, E. A., Fleischmann, R. M., Mohler, K., Widmer, M. B., and Blosch, C. M. (1997). Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75): Fc fusion protein. *N. Engl. J. Med.* **337**, 141–147.
648. Present, D. H., Rutgeerts, P., Targan, S., Hanauer, S. B., Mayer, L., van Hogezand, R. A., Podolsky, D. K., Sands, B. E., Braakman, T., DeWoody, K. L., Schaible, T. F., and van Deventer, S. J. (1999). Infliximab for the treatment of fistulas in patients with Crohn's disease. *N. Engl. J. Med.* **340**, 1398–1405.
649. Baert, F. J., D'Haens, G. R., Peeters, M., Hiele, M. I., Schaible, T. F., Shealy, D., Geboes, K., and Rutgeerts, P. J. (1999). Tumor necrosis factor alpha antibody (infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. *Gastroenterology* **116**, 22–28.
650. Fishman, P., Falach-Vaknine, E., Zigelman, R., Bakimer, R., Sredni, B., Djaldetti, M., and Shoenfeld, Y. (1993). Prevention of fetal loss in experimental antiphospholipid syndrome by in vivo administration of recombinant interleukin-3. *J. Clin. Invest.* **91**, 1834–1837.
651. Ganser, A., Seipelt, G., Lindemann, A., Ottmann, O. G., Falk, S., Eder, M., Herrmann, F., Becher, R., Hoffken, K., and Buchner, T. (1990). Effects of recombinant human interleukin-3 in patients with myelodysplastic syndromes. *Blood* **76**, 455–462.
652. Schattner, A. (1994). Ltmphokines in autoimmunty: A critical review. *Clin. Immmunol. Immunopathol.* **70**, 177–189.
653. Manzi, S., Rairie, J. E., Carpenter, A. B., Kelly, R. H., Jagarlapudi, S. P., Sereika, S. M., Medsger, T. A., Jr., and Ramsey-Goldman, R. (1996). Sensitivity and specificity of plasma and urine complement split products as indicators of lupus disease activity. *Arthritis Rheum.* **39**, 1178–1188.
654. Schulze, M., Donadio, J. V., Jr., Pruchno, C. J., Baker, P. J., Johnson, R. J., Stahl, R. A., Watkins, S., Martin, D. C., Wurzner, R., and Gotze, O. (1991). Elevated urinary excretion of the C5b-9 complex in membranous nephropathy. *Kidney Int.* **40**, 533–538.
655. Wang, Y., Hu, Q., Madri, J. A., Rollins, S. A., Chodera, A., and Matis, L. A. (1996). Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. *Proc. Natl. Acad. Sci. USA* **93**, 8563–8568.
656. Quigg, R. J., Kozono, Y., Berthiaume, D., Lim, A., Salant, D. J., Weinfeld, A., Griffin, P., Kremmer, E., and Holers, V. M. (1998). Blockade of antibody-induced glomerulonephritis with Crry-Ig, a soluble murine complement inhibitor. *J. Immunol.* **160**, 4553–4560.
657. Quigg, R. J., He, C., Lim, A., Berthiaume, D., Alexander, J. J., Kraus, D., and Holers, V. M. (1998). Transgenic mice overexpressing the complement inhibitor crry as a soluble protein are protected from antibody-induced glomerular injury. *J. Exp. Med.* **188**, 1321–1331.
658. Mihara, M., Tan, I., Chuzhin, Y., Reddy, B., Budhai, L., Holzer, A., Gu, Y., and Davidson, A. (2000). CTLA4Ig inhibits T cell-dependent B-cell maturation in murine systemic lupus erythematosus. *J. Clin. Invest.* **106**, 91–101.
659. Wofsy, D., and Daikh, D. I. (1998). Opportunities for future biological therapy in SLE. *Baillieres Clin. Rheumatol.* **12**, 529–541.
660. Daikh, D. I., and Wofsy, D. (1998). On the horizon: Clinical trials of new immunosuppressive strategies for autoimmune diseases. *Transplant. Proc.* **30**, 4027–4028.
661. Daikh, D. I., Finck, B. K., Linsley, P. S., Hollenbaugh, D., and Wofsy, D. (1997). Long-term inhibition of murine lupus by brief simultaneous blockade of the B7/CD28 and CD40/gp39 costimulation pathways. *J. Immunol.* **159**, 3104–3108.
662. Burt, R. K., and Traynor, A. (1998). Hematopoietic stem cell therapy of autoimmune diseases. *Curr. Opin. Hematol.* **5**, 472–477.
663. Burt, R. K., Traynor, A. E., Pope, R., Schroeder, J., Cohen, B., Karlin, K. H., Lobeck, L., Goolsby, C., Rowlings, P., Davis, F. A., Stefoski, D., Terry, C., Keever-Taylor, C., Rosen, S., Vesole, D., Fishman, M., Brush, M., Mujias, S., Villa, M., and Burns, W. H. (1998). Treatment of autoimmune disease by intense immunosuppressive conditioning and autologous hematopoietic stem cell transplantation. *Blood* **92**, 3505–3514.
664. van Vollenhoven, R. F., Park, J. L., Genovese, M. C., West, J. P., and McGuire, J. L. (1999). A double-blind, placebo-controlled, clinical trial of dehydroepiandrosterone in severe systemic lupus erythematosus. *Lupus* **8**, 181–187.
665. Nippoldt, T. B. and Nair, K. S. (1998). Is there a case for DHEA replacement? *Baillieres Clin. Endocrinol. Metab.* **12**, 507–520.
666. van Vollenhoven, R. F., Engleman, E. G., and McGuire, J. L. (1995). Dehydroepiandrosterone in systemic lupus erythematosus: Results of a double-blind, placebo-controlled, randomized clinical trial. *Arthritis Rheum.* **38**, 1826–1831.
667. Kaliyaperumal, A., Mohan, C., Wu, W., and Datta, S. K. (1996). Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. *J. Exp. Med.* **183**, 2459–2469.
668. Lu, L., Kaliyaperumal, A., Boumpas, D. T., and Datta, S. K. (1999). Major peptide autoepitopes for nucleosome-specific T cells of human lupus. *J. Clin. Invest.* **104**, 345–355.
669. Kaliyaperumal, A., Michaels, M. A., and Datta, S. K. (1999). Antigen-specific therapy of murine lupus nephritis using nucleosomal peptides: Tolerance spreading impairs pathogenic function of autoimmune T and B cells. *J. Immunol.* **162**, 5775–5783.
670. Weisman, M. H., Bluestein, H. G., Berner, C. M., and de Haan, H. A. (1997). Reduction in circulating dsDNA antibody titer after administration of LJP 394. *J. Rheumatol.* **24**, 314–318.
671. Zhang, J., Roschke, V., Baker, K. P., Wang, Z., Alarcon, G. S., Fessler, B. J., Bastian, H., Kimberly, R. P., and Zhou, T. (2001). Cutting edge: A role for B lymphocyte stimulator in systemic lupus erythematosus. *J. Immunol.* **166**, 6–10.

672. Cheema, G. S., Roschke, V., Hilbert, D. M., and Stohl, W. (2001). Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. *Arthritis Rheum*. **44**, 1313–1319.
673. Constantin, G., Laudanna, C., Brocke, S., and Butcher, E. C. (1999). Inhibition of experimental autoimmune encephalomyelitis by a tyrosine kinase inhibitor. *J. Immunol*. **162**, 1144–1149.
674. Constantin, G., Brocke, S., Izikson, A., Laudanna, C., and Butcher, E. C. (1998). Tyrphostin AG490, a tyrosine kinase inhibitor, blocks actively induced experimental autoimmune encephalomyelitis. *Eur. J. Immunol*. **28**, 3523–3529.
675. Hsu, H-C, Zhang, H-G, Zhou, T., and Mountz, J. D. (1999). Management of murine lupus by correction of Fas and Fas ligand-induced apoptosis: Therapeutic rationale and strategies. 671–693.
676. Lawson, B. R, Prud'Homme, G. J., Chang, Y., Gardner, H. A., Kuan, J., Kono, D. H., and Theofilopoulos, A. N. (2000). Non-viral gene therapy of mouce lupus with cDNA encoding IFN-gammaR/Fc. *J. Clin Invest*.
677. Fathman, C. G., Costa, G. L., and Seroogy, C. M. (2000). Gene therapy for autoimmune disease. *Clin. Immunol* **95**, S39-S43.
678. Evans, C. H., Rediske, J. J., Abramson, S. B., and Robbins, P. D. (1999). Joint efforts: Tackling arthritis using gene therapy. *Mol. Med. Today* **5**, 148–151.
679. Boyle, D. L., Nguyen, K. H., Zhuang, S., Shi, Y., McCormack, J. E., Chada, S., and Firestein, G. S. (1999). Intra-articular IL-4 gene therapy in arthritis: Anti-inflammatory effect and enhanced th2 activity. *Gene Ther*. **6**, 1911–1918.
680. Bessis, N., Boissier, M. C., Ferrara, P., Blankenstein, T., Fradelizi, D., and Fournier, C. (1996). Attenuation of collagen-induced arthritis in mice by treatment with vector cells engineered to secrete interleukin-13. *Eur. J. Immunol*. **26**, 2399–2403.
681. Zhang, H. G., Huang, N., Liu, D., Bilbao, L., Zhang, X., Yang, P., Zhou, T., Curiel, D. T., and Mountz, J. D. (2000). Gene therapy that inhibits nuclear translocation of nuclear factor kappaB results in tumor necrosis factor alpha-induced apoptosis of human synovial fibroblasts. *Arthritis Rheum*. **43**, 1094–1105.
682. Zambidis, E. T., Kurup, A., and Scott, D. W. (1997). Genetically transferred central and peripheral immune tolerance via retroviral-mediated expression of immunogenic epitopes in hematopoietic progenitors or peripheral B lymphocytes. *Mol. Med*. **3**, 212–224.
683. Kang, Y., Melo, M., Deng, E., Tisch, R., El Amine, M., and Scott, D. W. (1999). Induction of hyporesponsiveness to intact foreign protein via retroviral-mediated gene expression: The IgG scaffold is important for induction and maintenance of immune hyporesponsiveness. *Proc. Natl. Acad. Sci. USA* **96**, 8609–8614.
684. Mohan, C., Morel, L., Yang, P., and Wakeland, E. K. (1997). Genetic dissection of systemic lupus erythematosus pathogenesis: Sle2 on murine chromosome 4 leads to B cell hyperactivity. *J. Immunol*. **159**, 454–465.
685. Morel, L., Mohan, C., Yu, Y., Croker, B. P., Tian, N., Deng, A., and Wakeland, E. K. (1997). Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J. Immunol*. **158**, 6019–6028.
686. Sfikakis, P. P., Zografou, A., Viglis, V., Iniotaki-Theodoraki, A., Piskontaki, I., Tsokos, G. C., Sfikakis, P., and Choremi-Papadopoulou, H. (1996). CD28 expression on T cell subsets in vivo and CD28-mediated T cell response *in vitro* in patients with rheumatoid arthritis. *Arthritis Rheum*. **38**, 649–654.
687. Stohl, W. (1995). Impaired polyclonal T cell cytolytic activity: A possible risk factor for systemic lupus erythematosus. *Arthritis Rheum*. **38**, 506–516.
688. Linker-Israeli, M., Wallace, D. J., Prehn, J. L., Nand, R., Li, L., and Klinenberg, J. R. (1996). A greater variability in the 3' flanking region of the IL-6 gene in patients with systemic lupus erythematosus (SLE). *Autoimmunity* **23**, 199–209.

3

ETIOPATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Itineraries of Three Troikas

Donato Alarcón-Segovia
Marta E. Alarcón-Riquelme

Systemic lupus erythematosus (SLE) results from the interplay of multiple factors that vary in their participation among individuals with the disease, which, in turn makes SLE extremely variable. Actually, in normal individuals some of these factors may be present but are either not enough to cause the disease or have counterbalances or checks that prevent full disease expression. This may be particularly so in relatives of SLE patients.

To discuss the etiopathogenesis of SLE, one needs to subdivide an extremely complex process. We have elected to do this at three consecutive, but separable, levels: etiology, etiopathogenesis, and pathogenesis. The complexity of the subject, however, calls for further subdivision but, at the same time, it must be clear that both the levels and the various factors that compose them are unseparable. Actually, when having reached the third level we may find that some of its factors go back to influence the second or even the first level.

We have previously proposed that in the etiology of SLE there is the interplay of genetic, environmental, and hormonal factors. Because each one of these acts both independently and concurrently we have equated it to a troika, the three-horse Russian carriage where each horse pulls independently, but concertedly, to make it go [1].

The troika analogy has also been useful for the analysis of the subsequent levels of etiopathogenesis and pathogenesis. We therefore decided to present our chapter for the previous edition of this book as a tale of three troikas. The troika analogy continues to be useful to approach the complexity of our subject but many of the nine horses need to be reexamined or somewhat changed in order to keep the troikas tuned to current knowledge. We will now follow the troikas along their itinerary.

THE FIRST TROIKA: ETIOLOGY

As initially proposed [1], the first troika pertaining to the etiology of SLE continues to be pulled by genetic, environmental, and hormonal factors. That environment, hormones, or both have a role in the development of SLE does not exclude that genes are also involved. Thus, genetic factors may determine the way an individual copes with a viral infection or handles a certain drug (i.e., hepatic acetylation of drugs or multidrug resistance genes). In turn, hormonal factors may be modified by genes (i.e., the presence of an allele coding for a hormone receptor with higher affinity for a given hormone or of a mutation that makes estrogens more potent or testosterone less so).

Genetic Factors in SLE

From the genetic point of view, SLE probably results from the effects of a number of genes acting in an additive fashion [2, 3]. This may, however, be simplistic because an individual could have all the gene variants presumably required to develop the disease but still not develop it. This, in genetics, is called *penetrance*. SLE, as many other diseases with complex genetics, has a low penetrance. It can also be that the combination of genes needed to develop SLE in a given ethnic group is different from that required by another ethnic group. This is called *genetic heterogeneity*. A third level of complication in genetic studies occurs when all families studied are linked to a certain locus that is not represented by a single genetic variant of a gene, but by many different variants of the same gene, all of functional importance. This is called *allelic heterogeneity*. Work on the genetics of Crohn's disease [4] has shown that allelic heterogeneity is to be expected in complex diseases. Furthermore, some alleles may be so rare that unless they do affect the function of the protein in an obvious way, it is very difficult to statistically prove their association with the disease. Geneticists have had to find other means of studying the genetics of complex diseases such as SLE. As genes within a locus of interest may be regulated in *cis*, the analysis of haplotypes for association and gene expression may be needed to define the genetics of SLE.

Is SLE a genetic disease? Studies performed on twins suggest so. Comparisons made on the concordance between monozygotic and dizygotic twins revealed that the concordance in monozygotic twins is eight times higher than that in dizygotic twins and that the concordance is even higher if the presence of autoantibodies is considered (concordance may be as high as 92%) [5–7]. The lower concordance for the clinical expression of SLE may be due to the lack of an environmental factor triggering the development of an overt disease, whereas autoantibodies may represent "early" markers of a latent disease. The classical study of Moore and Lutz [8] where they found that patients with false-positive VDRL went on to develop SLE supports this notion. Also supportive is the study on sera stored by the Department of Defense of the United States where they had sera from 130 subjects who subsequently developed SLE. It was found that 62% of them had anti-dsDNA up to 9 years before the disease was diagnosed [9].

SLE is clinically very heterogeneous, but what determines this heterogeneity? Could it be the role of disease-modifying genes or stochastic mechanisms? The described association of the HLADQA1 and DQB1 alleles [10–16] with certain autoantibody specificities and, in turn, with defined clinical manifestations supports the former idea. How different genes play a role in the full-blown expression of SLE is the subject of intense research. What genes act as disease-predisposing genes and what genes act as disease modifiers? What epigenetic mechanisms take place? What genetic interactions are necessary? How do interactions between genes and environmental factors take place? For example, if the Epstein–Barr (EB) virus is indeed involved in SLE [17], what genetic predisposing factors are required for the virus to influence the immune system to promote disease development? Do B cells from some individuals express some type of receptors or molecules that make them handle the EB virus inadequately?

Two main approaches have been taken for studying the genetics of SLE. On the one hand, spontaneous animal models for SLE have been crossed and analyzed in their whole genome for linkage between lupus-related phenotypic traits and chromosomal markers (typically microsatellites). In particular, genome screens were performed on crosses of the NZM2410 recombinant strain (a recombinant of NZB × NZW) with the B6 mouse [18] or on backcrosses performed directly from the NZB × NZW hybrid [19]. Congenic strains have been developed and have led to interesting results. The first (the *nba1* locus) is identification of the susceptible variant of the *Ifi202* gene contributed by the NZB strain [20], and the second (the *sle1* locus) is the identification of a cluster of functionally related genes contributed by the NZW strain [21]. In this strain, however, no individual genetic variant has been detected. It was therefore suggested that as the genes formed by the cluster are all coregulated on lymphocytes, their role in SLE susceptibility could be related to their coregulation. It is therefore possible to hypothesize that the genetic variant could be many kilobases upstream of the gene cluster within an as yet unidentified regulatory element but still within the congenic fragment. Both of the susceptibility factors, the *Ifi202* gene and the cluster, are found close to each other (about 90 cM) in chromosome 1 of mice, but each could only be detected using the particular mouse crosses [18, 19]. This region is syntenic to the human chromosome 1q21–23 cytogenetic region. Previous studies in lupus patients suggested that FcGR genes located in that region were involved in SLE. In particular, the FcGRIIA functional polymorphism H131R was found to be associated to SLE or nephritis in numerous studies, but also as a functional polymorphism in IIIA [22–27]. The allele of IIA containing arginine (R131) has diminished IgG-binding capacity, supporting the hypothesis that FcGRs are involved in immune complex handling and disposal, a mechanism that is defective in

SLE. Researchers are, however, still not completely convinced of the role of this polymorphism. The FcGR family is a cluster of four genes (FcGRIIA, IIB, IIIA, and IIIB) and one pseudogene (FcGRIIC). The four genes vary in their function, and IIA and IIIA have been the main ones found to be associated with SLE, although IIB has not been studied probably because of its great similarity to IIA, despite its having a completely opposite role in cell regulation. Thus, as opposed to IIA, IIB inhibits antigen-specific B-cell activation through a tyrosine-based inhibitory motif in its intracytoplasmic tail. The mechanism leading to autoimmunity could thus be through a lack of inhibition of cellular activation. The close proximity of the mouse loci described earlier to the FcGR genes caused them to be considered important candidates. However, elegant studies by Morel *et al*. [21], together with discovery of the *ifi202* gene, have cast doubt on the role of FcGRs in the disease, at least in the mouse. The question being always whether we can extrapolate the findings made in mouse models to the human disease. Are FcGRs involved in human SLE but not in murine lupus? Alternatively, are *Ifi202* and the *sle1* gene cluster of mice lupus also involved in human SLE? Only intense study can give us answers to these queries, but animal models do give us clues as to pathogenic pathways of the human disease.

The second approach to the identification of genes for human disease is to directly use human material [28]. This implies collecting large cohorts of families with multiple cases of SLE. To date, five human genome scans have been published [29–33]. Table 1 describes the major loci identified in each study. All scans revealed loci in chromosome 1, but only one of these had linkage to the region where the FcGR genes are located (1q21–23). One genome scan performed in Nordic families, a population with little admixture, revealed a locus in chromosome 2q37 (*SLEB2*) [29, 34], whereas a third locus of apparent importance was found in chromosome 16, close to the newly described locus for Crohn's disease [4]. All studies identified the HLA in chromosome 6p also as a locus involved in SLE, as expected [31]. These studies require large numbers of families, and the strategies classically used for monogenic diseases cannot be fully applied for complex diseases. Linkage regions identified usually are many centimorgans in size and may include anywhere from 100 to 600 genes. A candidate gene approach can be taken, but it is difficult to decide what genes to start with. Is there prior evidence for any one gene? Are there many potential candidates? What type of mutations do we expect to find in complex diseases? How frequent are such mutations in the general population? The example of Crohn's disease is interesting [4]. Three rare amino acid substitutions were identified, each of them so rare that genetic association could not be performed and a haplotype test had to be implemented. However, as the polymorphisms were amino acid substitutions that modified the function of the NOD2/CAD15 gene, there was little reason to doubt their role in disease. Are we to expect similar mutations in SLE? We do not know. Each gene is a particular case depending on its function. One of the major problems in complex disease human genetics is the exclusion of genes within a region of linkage.

TABLE 1 Major Loci Identified in Genome scans of SLE

Human chromosome	LOD[a]	Population
1q23-24	3.37	African-American
1q41-42	3.50	African-American
2q37	4.24	Scandinavian
4p15	3.20	Icelandic
6p21-11	3.9	Mixed African-American, European-American
16q13	3.64	Mixed African-American, European-American

[a] LOD score (logarithm of the odds).

One of the approaches discussed in recent years is the identification of disease-associated haplotypes to which a gene expression or function can be related. Use of the publicly available databases of single nucleotide polymorphisms (SNPs) does not solve the issue completely because many of the disease variants we are looking for may be rare among normal individuals. Complete resequencing of genes within susceptibility loci has been proposed as an option with the use of new technologies for the identification of relevant SNPs forming common population haplotypes [35–37].

How different genes play a role in SLE, either through modification of other genes or in disease susceptibility, and what their relation is to disease expression, requires much research. On the one hand, there are germline genes that encode for natural autoantibodies that may become pathogenic when subject to mutation and/or idiotypic switch. On the other hand, there may be genes that predispose to the development of autoimmune disease in general that are evidenced by personal or familial associations with other diseases (e.g., autoimmune thyroiditis, rheumatoid arthritis, pernicious anemia, myasthenia gravis, autoimmune hepatitis).

Genes involved in SLE may participate in different immune pathways, including immune complex handling, B-cell signaling, regulation of apoptosis, antigen processing and presentation, T-cell receptor conformation,

and immunoglobulin structure. Of these, one of the most studied is the one related to inefficiency for immune complex handling by the immune system of lupus patients. Much of this pertains to the study of hereditary complement deficiencies that, when totally affecting the production of a complement component, particularly one of the early phases of complement activation that participate in stabilization and clearance of immune complexes, often result in a lupus-like syndrome.

Partial complement deficiencies cannot by themselves explain the disease but SLE does associate rather strongly with null alleles of C4A (C4AQ0), which in some populations is in linkage disequilibrium with the B8, DR3 haplotype of the HLA. In many instances the association of C4AQ0 has been found without association to DR3, but the reverse has also been found [38–44]. The strength of the role of HLA in SLE is yet undetermined. It should also be remembered that other potentially important genes [e.g., tumor necrosis factor (TNF)] are also located within the major histocompatibility complex (MHC) region.

Also related to immune complex handling are the FcG receptors of macrophages, natural killer (NK) or B cells that bind IgG subclasses with varying affinities. Quantitative differences in the binding of different alleles may be a predisposing factor to disease by influencing either B-cell activation or IgG levels, particularly those of autoantibodies or immune complex-mediated triggering of inflammatory processes. In addition, FcG receptors may participate in the handling of viral or bacterial infections. A strong genetic association has been found in Blacks and other ethnic groups between a polymorphic allele of FcGRIIa and lupus nephritis [45–49]. It could thus be that such association could represent that of a disease severity gene influencing development of nephritis within SLE, but having no role in primary genetic susceptibility to the development of the disease itself [50].

Genes related to the regulation of apoptosis may also be implicated. This has become apparent in the murine SLE model having the lymphoproliferation (*lpr*) that represents a defect in the *fas* gene that regulates apoptosis in lymphoid cells [51]. The *fas* gene has been implicated only in a familial recessive form of human SLE and does not seem to have a role in most SLE cases [52–55]. The *lpr* mutation behaves as an accelerating gene in certain genetic backgrounds. Thus, it seems to influence severe disease in MRL mice whereas it causes only lymphoproliferation in the C57BL/6 strain.

Strain differences provide important clues. The NZB mouse develops two bouts of hemolytic anemia, the second one being usually fatal and due to the interplay of natural autoantibodies to bromelain-treated erythrocytes wall and Coombs' antibody. When NZB mice are crossed with NZW mice, which is a generally normal strain, their F1 hybrids develop severe renal disease akin to lupus nephritis and numerous autoantibodies, including characteristic anti-dsDNA and anti-Sm [56]. Independent laboratories have studied crosses of (NZB/NZW)F1 mice and analyzed their complete genome using anonymous markers looking for loci that predispose to nephritis or to development of autoantibodies. Lupus nephritis, as well as other disease phenotypes, should be seen as a qualitative, dichotomous trait made up of various quantitative traits. When studyng quantitative traits, such as autoantibody titers or immunoglobulin levels of the various isotypes, a large number of loci with smaller effects are revealed. Each locus may act in an additive fashion to render a certain phenotype. However, the various genes may interact, a phenomenon known as epistasis, and modify each other's effects [57, 58] (Fig. 1). An example of this has been provided by crossing MRL-*lpr/lpr* with perforin-deficient mice (*pfp*–) [59]. Survival was reduced and renal IgG deposition was increased in *pfp-/fas-* as compared to *pfp* + /*fas-*,*pfp* + /*fas* +, and *pfp-/fas* + mice. This could indicate a role of the last complement components in disease severity.

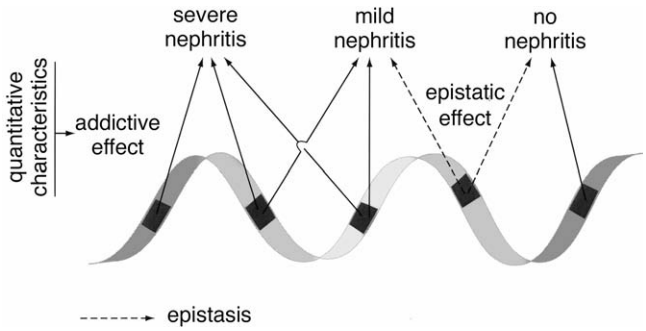


FIGURE 1 Interplay of additive and epistatic effects of genes participating in the development of lupus nephritis. The algebraic sum of these would yield the result, e.g., mild nephritis.

Figure 2 presents a scheme of potential genes that may participate in the development and/or the clinical and serologic picture of SLE. We also show how they may interplay with the other factors within the first troika as well as with the other two troikas. There are specific or general autoimmune disease-susceptibility genes, genes of disease severity or acceleration, and disease-modifying genes. There may be participation of genes for immune responses, complement components, immunoglobulin structure, T-cell receptor alleles, FcGRII, MHC, cytokine promoters, regulation of apoptosis and lymphoproliferation, and germline genes that encode for natural autoantibodies. There are also genes

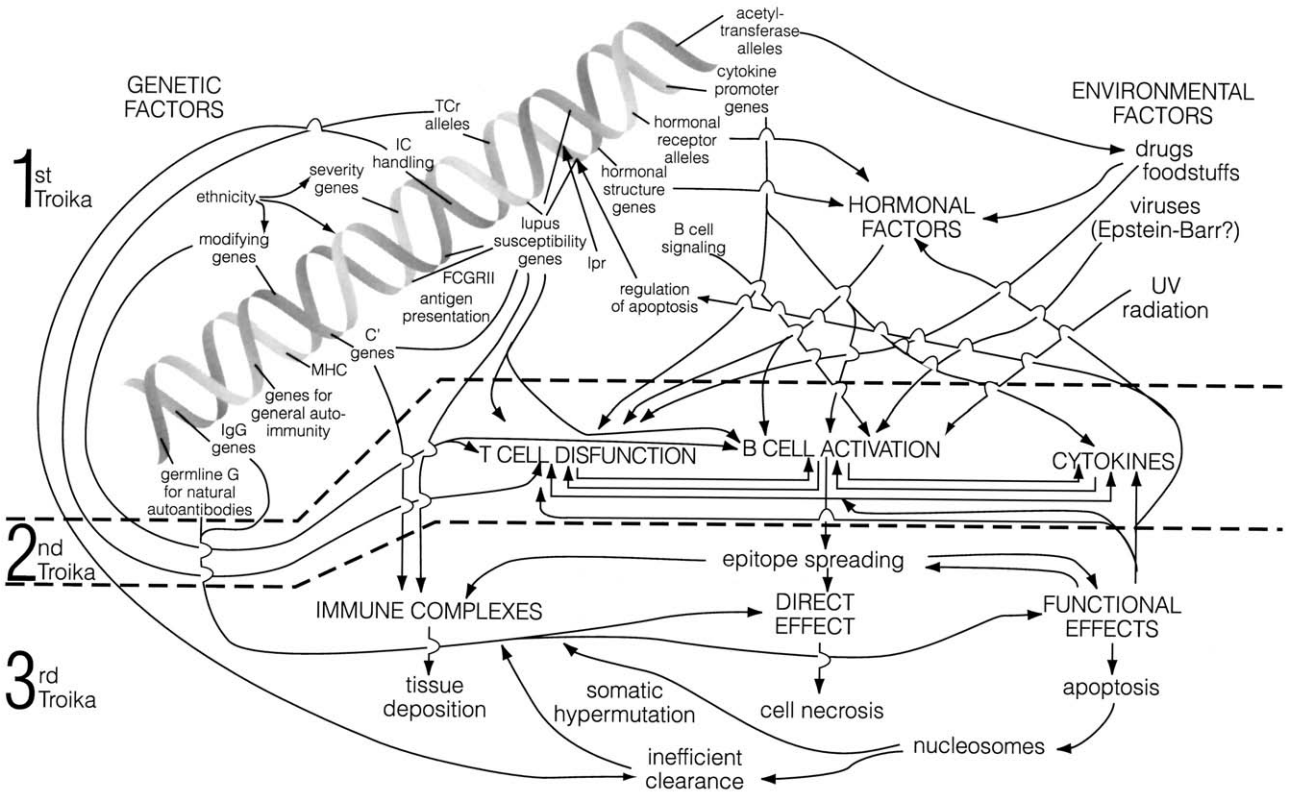


FIGURE 2 A tale of three troikas: Overview of the interplay of various factors at the etiologic (first troika) level. Etiopathogenic (second troika) and pathogenic (third troika) levels.

that regulate hormonal structure and hormone receptor alleles, as well as genes that participate in pharmacogenetics such as acetyltransferase alleles. Some of these genes exert their influence through the second and third horses of the first troika or extend their influence to the other two troikas.

In the field of lupus, human genetics is advancing and in the near future it is hoped that we will witness an explosion of findings that will result in new therapies that will improve the life of hundreds of thousands lupus patients. Identification of the genetic variants involved in disease susceptibility or expression should lead to better understanding of the mechanisms behind the other components of the troikas.

Hormonal Factors in SLE

That lupus occurs predominantly in females might relate to hormonal factors. Again, studies in lupus-prone mice have helped us define their role. Multiple experiments in castrated and noncastrated NZB/NZW mice have shown a disease-accelerating effect of estrogens that also causes H-2 related antibody production [60]. These effects of estrogens have also been encountered in MRL-*lp*-*lpr* and even in Balb/c mice immunized with the 16/6 pathogenic idiotype of anti-DNA antibodies [60–63]. Similar studies using testosterone instead of estrogens have shown a protective effect instead of a deleterious effect [64]. These contrary effects of the two groups of hormones could relate to their respective interactions with the immune system. In general, estrogens tend to have an activating effect on the immune system whereas testosterone has more of a quieting role. However, independent of the effect of hormones, the immune system of females is, in general, more powerful. In most species, thymuses of females are larger at birth. Another hormone that may have a role in the pathogenesis of SLE is prolactin, which can be found elevated in both males with SLE [65–66].

Prolactin participates in immune functions and there are receptors for prolactin on human lymphocytes and monocytes [67, 68]. Nonlupus patients with hyperprolactinemia tend to have lupus autoantibodies and may have a decreased production of, and response to, interleukin 2 (IL-2) that can be corrected with bromocriptine treatment that decreases prolactin levels [69]. It has been found that peripheral blood mononuclear cells from patients with SLE preferentially secrete a bioactive 60-kDa species of prolactin both in resting and in stimulated states [70]. This could point to an autocrine

loop of prolactin participating in the immune dysregulation of SLE. In support of this is the finding that prolactin may enhance the *in vitro* production of IgG by peripheral blood mononuclear cells from SLE patients as compared to those of normal controls [71]. Interestingly, mononuclear cells derived from patients who had hyperprolactinemia were the ones that produced more IgG. Whether the IgG thus formed consisted primarily of autoantibodies was not determined [71].

Although female patients with SLE may have normal estradiol levels, they have been shown to have increased 16α hydroxylation of estrone with a potent estrogenic activity [72–74]. Conversely, they tend to have low testosterone levels [75]. Males with lupus may have increased plasma 16α hydroxyestrone and decreased testosterone [76–78], and patients with Klinefelter's syndrome who have significantly increased 16-hydroxylation of estrone may be more prone to develop SLE [79]. This could also point to a role of genes on the X chromosome. As mentioned previously, hormonal factors could also be genetically influenced either at the level of hormonal structure or of hormonal receptor alleles. Some of these interactions between genetic and hormonal factors are also shown in Fig. 2.

Environmental Factors in SLE

That environmental factors play a role in the etiopathogenesis of SLE seems beyond dispute. Ultraviolet light, for instance, can clearly provoke the initiation or activation of the disease. A number of drugs, as well as some foodstuffs (e.g., alfalfa sprouts), have also been shown to cause lupus-like syndromes, and their lupus-inducing potential may be influenced by their pharmacogenetics. Individuals with a slow drug acetylator phenotype are more prone to have drug-induced lupus upon exposure to drugs that are a substrate for hepatic acetyl transferase [80]. That patients with spontaneously occurring SLE may also be predominantly slow acetylators has been controversial probably due to ethnic factors [81]. The potential role of viruses in the etiopathogenesis of SLE has long been suspected. A study of lupus patients of less than 20 years of age found a remarkable increased prevalence of Epstein–Barr virus infection as compared to matched controls [82]. These findings raise several possibilities, particularly that of triggering of the disease in individuals with the proper genetic background. The finding of increased EB virus infection could be significant in view of the well-known B-cell activation/immortalization properties of this virus on B cells. It should be kept in mind that in different ethnic groups, different geographies, or both, other viruses could be involved [83].

That normal individuals may have the genetic predisposition to develop SLE should an adequate triggering environmental factor come along, was amply discussed when dealing with genetic factors. Thus normal mice may contribute genes that increase the severity of lupus in their offspring. That an environmental factor may elicit a full-blown picture of SLE is exemplified by the pristane model of murine lupus where a single intraperitoneal injection of this compound into BALB/c mice can cause the disease with a corresponding cohort of autoantibodies [84]. Interestingly, the first autoantibodies to appear following an intraperitoneal pristane injection of mice are germline gene-encoded low-affinity IgM natural autoantibodies. This would also indicate the potential pathogenic importance of natural autoantibodies, whether subjected to mutations or not. Interactions of environmental factors with hormonal (e.g., estrogen administration) and genetic ones are also depicted in Fig. 2. From here we move to the second troika, which travels inside immune regulation territory.

THE SECOND TROIKA: ETIOPATHOGENESIS

The genetic, hormonal, and environmental factors that constitute the three horses of the first troika exert their influence on the three elements of the second troika. To pull this troika, we choose as its horses T-cell dysfunction, B-cell activation, and abnormal cytokine production, which are hallmarks of immune dysregulation present in SLE. Obviously all three are closely interrelated and it has been difficult to determine which, if any one, comes first. The end result, however, of T-cell dysfunction and abnormal cytokine production is the activation of B cells that produce pathogenic autoantibodies. These will pull our third troika. At the level of immune dysregulation of SLE there may be other participants. These include dendritic cells whose homeostasis is altered in murine lupus [85] and NK cells whose function is disrupted in human SLE [86], can regulate antibody production, and, through transforming growth factor-β (TGF-β), downregulate T-cell activity [87]. In turn, TGF-β has a role in the generation of $CD4^+CD25^+$ suppressor T cells that prevent $CD8^+$ T cells from proliferating in response to alloantigens and from becoming cytotoxic effector cells [88]. It should be recalled that a disturbed suppressor cell function was one of the first abnormalities of immune regulation encountered in SLE [89] and has reacquired attention for their potential role in autoimmunity [90].

T-Cell Dysfunction

That a T-cell dysfunction occurs in SLE has been amply demonstrated. In general, circulating blood

mononuclear cells from SLE patients show a decreased Th1 and an increased Th2 profile. T cells operating at the level of actively inflamed organs in SLE may belong to the Th1 and Th0 subsets [91], but in normal skin of lupus patients there is an accumulation of γδT cells that seems to correlate with disease activity [92]. In analogy to what has been described in the mouse, in human SLE there is a general decrease of cytokines produced by T cells to regulate other T cells. These include IL-2, TNF, IL-12, and interferon-γ [93]. In contrast, cytokines produced by Th2 cells are mostly B-cell growth, differentiation, and stimulatory factors (e.g., IL-4, IL-5, IL-6, IL-10). T-cell functions themselves are also affected, including the response to T-cell mitogens, decreased suppression of T cells on B cells, decreased contrasuppression, and decreased response in autologous mixed lymphocyte reactions [94]. The algebraic sum of these regulatory disturbances would tend to facilitate autoantibody production. Activation of T lymphocytes requires antigen-mediated signaling through the T-cell receptor as well as costimulatory signals transmitted through B7-1 and/or B7-2 with CD28. These costimulatory signals regulate autoantibody production in a murine lupus model as well as its ultimate renal pathology [95]. Impaired signaling in SLE T cells may, at least partly, be due to deficient protein kinase A phosphotransferase activity [96].

Polyclonal B-Cell Activation

Patients with SLE have hypergammaglobulinemia and characteristically produce a large number of autoantibodies. Whether these reflect passively the polyclonal B-cell activation itself, are antigen driven, or are the result of a somatic hypermutation of germline gene-encoded natural autoantibodies is difficult to tell at this time. The fate of autoreactive B cells within a monoclonal B-cell repertoire may be different from that in a polyclonal one [97]. If we return to the mice models, we find that anti-dsDNA B cells in nonautoimmune mice exit from the bone marrow to populate the spleen where they locate at the T–B interface of the splenic follicle where they have an increased *in vivo* turnover rate [98]. As opposed to anti-dsDNA B cells that locate in the spleen, those that produce natural autoantibodies to bromelain-treated erythrocytes, which may cause hemolytic anemia in NZB mice, locate in the peritonaeum [99]. It is thus possible that the various autoantibodies, including germline gene-encoded ones, emerge, escape deletion, or are subject to enhancement in SLE by different mechanisms. Also, as discussed in the first troika, genetic factors influence the appearance of particular autoantibodies in SLE.

Contrary to the way they had been considered in the past, B cells are not just the passive recipients of signals leading them to differentiate into antibody-producing plasma cells. Data on the autocrine production of cytokines by B cells that act as signals to themselves and to T lymphocytes, a recently described role in promoting the spontaneous activation of T cells [100], as well as the expression by them of lymphotoxin a/b, which is essential for the differentiation of follicular dendritic cells [101], indicate that B cells may have more active participation in the immune dysregulation of SLE than previously thought [102]. Whether this role is primary or results from the effects of T cells, monocytes, dendritic, or NK cells is yet unknown. A potential role for a B lymphocyte stimulator member of the TNF superfamily in triggering self-antigen-driven early B-cell activation in SLE has also been proposed [103]. It seems to be, however, that there are profound abnormalities in homeostasis in various B-cell compartments in SLE. Interestingly, B cells in each of these may respond differently to immunosuppressive therapy [104].

Cytokines

When we described the decreased production of, and response to, IL-2 in SLE [105], we thought this to be a primary event in the etiopathogenesis of SLE. We soon found that the production of, and response to, IL-1 is also defective [106]. It was found, however, that the production of IL-2 by lupus T cells would recover if these were allowed to rest in culture medium [107]. This suggested that decreased IL-2 production was the result of either exhaustion or secondary to the effect of other factors, including other cytokines and/or increased expression of increased of phosphorylated cAMP-responsive element modulator that binds to the –180 site of the IL-2 promoter [108]. The complexity of this became apparent when we and others found an increased production by T cells of B-cell-stimulating and differentiation factors in SLE patients [109, 110]. An important part of the riddle may have been solved when it was found that IL-10 both inhibits the production of IL-1 and IL-2 and enhances the production of Th2 cytokines (Fig. 3) and that monocytes and B cells from patients with SLE have an increased spontaneous production of IL-10 [111]. The role of IL-10 in B lymphocyte hyperactivity and autoantibody production of SLE was then tested both *in vitro* and *in vivo* and compared to those induced by IL-6, another cytokine suspected to play a role in these abnormalities [112]. The spontaneous *in vitro* production of immunoglobulins was increased modestly by recombinant (rIL)-6 but strongly by rIL-10 [112]. In severe combined immunodeficiency (SCID) mice injected with human SLE blood mononuclear cells, injection of the anti-IL-6 monoclonal antibody did not affect the serum concentration

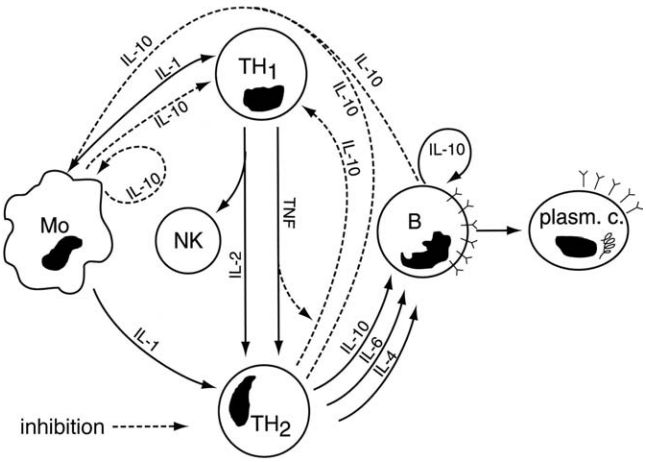


FIGURE 3 Cytokine networks in SLE, spontaneous production of IL-10 by monocytes, and B cells in SLE result in inhibition of IL-1 and IL-2 production with B-cell activation and autoantibody production.

of IgG or of anti-dsDNA IgG, whereas that of anti-IL-10 abrogated the production of human IgG anti-dsDNA without greatly decreasing the production of total human IgG. It would therefore seem that the production of anti-dsDNA by SLE B lymphocytes is largely IL-10 dependent and that the increased production of IL-10 by SLE monocytes and B cells is critical at the level of the second troika (etiopathogenesis). As a result of these observations, a pilot trial of the anti-IL-10 monoclonal antibody was carried out in six SLE patients whose disease required persistent corticosteroid treatment lest it be activated. The patients thus treated had remarkable responses to this form of treatment, supporting the notion of an important role of IL-10 in the immune dysregulation of SLE [113]. It was, however, found that monocytes and B cells from the healthy relatives of SLE patients also constitutively produce large amounts of IL-10 [114]. Interestingly, this increased production of IL-10 in lupus relatives was independent of age, sex, and of whether the relationship to the lupus patients was of first or second degree. This would pose two questions: if IL-10 is so important in the etiopathogenesis of SLE, how is it that lupus relatives who have high levels do not become ill? If we return to the first troika we find that a possible explanation could lie in the genetic makeup of patients as opposed to that of their relatives. These could have inherited the increased production of IL-10 but not the resulting effect on autoantibody production. In this regard it is of interest that the production of anti-ssDNA by mononuclear cells from SLE relatives in the SCID mice model is not affected by monoclonal anti-IL-10 (L. Llorente *et al.*, unpublished observations). The second and related question pertains to whether the IL-10 gene plays a role in the susceptibility to develop SLE. This gene has been located to chromosome q31–32 (the genome data base, www.gdb.org). Three studies have analyzed a microsatellite found in the promoter region of the IL-10 locus with controversial results. In one study there was association of polymorphisms in this locus [115], whereas in another study, associations with some alleles were only found when patients were subgrouped as per anti-Ro positivity or renal involvement [116].

In a third much larger study, no significant difference was found in the frequency of microsatellite alleles in the promoter region of IL-10 in 330 Mexican SLE patients compared to 368 controls of the same ethnic background [117]. Because ethnicity could explain the differences with the other two studies done in the United Kingdom, parametric linkage analyses were done in 22 northern European (14 Swedish and 8 Icelandic) and 13 Mexican multiplex families and again no linkage was revealed between the IL-10 gene promoter and SLE [117]. It seems, therefore, that as attractive as it might be, the hypothesis that the IL-10 gene promoter is implicated in SLE and explains the increased production of IL-10 in both patients and their relatives, the answer may lie elsewhere. One possibility could be that of associated altered production of a related cytokine. Increased serum levels of IL-13 and IL-15 have also been found in patients with SLE [94, 117].

Under the influences of the three elements of the first troika, the dysfunction of T cells, the activation of B cells, and the production of cytokines interact among themselves and lead to the production of pathogenic autoantibodies.

THE THIRD TROIKA: PATHOGENESIS

As a result of the immune dysregulation of the second troika and under the direct or indirect influence of the genetic, hormonal, and environmental factors described in the first Troika, a great variety of autoantibodies appear in the serum of patients with SLE. The nature of each of these autoantibodies may be reflecting the role of elements from these two Troikas (e.g., antihistones in drug-induced lupus, anticardiolipin antibodies by genes of the MHC, natural autoantibodies encoded by germline genes such as antibodies to phosphatidylcholine, or lack of arrest of autoreactive anti-dsDNA B cells in the spleen). Potential spreading of the immune reaction with a specific peptide segment of a molecule to other epitopes may also help explain the diversification of the immune response and the vast

array of autoantibodies. These, in turn, may have a pathogenic role. Their nature and variety, their immunochemical characteristics [118], and their potential to elicit anti-idiotypes will determine the resulting clinical manifestations or lupus subsets [119]. In turn, these characteristics, particularly their specificity and binding kinetics, may implicate different stimuli for their production [120]. There are too many variables to allow any one clinician to see two identical lupus patients. The third troika considers three mechanisms of potential damage done by autoantibodies: immune complex-mediated damage, a direct damaging effect, and functional effects of the autoantibodies that may either take place at this pathogenic level or revert to influence the second troika or even perhaps the first one.

Immune Complex-Mediated Damage

Lupus has long been considered as an immune complex-mediated disease (type III hypersensitivity of Coombs' and Gell classification) [121], but this is only one of the mechanisms whereby autoantibodies participate in the pathogenesis of the disease with a deposit of preformed circulating immune complexes at the site of damage. This is a rather passive or chance situation that will have no other specificity than that provided by the quantitative and qualitative (e.g., antigen excess) characteristics of complexes as it occurs in serum sickness. Higher specificity of their location is attained when the autoantibody seeks an antigen deposited in an organ (e.g., DNA attached to basement membranes of the skin or glomeruli) in an Arthus type of reaction. Either or both of these situations may participate in lupus nephritis, vasculitis, interstitial cystitis, pulmonary hemorrhage, neuronitis, and central nervous system involvement through immune complex deposition in the choroid plexus. The presence of a pathogenic idiotype in an autoantibody such as anti-dsDNA (e.g., the 16/6 idiotype) [122] may be another participating factor in this type of immune-mediated damage.

In vivo clearance of immune complexes may be influenced by Fcγ receptor polymorphisms that may be skewed toward genotypes yielding longer half-lives of IgG-coated erythrocytes [123]. This exemplifies the ample connections among the three troikas.

Direct Damaging Effects by Autoantibodies

Hemolytic anemia, thrombocytopenia, neutropenia, and perhaps some aspects of the lymphopenia of SLE are more likely due to a direct lytic effect by autoantibodies and/or facilitation of removal of these cells by the phagocytic system (type II hypersensitivity of Gell and Coombs' classification) [121]. There are two general types of autoantibody systems in lupus that may lead to this: autoantibodies directed to specific antigens on the cell surface and autoantibodies directed to more ubiquitous antigens, such as phospholipids and/or their protein cofactors [124]. Recognition of platelets by autoantibodies detected by conventional anticardiolipin assays requires platelet activation or aggregation, which causes the translocation of anionic phospholipids to the outer leaflet of the platelet wall. These anionic phospholipids are subsequently bound by proteins with a high affinity for anionic compounds, such as β2-glycoprotein-I (β2GPI). Upon binding to the anionic phospholipid now available on the surface of the platelet, β2GPI seems to suffer a conformation change that may expose cryptic epitopes in this protein. These are, in turn, recognized by autoantibodies putatively directed to cardiolipin but actually reactive with the protein at this epitopic site [124]. It is also possible that reactivity of β2GPI with the anionic phospholipid or even with an oxygenated plate causes it to be bound by antibodies with lower affinity that would otherwise not recognize the native molecule [125]. Other protein cofactors that have been implicated in the binding of these autoantibodies include prothrombin, thrombin, kininogen or kininogen-binding protein, annexin V, and perhaps protein C [124].

The occurrence of hemolytic anemia, both in SLE and as a variant of the primary antiphospholipid syndrome [126], as well as the terminal event in NZB mice, involves a different mechanism. Here a natural autoantibody to PTC recognizes this zwitterionic phospholipid on the erythrocyte surface when a glycoprotein that would impede it is removed either by the effect of proteolytic enzymes (e.g., bromelain) or upon aging of erythrocytes [127]. Because this antibody is normally present in all mammals tested so far, it is possible that for it to become pathogenic, either a subtle mutation or the participation of another autoantibody (e.g., Coombs' antibody) is required.

Neutropenia in SLE patients has also been found to associate with antibodies to phospholipids and/or its protein confactors [128]. The possible mechanism and specific autoantibody that may cause this have not been determined. Although the association has been found with anticardiolipin antibodies as detected in conventional enzyme-linked immunosorbent assays (ELISA), there may be ample cross-reactivity of these antibodies with those directed primarily to other phospholipids, including zwitterionic ones [129].

Functional Effects of Autoantibodies

In addition to the potential to cause cell or tissue damage, serum autoantibodies present in patients with

SLE can cause functional alterations that manifest themselves in the clinical spectrum of lupus patients or go back to the other troikas to influence immune regulation or even hormonal factors of the first troika (Fig. 2). Thus, antiprolactin antibodies have been found in patients with SLE having hyperprolactinemia [130] (Fig. 3).

The aforementioned antibodies to phospholipids or their protein cofactors can participate in phospholipid-dependent coagulation, natural anticoagulation processes, or both. As a result of this, an acquired (phenotypic) defect of protein C activation may occur in these patients that could lead to increased thrombogenesis [131]. In addition, because β2GPI may itself be a natural anticoagulant, antibodies to this protein could also favor thromboses.

Another mechanism whereby autoantibodies may cause functional disturbances is by their penetration into live cells. This mechanism was described in 1978 [132], but despite having being studied extensively by our group, it has been until only recently recognized as a potentially important phenomenon from both the standpoint of immunobiology and of immunopathology [133]. In our initial studies we were able to determine that the penetration of antibodies to RNP could cause immunoregulatory disturbances [134]. We also found that anti-RNP and anti-DNA antibodies could cause cell cycle arrest at different levels [135, 136] and that both could cause protracted cell death. It was later found that cell death caused by antibody penetration is due to apoptosis [137, 138]. Increased apoptosis in SLE could lead to an increased availability of nucleosomes, which because of inherent phagocytic disturbances may not be removed promptly and thus be capable of stimulating the antigen-driven production of anti-DNA/nucleosome antibodies. The LE cell phenomenon itself is an example of these mechanisms: causation of apoptosis by the penetration of the anti-DNA antibody and phagocytosis of the apoptotic body by a polymorphonuclear leukocyte [139]. Antibodies to ribosomal P proteins can also penetrate into live hepatocytes and cause cellular dysfunction [140]. This could explain the association of antiribosomal protein P antibodies and hepatic disease in SLE patients [141]. It is possible that the heart block caused by anti-Ro and/or anti-La antibodies to the offspring of pregnant lupus patients results from this same mechanism because antibodies penetrate best into immature cells [142]. A role for calreticulin as a surface receptor allowing the penetration of autoantibodies has been postulated [143], and increased expression of this protein has been found to be associated with congenital heart block [144].

SUMMARY

Our previous division of the etiopathogenesis of SLE into three consecutive levels continues to be pertinent, as is their respective subdivision making use of the troika analogy to emphasize how each of these are pulled concomitantly by at least three factors riding together. At the etiology level, genetic, hormonal, and environmental factors participate concertedly. In turn, genes involved in SLE may be multiple, behaving in an additive fashion, some acting as lupus susceptibility genes proper and others as disease-modifying genes. Some may have an epistatic role modulating the effect of other genes. As a result of the interplay of genetic, hormonal, and environmental factors, a level is reached where T-cell dysfunction, B-cell activation, and imbalance of cytokines cause a complex immune dysregulation. A result of this is the production of a vast array of autoantibodies that will be the main determinants of the clinicopathologic manifestations of SLE. At this pathogenic level autoantibodies may cause damage by forming immune complexes, by producing cell lysis, or by causing functional disturbances. These include, among others, an imbalance of coagulation–anticoagulation systems with resulting thrombophilia and penetration of autoantibodies into live cells, a process that may cause immune regulation disturbances, cellular dysfunction of specific cells, or apoptosis. A potential treatment of SLE is currently being sought at these various levels of disturbance.

References

1. Alarcón-Segovia, D. (1984). The pathogenesis of immune dysregulation in systemic lupus erythematosus: A Troika *J. Rheumatol*. **11**, 588.
2. Hochberg, M. C. (1987). The application of genetic epidemiology to systemic lupus erythematosus. *J. Rheumatol*. **14**, 867.
3. Lawrence, J. S., Martins, C. L., and Drake, G. L. (1987). A family survey of lupus erythematosus. 1. Heritability. *J. Rheumatol*. **14**, 913.
4. Hugot, J. P., Chamaillard, M., and Zouali, H. (2001). Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* **411**, 599.
5. Block, S. R. (1993). Twin studies: Genetic factors are important. *Arthritis Rheum*. **36**, 135.
6. Deapen, D., Escalante, A., Weinrib, L., *et al*. (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum*. **35**, 311.
7. Reichlin, M., Harley, J. B., and Lockshin, M. D. (1992). Serologic studies of monozygotic twins with systemic lupus erythematosus. *Arthritis Rheum*. **35**, 457.
8. Moore, J. E., and Lutz, W. B. (1955). The natural history of systemic lupus erythematosus: An approach to its

study through chronic biologic false positive reactors. *J. Chron. Dis*. **1**, 297.
9. Arbuckle, M. R., James, J. A., Kohalhase, K. F., *et al*. (2001). Development of anti-dsDNA antibodies before the clinical diagnosis of systemic lupus erythematosus. *Scand. J. Immunol*. **54**, 211.
10. Reveille, J. D. (1991). The molecular genetics of systemic lupus erythematosus and Sjogren's syndrome. *Curr. Opin. Rheumatol*. **3**, 722.
11. Arnett, F. C., and Reveille, J. D. (1992). Genetics of systemic lupus erythematosus. *Rheum. Dis. Clin. North. Am*. **18**, 865.
12. Grumet, F. C., Coukell, A., Bodmer, J. G., Bodmer, W. F., and McDevitt, H. O. (1971). Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus: A possible genetic predisposition to disease. *N. Engl. J. Med*. **285**, 193.
13. Gibofsky, A., Winchester, R. J., Patarroyo, M., Fotino, M., and Kunkel, H. G. (1978). Disease associations of the Ia-like human alloantigens: Contrasting patterns in rheumatoid arthritis and systemic lupus erythematosus. *J. Exp. Med*. **148**, 1728.
14. Reinertsen, J. L., Klippel, J. H., Johnson, A. H., Steinberg, A. D., Decker, J. L., and Mann, D. L. (1978). B- lymphocyte alloantigens associated with systemic lupus erythematosus. *N. Engl. J. Med*. **299**, 515.
15. Schur, P. H., Meyer, I., Garovoy, M., and Carpenter, C. B. (1982). Associations between systemic lupus erythematosus and the major histocompatibility complex: Clinical and immunological considerations. *Clin. Immunol. Immunopathol*. **24**, 263.
16. Arnett, F. C. (1985). HLA and genetic predisposition to lupus erythematosus and other dermatologic disorders. *J. Am. Acad. Dermatol*. **13**, 472.
17. James, J. A., Kaufman, K. M., Farris, A. D., Taylor-Albert, E., Lehman, T. J., and Harley, J. B. (1997). An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J. Clin. Invest*. **100**, 3019.
18. Morel, L., Rudofsky, U. H., Longmate, J. A., Schiffenbauer, J., and Wakeland, E. K. (1994). Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* **1**, 219.
19. Kono, D. H., Burlingame, R. W., and Owens, D. G. (1994). Lupus susceptibility loci in New Zealand mice. *Proc. Natl. Acad. Sci. USA* **91**, 10168.
20. Rozzo, S. J., Allard, J. D., Choubey, D., *et al*. (2001). Evidence for an interferon-inducible gene, *Ifi*202, in the susceptibility to systemic lupus. *Immunity* **15**, 435.
21. Morel, L., Blenman, K. R., Croker, B. P., and Wakeland, E. (2001). The major murine systemic lupus erythematosus susceptibility locus, *Sle1*, is a cluster of functionally related genes. *Proc. Natl. Acad. Sci. USA* **98**, 1787.
22. Song, Y. W., Han, C. W., Kang, S. W., *et al*. (1998). Abnormal distribution of Fc gamma receptor type IIa polymorphisms in Korean patients with systemic lupus erythematosus. *Arthritis Rheum*. **41**, 421.
23. Duits, A. J., Bootsma, H., Derksen, R. H., *et al*. (1995). Skewed distribution of IgG Fc receptor IIa (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. *Arthritis Rheum*. **38**, 1832.
24. Manger, K., Repp, R., Spriewald, B. M., *et al*. (1998). Fc gamma receptor IIa polymorphism in Caucasian patients with systemic lupus erythematosus: Association with clinical symptoms. *Arthritis Rheum*. **41**, 1181.
25. Botto, M., Theodoridis, E., Thompson, E. M., *et al*. (1996). Fc gamma RIIa polymorphism in systemic lupus erythematosus (SLE): No association with disease. *Clin. Exp. Immunol*. **104**, 264.
26. Wu, J., Edberg, J. C., Redecha, P. B., *et al*. (1997). A novel polymorphism of Fc gammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest*. **100**, 1059.
27. Koene, H. R., Kleijer, M., Swaak, A. J., *et al*. (1998). The Fc gammaRIIIA-158F allele is a risk factor for systemic lupus erythematosus. *Arthritis Rheum*. **41**, 1813.
28. Lander, E., and Kruglyak, L. (1995). Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. *Nature Genet*. **11**, 241.
29. Lindqvist, A. K., Steinsson, K., Johanneson, B., *et al*. (2000). A susceptibility locus for human systemic lupus erythematosus (hSLE1) on chromosome 2q. *J. Autoimmun*. **14**, 169.
30. Moser, K. L., Neas, B. R., Salmon, J. E., *et al*. (1998). Genome scan of human systemic lupus erythematosus: Evidence for linkage on chromosome 1q in African-American pedigrees. *Proc. Natl. Acad. Sci. USA* **95**, 14869.
31. Gaffney, P. M., Kearns, G. M., Shark, K. B., *et al*. (1998). A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc. Natl. Acad. Sci. USA* **95**, 14875.
32. Gaffney, P. M., Ortmann, W. A., Selby, S. A., *et al*. (2000). Genome screening in human systemic lupus erythematosus: Results from a second Minnesota cohort and combined analyses of 187 sib-pair families. *Am. J. Hum. Genet*. **66**, 547.
33. Shai, R., Quismorio, F. P., Jr., Li, L., *et al*. (1999). Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum. Mol. Genet*. **8**, 639.
34. Magnusson, V., Lindqvist, A. K., Castillejo-Lopez, C., *et al*. (2000). Fine mapping of the SLEB2 locus involved in susceptibility to systemic lupus erythematosus. *Genomics* **70**, 307.
35. Daly, M. J., Rioux, J. D., Schaffner, S. F., Hudson, T. J., and Lander, E. S. (2001). High-resolution haplotype structure in the human genome. *Nature Genet*. **29**, 229.
36. Weeks, D. E., Sobel, E., O'Connell, J. R., and Lange, K. (1995). Computer programs for multilocus haplotyping of general pedigrees. *Am. J. Hum. Genet*. **56**, 1506.
37. Patil, N., Berno, A. J., *et al*. (2001). Blocks of limited haplotype diversity revealed by high-resolution scanning of human chromosome 21. *Science* **294**, 1719.

38. Davies, E. J., Steers, G., Olliver, W. E., *et al*. (1995). Relative contributions of HLA-DQA and complement C4A loci in determining susceptibility to systemic lupus erythematosus. *Br. J. Rheumatol*. **34**, 221.
39. Hong, G. H., Kim, H. Y., Takeuchi, F., *et al*. (1994). Association of complement C4 and HLA-DR alleles with systemic lupus erythematosus in Koreans. *J. Rheumatol*. **21**, 442.
40. Skarsvag, S. (1995). The importance of C4A null genes in Norwegian patients with systemic lupus erythematosus. *Scand. J. Immunol*. **42**, 572.
41. Granados, J., Vargas-Alarcón, G., Andrade, F., *et al*. (1996). The role of HLA-DR alleles and complotypes though the ethnic barrier in systemic lupus erythematosus in Mexicans. *Lupus* **5**, 182.
42. Kaneoka, H., Hsu, K. C., Takeda, Y. L., Sharp, G. C., and Hoffman, R. W. (1992). Molecular genetic analysis of HLA-DR and HLA-DQ genes among anti-U1-70-kd autoantibody positive connective tissue disease patients. *Arthritis Rheum*. **35**, 83.
43. Kemp, M. E., Atkinson, J. P., and Skanes, V. M. (1987). Deletion of C4A genes in patients with systemic lupus erythematosus. *Arthritis Rheum*. **30**, 1015.
44. Reveille, J. D., MacLeod, M. J., and Whitington, K. (1991). Specific amino acids residues in the second hypervariable region of HLA-DQA1 and DQB1 chain genes promote Ro (SS-A/La(SS-B autoantibody responses. *J. Immunol*. **146**, 3871.
45. Rascu, A., Repp, R., Westerdaal, N. A, Alden, J. R., and van de Winkel, J. G. (1997). Clinical relevance of Fc gamma receptor polymorphism. *Ann. N.Y. Acad. Sci*. **815**, 382.
46. Salmon, J. E., Millard, S., Schachter, L. A., *et al*. (1996). FcγRIIA alleles are heritable risk factors for lupus nephritis in African Americans. *J. Clin. Invest*. **97**, 1348.
47. Duits, A. J., Bootsman, H., Derksen, R. H., *et al*. (1995). Sw?kewed distribution of IgG Fc receptor lia (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus. *Arthritis Rheum*. **18**, 1832.
48. Botto, M., Theodoridis, E., Thompson, E. M., *et al*. (1996). Fc gamma RIIa polymorphism in systemic lupus erythematosus (SLE): No association with disease. *Clin. Exp. Immunol*. **104**, 264.
49. Wu, J., Edberg, J. C., Redecha, P. B., *et al*. (1997). A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest*. **100**, 1059.
50. Clynes, R., Dumitru, C., and Ravetch, J. V. (1998). Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* **279**, 1052.
51. Watanabe-Fukunaga, R., Brennan, C. I., Copeland, N. G., *et al*. (1992). Lymphoproliferative disorder in mice explained by defects in as antigen that mediates apoptosis. *Nature* **356**, 314.
52. Rieux-Laucat, F., Le Deist, F., Hivroz, C., *et al*. (1995). Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* **268**, 1347.
53. Fisher, G. H., Rosenberg, F. J., Straus, S. E., *et al*. (1995). Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* **81**, 935.
54. Mysler, E., Bini, P., Drappa, J., *et al*. (1994). The apoptosis-1/fas protein in human systemic lupus erythematosus. *J. Clin. Invest*. **93**, 1029.
55. Howie, J. B., and Helyer, B. J. (1968). The immunology and pathology of NZB mice. *Adv. Immunol*. **9**, 215.
56. Lander, E. S., and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185.
57. Kruglyak, L., and Lander, E. S. (1995). High resolution genetic mapping of complex traits. *Am. J. Hum. Genet*. **56**, 1212.
58. Peng, S. L., Moslehi, J., Robert, M. E., and Craft, J. (1998). Perforin protects against autoimmunity in lupus-prone mice. *J. Immunol*. **160**, 652.
59. Watson, M. L., Rao, J. K., Gilkeson, G. S., *et al*. (1992). Genetic analysis of MRL-1pr mice: Relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. *J. Exp. Med*. **176**, 1645.
60. Carlsten, H., Holmdahl, R., and Tarkowski, A. (1991). Analysis of the genetic encoding of oestradiol suppression of delayed-type hypersensitivity in (NZBxNZW) F1 mice. *Immunology* **73**, 186.
61. Brick, J. E., Wilson, D. A., and Walker, S. E. (1985). Hormonal modulation of responses to thymus-independent and thymus-dependent antigens in autoimmune NZB/W mice. *J. Immunol*. **134**, 4693.
62. Blank, M., Mendlovic, S., Fricke, H., *et al*. (1990). Sex hormone involvement in the induction of experimental systemic lupus erythematosus by a pathogenic anti-DNA idiotype in naive mice. *J. Rheumatol*. **17**, 311.
63. Carlsten, H., Nilsson, N., Jonsson, R., and Tarkowski, A. (1991). Differential effects of estrogen in murine lupus: Acceleration of glomerulonephritis and amelioration of T cell-mediated lesions. *J. Autoimmun*. **4**, 845.
64. Melez, K. A., Reeves, J. P., and Steinberg, A. D. (1978). Modification of murine lupus by sex hormones. *Ann. Immunol.* (*Paris*) **129C**, 707.
65. Lavalle, C., Loyo, E., Paniagua, R., *et al*. (1990). Correlation study between prolactin and androgens in male patients with systemic lupus erythematosus. *J. Rheumatol*. **14**, 268.
66. Folomeev, M., Prokaeva, T., Nassonova, V., *et al*. (1990). Prolactin levels in men with SLE and RA. *J. Rheumatol*. **17**, 1569.
67. Russell, D. H., Kibler, R., Matrisian, L., *et al*. (1985). Prolactin receptors on human T and B lymphocytes: Antagonism of prolactin binding by cyclosporine. *J. Immunol*. **134**, 3027.
68. Pellegrini, Y., Lebrun, J. J., Ali, S., and Kelly, P. A. (1992). Expression of prolactin and its receptor in human lymphoid cells. *Mol. Endocrinol*. **6**, 1023.
69. Vidaller, A., Llorente, L., Larrea, F., *et al*. (1986). T-cell dysregulation in patients with hyperprolactinemia: Effect of bromocriptine treatment. *Clin. Immunol. Immunopathol*. **38**, 337.

70. Larrea, F., Martínez-Castillo, A., Cabrera, V., *et al*. (1997). A bioactive 60-kilodalton prolactin species is preferentially secreted in cultures of mitogen-stimulated and non-stimulated peripheral blood mononuclear cells from subjects with systemic lupus erythematosus. *J. Clin. Endocrinol. Metab*. **82**, 3664.
71. Jacobi, A. M., Rohde, W., Volk, H.-D., *et al*. (2001). Prolactin enhances the *in vitro* production of IgG in peripheral blood mononuclear cells from patients with systemic lupus erythematosus but not from healthy controls. *Ann. Rheum. Dis*. **60**, 242.
72. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1979). Alterations of estrogen metabolism in systemic lupus erythematosus. *Arthritis Rheum*. **22**, 1195.
73. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1981). Icreased 16α-hydroxylation of estradiol in systemic lupus erythematosus. *J. Clin. Endocrinol. Metab*. **53**, 174.
74. Bucala, R., Lahita, R. G., Fishman, J., and Cerami, A. (1985). Increased levels of 16 alpha-hydroxyestrone-modified protein in pregnancy and systemic lupus erythematosus. *J. Clin. Endocrinol. Metab*. **60**, 841.
75. Lahita, R. G., Bradlow, H. L., Ginzler, E., and Pang, S. (1987). New M: Low plasma androgens in women with systemic lupus erythematosus. *Arthritis Rheum*. **30**, 241.
76. Lahita, R. G., Bradlow, H. L., Fishman, J., and Kunkel, H. G. (1982). Abnormal estrogen and androgen metabolism in the human with systemic lupus erythematosus. *Am. J. Kidney Dis. Suppl*. **2**, 206.
77. Mackworth-Young, C. G., Parke, A. L., Morley, K. D., Fotherby, K., and Hughes, G. R. V. (1983). Sex hormones in male patients with systemic lupus erythematosus: A comparison with other disease groups. *Eur. J. Rheum. Inflamm*. **6**, 228.
78. Sequeira, J. F., Keser, G., Greenstein, B., *et al*. (1993). Systemic lupus eryematosus: Sex hormones in male patients. *Lupus* **2**, 315.
79. Lahita, R. G., and Bradlow, H. L. (1987). Klinefelter's syndrome: Hormone metabolism in hypogonadal males with systemic lupus erithematosus. *J. Rheumatol*. **14**(Suppl. 3), 154.
80. Woosley, R. L., Drayer, D. E., Reidenberg, M. D., *et al*. (1978). Effect of acetylatorphenotype on the rate at which procainamide induces antinuclear antibodies and the lupus syndrome. *N. Engl. J. Med*. **298**, 1157.
81. Reidenberg, M. M., Levy, M., Drayer, D. E., Zylber-Katz, E., and Robbins, W. C. (1980). Acetylator phenotype in idiopathic systemic lupus erythematosus. *Arthritis Rheum*. **23**, 569.
82. James, J. A., Kaufman, K. M., Farris, A. D., *et al*. (1997). An increased prevalence of Epstein-Barr virus infection in young patients suggest a possible etiology for systemic lupus erythematosus. *J. Clin. Invest*. **100**, 3019.
83. Vaughan, J. H. (1997). The Epstein-Barr virus and systemic lupus erythematosus. *J. Clin. Invest*. **100**, 2939.
84. Satoh, M., and Reeves, W. H. (1994). Induction of lupus-associated autoantibodies in BALB/c mice by intraperitoneal injection of pristane. *J. Exp. Med*. **180**, 2341.
85. Kalled, S. L., Cutler, A. H., and Burkly, L. C. (2001). Apoptosis and altered dentritic cell homeostasis in lupus nephritis are limited by anti-CD154 treatment. *J. Immunol*. **167**, 1740.
86. Gonzáez-Amaro, R., Alcocer-Varela, J., and Alarcón-Segovia, D. (1988). Natural killer cell activity in the systemic connective tissue disease. *J. Rheumatol*. **15**, 223.
87. Horwitz, D. A., Gray, J. D., Ohtsuka, K., *et al*. (1997). The immunoregulatory effects of NK cells: The role of TGF-β and implications for autoimmunity. *Immunol. Today* **18**, 538.
88. Yamagiwa, S., Gray, J. D., Hashimoto, S., and Horwitz, D. A. (2001). A role of TGF-β in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. *J. Immunol*. **166**, 7282.
89. Ruíz-Argüelles, A., Alarcón-Segovia, D., Llorente, L., and Del Giudice-Knipping, J. A. (1980). Heterogeneity of the spontaneously expanded and mitogen-induced generation of suppressor cell function of cells on B cells in systemic lupus erythematosus. *Arthritis Rheum*. **23**, 1004.
90. Shevach, E. M. (2000). Regulatory T cells in autoimmunity. *Annu. Rev. Immunol*. **18**, 423.
91. Converso, M., Bertero, M. T., Vallario, A., and Caligaris-Cappio, F. (2000). Analysis of T cell clones in systemic lupus erythematosus. *Haematologica* **85**, 118.
92. Robak, E., Niewiadomska, H., Robak, T., Bartkowiak, J., Pomorski, L., and Sysa-Jedrzejowska (2001). Lymphocytes Tγδ in clinically normal skin and peripheral bood of patients with systemic lupus erythematosus and their correlation with disease activity. *Mediators Inflamm*. **10**, 179.
93. Horwitz, D. A., Gray, J. D., Behredsen, S. C., Kubin, M., *et al*. (1998). Decreased production of interleukin-12 and other Th-1-type cytokines in patients with recent-onset systemic lupus erythematosus. *Arthritis Rheum*. **41**, 838.
94. Alarcón-Segovia, D., Alcocer-Varela, J., and Díaz-Jouanen, E. (1985). The connective tissue diseases as disorders of immune regulation. *Clin. Rheum. Dis*. **11**, 451.
95. Liang, B., Kashgarian, M. J., Sharpe, A. H., and Mamula, M. J. (2000). Autoantibody responses and pathology regulated by B7-1 and B7-2 costimulation in MRL/lpr lupus. *J. Immunol*. **165**, 3436.
96. Mishra, N., Khan, I. U., Tsokos, G. C., and Kammer, G. M. (2000). Association of deficient type II protein kinase A activity with aberrant nuclear translocation of the RIIB subunit in systemic lupus erythematosus T lymphocytes. *J. Immunol*. **165**, 2830.
97. Cyster, J. G., and Godnow, C. C. (1995). Antigen-induced exclusion from follicles and anergy are separate and complementary processes that influence peripheral B cell fate. *Immunity* **3**, 691.
98. Mandik-Nayak, L., Bui, A., Noorchashm, H., Eaton, A., and Erikson, J. (1997). Regulation of anti-double-stranded DNA B cells in nonautoimmune mice: Localization to the T-B interface of the splenic follicle. *J. Exp. Med*. **186**, 1257.
99. Cabral, A. R., and Alarcón-Segovia, E. (1996). Bromelain-treat erythrocyte autoantibodies. *In* "Auto-

antibodies" (J. B. Peter and Y. Shoenfeld, eds.), pp. 120–125. Elsevier Science, Amsterdam.
100. Chan, O., and Shlomchlk, M. J. (1998). A new role for B cells in systemic autoimmunity: B cells promote spontaneous T cell activation in MRL-*lpr/lpr* mice. *J. Immunol*. **160**, 51.
101. González, M., Mackay, F., Browning, J. L., Kosko-Vilbois, M. H., and Noelle, R. J. (1999). The sequential role of lymphotoxin and B cell in the development of splenic follicles. *J. Exp. Med*. **187**, 997.
102. Lipsky, P. E. (2001). Systemic lupus erythematosus: An autoimmune disease of B cell hyperactivity. *Nature Immunol*. **2**, 764.
103. Zhang, J., Roschke, V., Baker, K. P., *et al*. (2001). A role for B lymphocyte stimulator in systemic lupus erythematosus. *J. Immunol.* **166**, 6.
104. Odendahl, M., Jacobi, A., Hansen, A., *et al*. (2000). Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J. Immunol.* **165**, 5970.
105. Alcocer-Varela, J., and Alarcón-Segovia, D. (1982). Decreased production of and response to interleukin-2 by cultured lymphocytes from patients with systemic lupus erythematosus. *J. Clin. Invest*. **69**, 1388.
106. Alcocer-Varela, J., Laffón, A., and Alarcón-Segovia, D. (1984). Defective monocyte production of and T lymphocyte response to, interleukin-1 in the peripheral blood of patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **55**, 125.
107. Huang, Y. P., Pertin, L. H., Miescher, P. A., and Zubler, R. H. (1988). Correlation of T and B cell activities *in vitro* and serum IL-2 levels in systemic lupus erythematosus. *J. Immunol.* **141**, 827.
108. Solomou, E. E., Juang, Y.-T., Gourley, M. F., Kammer, G. M., and Tsokos, G. C. (2001). Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus. *J. Immunol.* **166**, 4216.
109. Hirose, S., Ueda, G., Noguchi, K., *et al*. (1986). Requirement of H-2 heterozygozity for autoimmunity in (NZBxNZW)F1 hybrid mice. *Eur. J. Immunol.* **16**, 1631.
110. Martínez-Cordero, E., Alcocer-Varela, J., and Alarcón-Segovia, D. (1986). Stimulating and differentiation factors for human B lymphocytes in systemic lupus erythematosus. *Clin. Exp. Immunol*. **65**, 598.
111. Llorente, L., Richaud-Patin, Y., Wijdenes, J., *et al*. (1993). Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur. Cytokine. Netw*. **4**, 421.
112. Llorente, L., Zou, W., Levy, Y., *et al*. (1995). Role of interleukin-10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med*. **181**, 839.
113. Llorente, L., Richaud-Patin, Y., and García-Padilla, C. (2000). Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. *Arthritis Rheum*. **43**, 1790.
114. Llorente, L., Richaud-Patin, Y., Coudere, J., *et al*. (1997). Dysregulation of interleukin-10 production in relatives of patients with systemic lupus erythematosus. *Arthritis Rheum*. **40**, 1429.
115. Eskdale, J., Wordsworth, P., Bowman, S., Field, M., and Gallagher, G. (1977). Association between polymorphisms at the human IL-10 locus and systemic lupus erythematosus. *Tissue Antigen* **49**, 635.
116. Lazarus, M., Hajeer, A. H., Turner, D., *et al*. (1997). Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. *J. Rheumatol*. **24**, 2314.
117. Alarcón-Riquelme, M. E., Lindqvist, A. K. B., Jonasson, Y., *et al*. (1999). Genetic analysis of the contribution of IL-10 to systemic lupus erythematosus. *J. Rheumatol*. **26**, 2148.
118. Vargas, M. T., Gustilo, K., Andrea, D. M., *et al*. (1997). Structural features of nephritogenic lupus autoantibodies. *Immun. Methods* **11**, 62.
119. Alarcón-Segovia, D., and Díaz-Jouanen, E. (1980). Lupus subsets: Relationship to genetic and environmental factors. *Semin. Arthritis Rheum*. **10**, 18.
120. Eivazova, E. R., McDonnell, J. M., Sutton, B. J., and Staines, N. A. (2001). Specificity and binding kinetics of murine lupus anti-DNA monoclonal antibodies implicate different stimuli for their production. *Immunology* **101**, 171.
121. Male, D. (1996). Hypersensitivity: Type II. *In* "Immunology" (Y. Roitt, J. Brostoff, and D. Male, eds.), pp. 23.1–23.10. Mosby, London.
122. Buskila, D., and Shoenfeld, Y. (1994). Anti-DNA antibodies: Their idiotypes and SLE. *Clin. Rev. Allergy*. **12**, 237.
123. Dijstelbloem, H. M., Bijl, M., Fijnheer, R., *et al*. (2001). Fcγ receptor polymorphisms in systemic lupus erythematosus: Association with disease and in clearance of immune complexes. *Arthritis Rheum*. **43**, 2793.
124. Alarcón-Segovia, D., and Cabral A. R. (1994). Antiphospholipid antibodies: Where do they come from? Where do they go? *J. Rheumatol*. **21**, 982.
125. Roubey, R. A. S., Eisenberg, R. A., Harper, M. F., and Winfield, J. B. (1995). "Anticardiolipin" autoantibodies recognize B2-glycoprotein I in the absence of phospholipid: Importance of antigen density and bivalent binding. *J. Immunol*. **154**, 954.
126. Alarcón-Segovia, D., and Cabral, A. R. (1996). The antiphospholipid/cofactor syndromes. *J. Rheumatol*. **23**, 1319.
127. Cabral, A. R., Cabiedes, J., and Alarcón-Segovia, D. (1990). Hemolytic anemia related to an IgM autoantibody to phosphatidylcholine that binds *in vitro* to stored and to bromelain-treated human erythrocytes. *J. Autoimmun*. **3**, 773.
128. Alarcón-Segovia, D., Pérez-Vázquez, M. E., Villa, A. R., Drenkard, C., and Cabiedes, J. (1992). Preliminary classification criteria for the antiphospholipid syndrome within systemic lupus erythematosus. *Semin. Arthritis Rheum*. **21**, 275.
129. Cabral, A. R., Cabiedes, J., and Alarcón-Segovia, D. (1992). Phospholipid specificity and requirement of β2-glycoprotein-1 for reactivity of antibodies from patients with primary antiphospholipid syndrome. *J. Autoimmun*. **5**, 787.

130. Leanos, A., Pascoe, D., Fraga, A., and Blanco-Favela, F. (1998). Anti-prolactin autoantibodies in systemic lupus erythematosus patients with associated hyperprolactinemia. *Lupus* **7**, 398.
131. Ruíz-Argüelles, G. J., Garcés-Eisele, J., Alarcón-Segovia, D., and Ruíz-Argüelles, A. (1996). Activated protein C resistance phenotype and genotype in patients with primary antiphospholipid syndrome. *Blood Coagul. Fibrinol*. **7**, 344.
132. Alarcón-Segovia, D., Ruíz-Argüelles, A., and Fishbein, E. (1978). Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors. *Nature* **271**, 67.
133. Alarcón-Segovia, D., Ruíz-Argüelles, A., and Llorente, L. (1996). Broken dogma: Penetration of autoantibodies into living cells. *Immunol. Today* **17**, 163.
134. Alarcón-Segovia, D., Ruíz-Argüelles, A., and Llorente, L. (1979). Antibody penetration into living cells. II. Anti-ribonucleoprotein IgG penetrates into T lymphocytes causing their deletion and the abrogation of suppressor function. *J. Immunol*. **122**, 1855.
135. Alarcón-Segovia, D., Llorente, L., and Ruíz-Argüelles, A. (1982). Antibody penetration into living cells. III. Effect of antiribonucleoprotein IgG on the cell cycle of human peripheral blood mononuclear cells. *Clin. Immunol. Immunopathol*. **23**, 22.
136. Alarcón-Segovia, D., Llorente, L., Fishbein, E., and Díaz-Jouanen, E. (1982). Abnormalities in the content of nucleic acids of peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Arthritis Rheum*. **25**, 304.
137. Alarcón-Segovia, D., Llorente, L., Ruíz-Argüelles, A., Richaud-Patin, Y., and Pérez-Romano, B. (1995). Penetration of anti-DNA antibodies into mononuclear cells (MNC) causes apoptosis. *Arthritis Rheum*. **38**, S182. [Abstract]
138. Alarcón-Segovia, D., Llorente, L., and Ruíz-Argüelles, A. (1996). The penetration of autoantibodies into cell may induce tolerance to self by apoptosis of autoreactive lymphocytes and cause autoimmune disease by dysregulation and/or of cell damage. *J. Autoimmun*. **9**, 295.
139. Schmid-Acevedo, S., Pérez-Romano, B., and Ruíz-Argüelles, A. (2000). LE cells result from phagocytosis of apoptotic bodies induced by antinuclear antibodies. *J. Autoimmun*. **15**, 15.
140. Koscec, M., Koren, E., Wolfson-Reichlin, M., *et al*. (1997). Autoantibodies to ribosomal P proteins penetrate into live hepatocytes and cause cellular dysfunction in culture. *J. Immunol*. **159**, 2033.
141. Hulsey, M., Goldstein, R., Scully, S., Surbeck, W., and Reichlin, M. (1995). Antiribosomal P antibodies in systemic lupus erythematosus: A case-control study correlating hepatic and renal disease. *Clin. Immunol. Immunopathol*. **74**, 252.
142. Alarcón-Segovia, D., Llorente, L., and Ruíz-Argüelles, A. (1996). Autoantibodies that penetrate into living cells. *In* "Autoantibodies" (J. B. Peter and Y. Shoenfeld, eds.), pp. 96–102. Elsevier Science, Amsterdam.
143. Seddiki, N., Nato, F., Lafaye, P., Amoura, Z., Piette, J.-C. H., and Mazié, J.-C. (2001). Calreticulin, a potential cell surface receptor involved in cell penetration of anti-DNA antibodies. *J. Immunol*. **166**, 6423.
144. Nakamura, K., Robertson, M., Liu, G., Dickie, P., Nakamura, K., Qing-Guo, J., Duff, H., Opas, M., Kavanagh, K., and Michalak, M. (2001). Complete heart block and sudden death in mice overexpressing calreticulin. *J. Clin. Invest*. **107**, 1245.

4

MHC CLASS II AND NON-MHC GENES IN THE PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

John D. Reveille
George S. Bruce Jr.

INTRODUCTION

The actual cause of systemic lupus erythematosus (SLE) is unknown. No environmental agent, whether chemical, infectious, or otherwise, has been implicated. However, the importance of genetic influences is being increasingly recognized. After numerous reports of familial aggregation in this disease, the first genetic factors to be identified as important in the pathogenesis of SLE were those of the major histocompatibility complex (MHC) on chromosome 6 in the early 1970s. Until recently, most of the studies of markers in SLE have focused on this region.

It is most widely accepted, however, that MHC genes constitute only a part of the genetic susceptibility to SLE. The past few years have seen a great deal of effort in defining other genes outside the MHC, and the results from such searches have already pinpointed other chromosomal regions, where numerous genes reside whose products are critical in the immune response.

In the previous edition of this book [1], this author stated: "by the time the next edition of this book is published, the genetic contribution to SLE probably will be almost completely defined." Despite the huge number of studies (with over 150 new investigations included in this chapter since the last edition in early 1998), the past 4 years have, unfortunately, fallen short of that prediction. One of the best explanations is the clinical heterogeneity of SLE, which requires such large numbers of patients and families to attain the necessary statistical power that few studies have approached this.

This chapter reviews the genetic basis for SLE, the familial aggregation in SLE (in way of historical perspective and in establishing the genetic basis of this disease), the current status of genome-wide scans in families of SLE patients (including defining the terminology relevant to genetic studies), organization of the MHC, whose numerous genes have been studied extensively in their relation to SLE, the association of MHC genes and susceptibility to SLE, the autoantibody subsets of SLE and their MHC class II associations, and finally the status of the ongoing search for non-MHC genes in SLE susceptibility.

FAMILIAL AGGREGATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

The classic method to show a genetic basis for any disorder is to demonstrate that it occurs more commonly in family members than would be expected by its frequency in the general population. The major *caveat* in this rationale is that environmentally triggered diseases would also be more likely to occur at the same location, such as in a single household. Therefore,

proving familial aggregation *per se* does not establish that a disease is transmitted genetically. Instead, where familial aggregation is established, showing that nonconsanguinous household contacts (such as spouses) are not as likely to develop the disease in question, that affected family members are more likely to develop the disorder around the same age and not at the same time chronologically, and, finally, in twin studies, that monozygotic (identical) twins are more likely to concordant for the disease than dizygotic (fraternal) twins indicates nature instead of nurture.

Family Studies

Familial Aggregation in SLE

Reports of familial aggregation in SLE date from as early as 1903, when Sequera [2] reported its occurrence in two sisters. Since then, there have been numerous other reports (reviewed in Reveille [1]). Siegel *et al*. [3] were the first to examine the prevalence of SLE in relatives of SLE patients, finding no evidence of significant familial clustering. Estes and Christian [4] reported seven (10%) of their cohort of 150 North American SLE patients to have first-degree relatives similarly affected. Buchman *et al*. [5] reported, from a cohort of 340 patients with SLE, that 41 (12%) had affected relatives of whom 5 had multiply affected family members. Hochberg [6] carried out a case–control study of 77 SLE patients and 77 matched controls, and found significant familial aggregation, with eight of the patients (10%) vs one of the controls (1%) having a similarly affected first-degree relative. Lawrence *et al*. [7] examined first-degree relatives and spouses of 36 patients with SLE and 37 with discoid LE, finding definite SLE in 3.9% of SLE probands and in 0.3% of controls, but not at all in spouses of SLE patients. Koskenmies *et al*. [8] reported a cross-sectional nation-wide clinical study of familial systemic lupus erythematosus in Finland and identified a prevalence of familial SLE of 4–5%. No differences were found in the clinical presentation of SLE between familial and sporadic cases. More recently, Michel *et al*. [9] reported a frequency of SLE in first-degree relatives in 8% of 137 French-Caucasian SLE probands.

Relationships between Affected Family Members

Virtually every kind of familial relationship between relatives with SLE has been reported (reviewed in Reveille [1]). As would be expected from the propensity of SLE to occur in women, the overwhelming majority of familial cases involved sister:sister or mother:daughter pairs. While this might raise the possibility of X-linked transmission, Lahita *et al*. [10] presented four kindreds in whom there were father–son pairs affected with SLE, suggesting that in these families genetic transmission may have been similar to that in the BXSB mouse, where there is a disease-linked gene on the Y chromosome, with resultant male–male transmission.

Concordance for SLE Features and Other Autoimmune Diseases in Family Members of SLE Patients

Arnett *et al*. [11] examined eight families multiplex for SLE and reviewed previously reported familial cases and found that an impressive concordance for disease features was found in identical twin and parent : offspring pairs and not for sib pairs (suggesting more of a genetic than an environmental contribution). However, studies of the *timing* of disease onset between family members with SLE have suggested that environmental factors may also be at work in triggering disease. In the same study of Arnett *et al*. [11], the average difference in *time* at disease onset in nontwin sibling pairs was only 3 years, whereas the average difference between *ages* at onset was 9 years. Kaplan [12] also found a shorter interval between the times of onset compared with the ages at SLE onset in the affected siblings, underscoring the importance of nongenetic factors.

The higher frequency of other autoimmune diseases in family members of SLE patients has also been long observed [13, 14–16], particularly autoimmune thyroid disease [13, 14], inflammatory arthritis [especially rheumatoid arthritis (RA)] [1, 17, 18], and even scleroderma [16–19]. Larsen [15] found that thyroid disease was four times more frequent in family members of SLE patients who themselves also had thyroid disease than in relatives of SLE patients who did not have this complication. Dubois *et al*. [17] found 5.5% of his cohort of 520 SLE patients had a family member with RA. However, Siegel *et al*. [3] could not document an increased frequency of RA in 155 relatives of 66 SLE patients. Flores *et al*. [20] described eight families where one family member had scleroderma and the other SLE. What was particularly noteworthy about this latter report was that even though five of the six pairs studied for HLA antigens shared haplotypes, seven of the eight pairs did not live in the same household at the time disease developed, suggesting a genetic predisposition. Arnett *et al*. [21] reported 19 extended multiplex SLE families, finding 13% of the relatives to have other immune-mediated disorders and 26% to express autoantibodies [including antinuclear antibodies (ANA), anti-ss DNA, and/or biologic false-positive tests for syphilis (BFP-STS)]. Considering the presence of other autoimmune disease or these autoantibodies as an

TABLE 1 Serologic Abnormalities in Family Members of SLE Patients[a]

Antibody	Technique	Patients	Relatives	Controls
Antinuclear antibodies (ANA)	IIF on tissue substrate	72–100%	4–50%	4–14%
ANA	IIF on Hep-2 cells	75–100%	17–33%	19%
Anti-ssDNA	Modified Farr	70%	23%	n.a.[b]
Anti-ssDNA	ELISA	40%	20%	0%
Anti-dsDNA	Farr	74%	5%	0%
Anti-dsDNA	*Crithidia lucilae*	14–56%	1%	0%
Anti-Ro (SS-A)	Immunodiffusion	25%	1%	0%
Anti-Ro (SS-A)	ELISA	60–80%	28%	6%
Anti-RNP	Immunodiffusion	8–40%	0%	0%
Anti-Sm	Immunodiffusion	5–12%	1%	0%
Anti-Sm	ELISA	n.a.[b]	12%	2%

[a] modified from a review of previous studies by Miles and Isenberg [36].
[b] Not available.

autoimmune "trait," formal genetic analysis of these kindreds suggested the presence of an autosomal-dominant non-MHC-linked "autoimmune gene" that is epistatic to other "secondary" genes (such as HLA class II) in influencing the tendency to develop autoimmunity [22]. This consideration has been largely replaced by more sophisticated genome-wide scans covered later in this chapter.

Other Immunologic Abnormalities in Family Members of SLE Patients (Table 1)

Antinuclear Antibodies

Since the initial reports of antinuclear antibodies in family members of SLE patients in 1960–1961 using tissue substrates [9, 23–34], the increased frequency of a positive ANA has been well documented [1] with frequencies ranging from 4% [3, 28] to 50% [34]. In most studies, the presence of ANA did not correlate with clinical abnormalities in the non-SLE family members; however, in a study by Sato *et al*. [35] of 120 first-degree relatives of 25 SLE patients, the presence of ANA in non-SLE family members (as determined by a *Hep2* assay) was associated with a higher frequency of arthralgias and Raynaud's phenomenon. These and most of the following data on serologic abnormalities in relatives of SLE patients have been reviewed [36].

Antiextractable Nuclear Antigen Antibodies (ENA) and Anticardiolipin Antibodies (aCL)

In contrast to antinuclear antibodies, anti-Ro, La, Sm, and RNP autoantibodies occur uncommonly in healthy family members of SLE patients. Their frequency varies as to the technique utilized. Studies examining these autoantibodies using less sensitive immunodiffusion technique found them to occur rarely in healthy relatives [13, 35]. The use of more sensitive ELISAs in determining ENA autoantibodies has increased the frequency of anti-Ro antibodies in healthy relatives to 29% and of anti-Sm to 13% [37]. The clinical significance of this, however, is unclear. More recently, Van der Linden *et al*. [38] tested plasma from 50 patients with SLE, 154 unaffected first-degree family members, and 330 healthy controls for the presence of autoantibodies (by ELISA). Seventy-four percent of patients, 32% of first-degree family members, and 1.5% of healthy controls had antibodies against any nuclear antigen. Most frequent autoantibodies in the patients were antihistone and anti-Ro (SS-A), whereas in family members these were anti-RNP-C and anti-Topo-I/Scl. The presence and specificity of autoantibodies in family members were independent of the presence or absence of that autoantibody in the probands with SLE.

Radway-Bright *et al*. [39] reported an increased frequency of IgG antiphospholipid antibodies in healthy first-degree relatives of SLE patients with antiphospholipid antibody syndrome relative to both nonconsanguinous household contacts and unrelated controls.

Antipolynucleotide Antibodies and Lymphocytotoxic Antibodies

DeHoratius *et al*. [40, 41] examined North American SLE patients, their family members, and nonconsanguinous household contacts for the presence of anti-DNA and RNA and lymphocytotoxic autoantibodies. Anti-DNA and RNA autoantibodies were found in the SLE patients and their relatives, but not in household contacts. However, lymphocytotoxic antibodies were

found in all, including close nonconsanguinous household contacts, leading to the conclusion that both genetic and environmental influences were at work. Cleland *et al*. [42] examined 103 members of four multiplex Canadian SLE kindreds. Over 50% of SLE family members and nonconsanguinous household contacts had positive ANA as compared to 5% of controls, further suggesting environmental agents. Lymphocytotoxic antibodies, however, were not increased in non-SLE family members or controls. Folomeeva *et al*. [43] confirmed the increased frequency of antipolynucleotide antibodies in 15 Russian SLE probands and 41 of their relatives, finding an increased frequency compared to control families. However, nonconsanguinous household contacts were not examined. Eroglu and Kohler [44] examined 26 North American families and found antilymphocytotoxic antibodies in 83% of SLE patients, 50% of family members, and only 11% of nonconsanguinous household members. Malave *et al*. [45] examined 50 Venezuelan SLE patients and 109 of their relatives, finding an increased frequency of antilymphocytotoxic antibodies in consanguineous relatives and in unrelated spouses compared to controls. Hazelton [46], as well as LePage *et al*. [47], however, could not confirm an increase in antilymphocyte or lymphocytotoxic antibodies in British studies of SLE families.

Antisingle-stranded DNA antibodies commonly occur in family members of lupus patients, having been in 20–30% [13, 31, 47, 51], whereas anti-dsDNA antibodies are rare. Studies employing the less specific Farr or ELISA assays report frequencies of anti-dsDNA antibodies up to 23%, respectively [34, 47, 48]. However, using the more specific *Crithidia lucillae* assay, the frequency approaches zero [13, 31, 49].

Abnormalities in T- and B-Cell Function and IL-10 Production

Miller and Schwartz [50] documented abnormalities in suppressor T-cell function in 11 of 15 SLE patients and in 26% of their female relatives, which did not correlate with the presence of antilymphocytotoxic antibodies. Mendlovic *et al*. [51] examined proliferative T-cell responses to an anti-DNA idiotypic DNA antibody in 38 Israeli SLE patients and 18 of their first-degree relatives, finding a lower response in both patients and family members. Haug *et al*. [33] found the healthy relatives of SLE patients to have a decrease in the synthesis of poly(ADP-ribose), a nucleic acid polymer involved in several cell functions, and suggested that abnormalities in the function of the poly(ADP-ribose) polymerase may be a predisposing factor to SLE. Isenberg *et al*. [31] examined 44 SLE patients and 147 healthy first-degree relatives for the presence of anti-DNA antibody idiotypes, finding elevated levels in 40% of patients and 24% of relatives, suggesting a higher frequency of certain disease-relevant immunoglobulin genes to be present in SLE families. Tomana *et al*. [52] examined 30 patients with SLE and 58 members of an additional five families with multiple members with SLE and other autoimmune diseases, finding abnormal galactosylation of IgG in 60% of the patients and family members. The importance of this finding has been underscored by the discovery that a mutation of the gene encoding α-mannosidase II, which regulates protein glycosylation, results in a systemic autoimmune disease in mice resembling SLE [53].

van der Linden *et al*. [54] determined IL-10 production after endotoxin stimulation in 163 first-degree relatives of patients with systemic lupus, 50 first-degree relatives of patients with chronic cutaneous lupus, and 133 control persons. IL-10 production was higher in the families of patients with systemic lupus than in the control families. Similarly, Grondal *et al*. [55] studied IL-10 as well as levels of IgG and ANA in SLE patients and their first-degree relatives and spouses in Icelandic SLE multicase families. The SLE patients had a significantly higher number of IL-10-producing cells compared with both first-degree relatives and healthy controls, although first-degree relatives also had a significantly higher number of IL-10-producing cells compared with healthy controls. Conversely, this was also true for the spouses of SLE patients, who had a higher number of IL-10-producing cells compared with matched healthy controls, which suggests both genetic and environmental factors influencing the development of immunologic abnormalities in family members of patients with SLE.

Twin Studies

Block *et al*. [56, 57] reported the first series of nine North American twin pairs, with a concordance rate of 67%. Reichlin *et al*. [58] subsequently analyzed eight of these twin pairs and found serologic concordance for autoantibodies (anti-Ro, La, Sm, and RNP determined by ELISA) in both concordant and discordant monozygotic twin pairs. Deapen *et al*. [59], however, found a lower rate of concordance (24%) in a large North American cohort of 100 identical twins. One concern that has been raised about series of twins is a bias from clinical selection, where families with multiple cases of a given disease would be more likely to be ascertained than families with single cases [7]. Jarvinen *et al*. [60] examined nine sets of identical twins and 10 sets of dizygotic twins in Finland, where every citizen is assigned a code and complete disease ascertainment is thus possible, and found only a 11% concordance rate for SLE in

the identical and none concordant in the dizygotic twins. One of the most intriguing findings came from the study of Grennan *et al*. [61] where one of four sets of Australian monozygotic twins and none of six sets of dizygotic twins were concordant for SLE. A further analysis of HLA haplotypes in the families of 38 SLE patients found none of 18 HLA identical, same-sex siblings to have definite SLE, suggesting that most of the genetic predisposition to SLE is attributable to genes outside the HLA region.

Genetic Studies in Familial SLE

Basic Genetic Terms

One of the most daunting tasks facing the nongeneticist is understanding the difficult and often changing terminology used in genetic studies. An HLA *specificity* refers to the HLA protein as expressed on the cell surface, which is detected most commonly by serologic typing. HLA specificities are thus designated by terms such as HLA-DR1 or DR4. An *epitope* is literally defined as an antigenic determinant on a complex antigenic molecule. A *locus* is a site on a chromosome occupied by a specific gene. An HLA *allele* refers to one of potentially many forms of the HLA gene itself. Because this term refers to differences in the DNA sequence of the gene, different alleles (or forms) of an HLA gene are usually detected by examination of the DNA sequence, which is now possible by a variety of techniques. *Linkage disequilibrium* refers to alleles of two genes that are physically spaced close together on the same chromosome that tend to be passed from generation to generation *en bloc* and thus occur together more frequently than would be expected from the chance frequencies of the individual genes. A *haplotype* is a set of genes, usually linked, which are passed *en bloc* from generation to generation (the presence of haplotypes is usually established by conducting family studies, wherein the inheritance pattern and linkage can be determined). Centimorgan (cM) is a measure of genetic distance between two loci; 1 cM is the distance between two loci that will recombine with a frequency of exactly 1%. *Linkage analysis* examines the phenomenon of linkage whereby loci that are are close to one another on the same chromosome tend to be transmitted together more frequently than chance, such as a disease or disease trait and a genetic marker. *Linkage analysis* tests whether affected relatives inherited a region identical by descent more often than expected under random Mendelian segregation. A quantitation of the support for linkage (versus absence of linkage) is provided by the *lod score*, which is the common logarithm (to the base 10) of the likelihood ratio. Although linkage analysis can be carried out in large families with multiple affected members, the easiest and most effective method is by *affected sib pair analysis*, whereby an affected sib pair may share 0, 1, or 2 alleles identical by descent (IBD) with probabilities of 0.25, 0.5, or 0.25, respectively, at any marker locus if by chance alone. If the ratios differ significantly from this, then linkage between the two marker alleles or traits in the siblings exists.

Genome-Wide Scans in Familial SLE

Genome-wide scans in familial SLE have been underway since the mid-1990s in at least five different locations [62, 64–75]: two in California [63, 64], one in Minneapolis [65–68], one in Oklahoma City [68–72], which is the only site to focus on African-American kindreds, and one in Uppsala [73–75], which not only includes families from Sweden, but also from Iceland and Mexico City) (Figs. 1 and 2). The first report was from Tsao *et al*. [62] in Los Angeles, who analyzed seven microsatellite markers from human chromosome 1q31–1q42 in 43 families with at last two siblings affected with SLE and found five markers located at 1q42–1q42 to show linkage. This region is syntenic with the murine chromosomal region containing the gene *SLE1* [63].

This was followed by Gaffney *et al*. [65] in Minneapolis, who reported the results of a genome-wide scan in 105 sib pair families, finding the strongest evidence for linkage in the HLA region (lod score 3.9) and at three additional regions—16p11–21(lod score 3.64); 14q21–23 (lod score 2.81), and 20p12 (lod score 2.62). Subsequent to this, this group reported the results of a second scan on an additional 82 families, finding evidence of linkage at 7p22 (lod score 2.87), 7q21 (lod score 2.3), 10p13 (lod score 2.24), and 7q36 (lod score 2.15). A combined analysis of both scans 6p11–p21 and 16q13 met criteria for linkage (LOD scores 4.19 and 3.85, respectively). In addition to this, four intervals had weaker evidence for linkage, including 2p15, 7q36, 1q42 [67] and 4p16–15.2 [68].

At the same time, Moser *et al*. [69] reported the results of a scan on 94 extended multiplex kindreds, finding evidence of linkage at 1q41, 1q23 [70], and 11q14–23 in 40 African-American kindreds and 14q11, 4p15 [68], 11q25, 2q32, 19q13, 6q26–27, and 12p12–11 in 40 Caucasian families. Combining both races, 1q23, 13q32, 20q13, and 1q31 were identified. Subsequent to this observation, Gray-McGuire *et al*. [68] presented data on an additional 32 pedigrees from this group, which, when combined with the original families studied, provided stronger evidence of linkage to 4p16 (lod score 3.84), with additional evidence for epistasis at 5p15. Nath *et al*. [71] and Rao *et al*. [72] analyzed 101

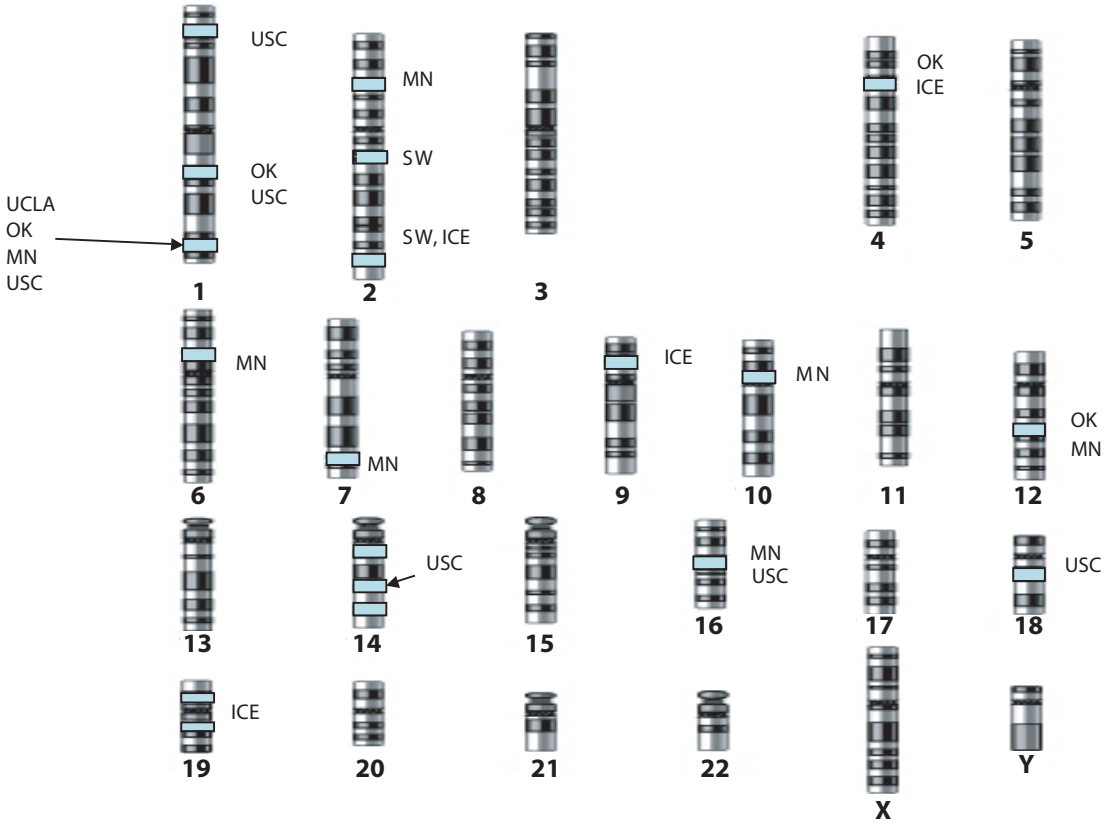


FIGURE 1 Locations of SLE susceptibility regions identified on genome-wide scans. Included are regions seen in the scan from the University of Southern California (USC) [64], Oklahoma Medical Research Foundation (OK) [68–72], the University of Minnesota (MN) [65–68], the University of California, Los Angeles (UCLA) [62], and Uppsala University, including families from Sweden (SW), Iceland (ICE), and Mexico City (MEX) [73–75].

SLE-affected sib pairs with respect to dermatologic, renal, immunologic, hematologic, neurologic, cardiopulmonary, and arthritic characteristics. Significant linkage with vitiligo was identified at 17p13. In addition to this, significant linkage for an individual trait was on chromosome 2 at 228 cM (immunologic; $P = 0.00048$), and the most significant linkage to an individual principal component was on chromosome 4 at 208 cM ($P = 0.00007$). The largest multivariate linkage was on chromosome 7 at 69 cM ($P = 0.0001$). Of the individual organ systems, dermatologic involvement had the largest effect ($P = 0.0083$) at this peak at 7p13 on chromosome 7. Further analyses revealed that malar rash, a subtype of dermatologic involvement, was linked significantly ($P = 0.00458$) to this location.

Shai *et al.* [64] examined 80 SLE families from southern California with two or more affected relatives, finding evidence of linkage at 1p21, 1q24, 1q44, 14q23, 16q13, 18q21, and 20p13.

Johanneson *et al.* [73], Lindquist *et al.* [74], and Magnusson *et al.* [75] reported genome-wide scans performed in multicase extended families from three different population groups: Sweden (11 families containing 54 individuals, including 28 SLE patients), Iceland (8 families composed of 147 individuals, including 16 SLE patients, with a high degree of homogeneity), and a recently admixed population of Mexican Mestizos, with 14 families containing 32 patients with SLE. A number of chromosomal regions showed maximum lod scores indicating possible linkage to SLE in both Icelandic and Swedish families. In the Icelandic families, five regions showed lod scores greater than 2.0, three of which (4p15–13, 9p22, and 19q13) are homologous to the murine regions containing the lmb2, sle2, and sle3 loci, respectively. The fourth region is located on 19p13 and the fifth on 2q37. Only two regions showed lod scores above 2.0 in the Swedish families: on chromosome 2q11 and 2q37. Thirteen Mexican families were analyzed separately and found not to have linkage to this region (Table 2).

From these at times confusing and confounding results the following chromosomal regions appear to

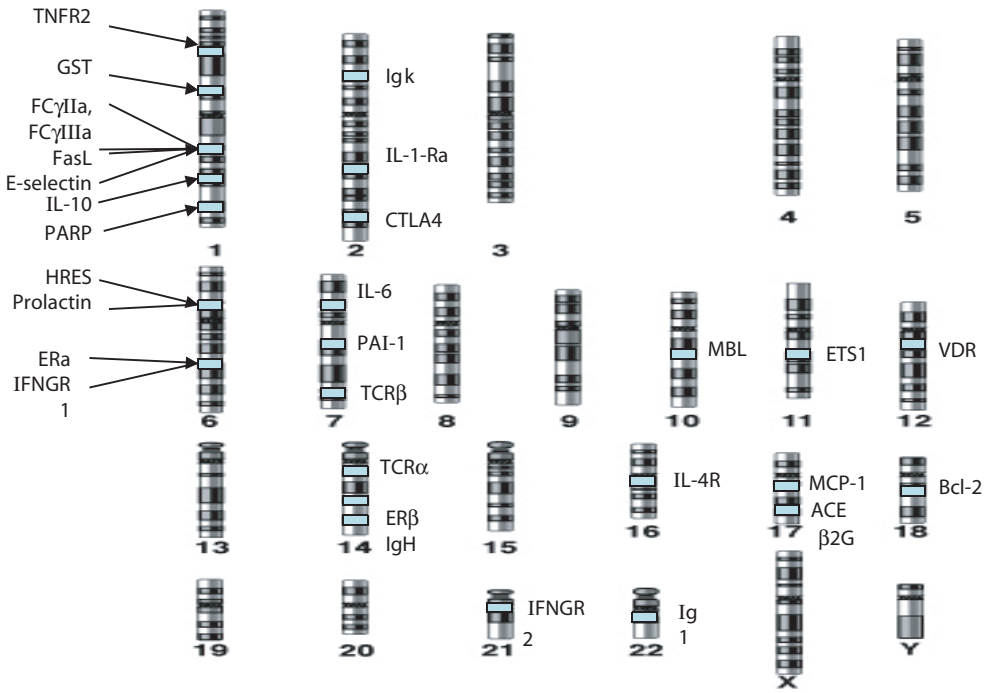


FIGURE 2 Locations of genes implicated in susceptibility to SLE. Included are only genes implicated in at least one study, whether predisposing to SLE or a clinical subset thereof.

TABLE 2 LOD Scores for Chromosomal Regions Implicated on Genome-Wide Scans

	Minnesota (n = 187)	Oklahoma (n = 126)	USC[a] (n = 80)	Uppsala, Sweden (n = 11)	Uppsala–Iceland (n = 8)
1p21			2.26		
1q23,24		3.45	2.56		
1q31					
1q41–42	1.92	3.50	3.33		
2p15	2.06				
2q11				2.13	
2q37				2.18	2.06
4p15–13		3.84			3.20
6p21	4.19				
7q36	2.06				
9p22					2.27
14q23			2.02		
16q13	3.85		2.14		
18q21			2.54		
19p13					2.58
19q13					2.06
20p13			2.28		

[a] Results are given as NPL values, which correlate with LOD scores.

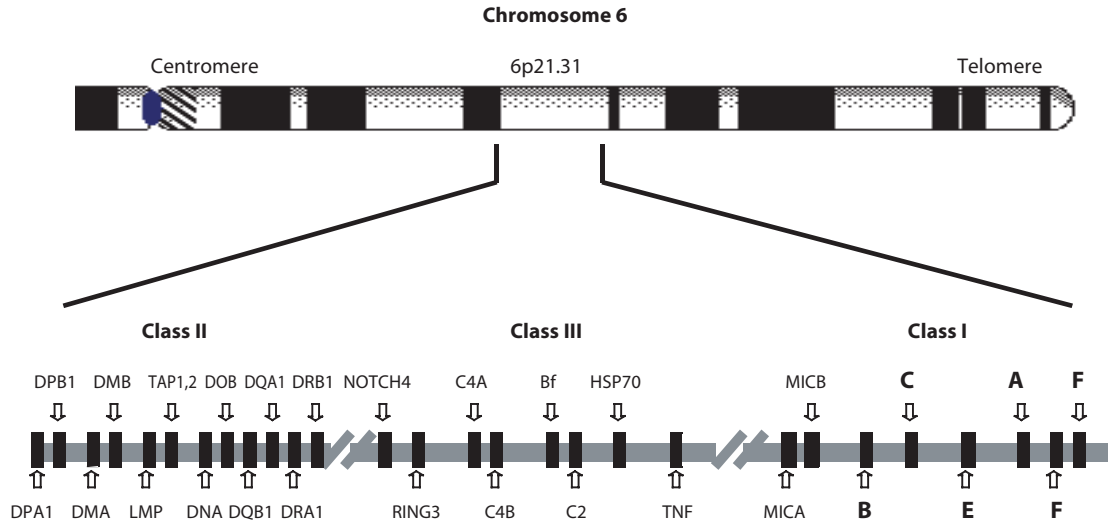


FIGURE 3 Schematic of the major histocompatibility complex. The 3.6 Mb MHC contains over 220 genes and is divided into three classes. Only genes with known (or presumed) immunological function are shown.

have been confirmed by more tham one group: (1) chromosome 6p11–21, near the MHC; (2) chromosome 1q42 and (3) chromosome 4p15, whose disease relevant genes have yet to be identified. It is hoped that with more families collected, pooling of data, and stratification for clinical subsets of SLE may provide more clues by the publication of the next edition of this book.

STUDIES OF CANDIDATE GENES IN SLE

Genes of the Major Histocompatibility Complex

Organization of the MHC (Fig. 3)

HLA-DP Alleles

The last and most centromeric lying of the "classical" class II loci to be described, this region is organized much like DQ, with two α and two β chain genes, only one of each being functional. HLA-DPA1 exhibits limited polymorphism, while over 86 alleles now have been identified for HLA-DPB1.

HLA-DM

While the newest discovered of the functional loci within the MHC class II region [76], HLA-DM has nonetheless been shown to have a unique and important function in the immune system, acting as both a chaperone and an editor in the loading of the peptides onto the HLA-class II heterodimer in the cytosol. It exerts its effect mainly in the acidic lysosomal or late endosomal compartments, but also (about 10% of total) can be found at the cell surface. HLA-DM heterodimers, however, cannot bind peptides on its own [77]. Genes encoding DMA and DMB lie between HLA-DQ and the TAP/LMP gene complex. DMA alleles are defined by variants at three nucleotide positions involving codons 140, 155, and 184 of exon 3 and seven DMB alleles by variants at two nucleotide positions involving codons 144 and 179 of exon 3 [78–80].

TAP-1 and -2 Genes

These genes together encode a heterodimeric protein that transports peptides from the cytosol into the lumen of the endoplasmic reticulum. Newly synthesized HLA class I molecules (MHC peptide plus β_2-microglobulin) are associated with the TAP heterodimer before binding to endogenous peptides. Once the latter occurs, class I molecules disassociate from the TAP molecule and move to the cell surface for presentation of the bound peptide to the appropriate T-cell receptor [81–84].

LMP 2 and 7 Genes

These genes encode proteosomes that degrade endogenous products (plus viral and tumor proteins) to generate peptide fragments that bind to MHC class I molecules [85].

HLA-DO

HLA-DO is a nonpolymorphic MHC class II-like heterodimer composed of the products of the genes DNA and DOB, which lie beween the TAP/LMP complex and the HLADQ complex. The HLA-DO

heterodimer appears to function in B cells and modulates the function of DM. In fact, DO is stably complexed with DM during intracellular transport and in endosomal/lysosomal compartments [77, 86]. Like DM, it does not bind peptides on its own.

HLA-DQ Alleles

This region is composed of two DQA and two DQB genes, only one of each encoding a functional product. The DQA1 locus has over 9 known alleles, whereas DQB1 has over 17. HLA-DQA2 and DQB2 genes are not known to be functional.

HLA-DR Alleles

This region is composed of one nonpolymorphic DRA gene whose product combines with the product of numerous DRB genes to form HLA-DR heterodimers. Most of the polymorphism of classic HLA-DR molecules is derived from the DRB1 gene, where over 150 polymorphisms are now recognized. The presence and number of other DRB loci vary on different HLA-DR haplotypes, e.g., DRB3, found on DR3, DR5 (DR11, 12), and DR6 (DR13, DR14) haplotypes, encodes, in conjunction with DRA, the DR52 molecule. The HLA-DRB4 gene is found on HLA-DR4, DR7, and DR9 haplotypes and, in conjunction with DRA, encodes HLA-DR53 specificity. HLA-DRB5 is found on DR2 haplotypes and with DRA encodes what is now recognized serologically as HLA-DR51.

MHC Class III Region

Here lie the early components of the classical and alternate complement cascade (C4, which has undergone a gene duplication resulting in C4A and C4B, C2 and properdin factor B), as well as heat shock 70 gene complexes and tumor necrosis factor (TNF) complexes. In addition to these genes are newly discovered genes RING3 and Notch 4, which lie between C4 and HLA-DRA. RING3 a is nuclear-localized, serine-threonine kinase, and Notch 4 a receptor that controls differentiation or proliferation in various cells. Also found in the MHC class III region are other genes which either do not have a known immunologic function (21-hydroxylase B) or whose function is not well understood [RAGE, RD, and the B-associated transcript (BAT) complex]. This region and its relevance to SLE are better covered in Chapter 5.

MHC Class I Region

Located most telomeric in the MHC and spanning two megabases of DNA [87], the MHC class I region contains HLA-A, B, and C genes (class Ia), a number of other class I-like genes, HLA-E, F, and G (class Ib), and MIC genes. MIC genes include MIC A and B, which are located between HLA-B and the TNF complex [87]. MIC proteins are considered to be markers of stress in the epithelia and act as ligands for cells expressing a common activatory natural killer cell receptor NKG2D. HLA-E is widely expressed on different tissues but displays limited allelic polymorphism. It presents hydrophobic peptides from the leader sequences of other class I molecules. HLA-E:peptide complexes constitute the only known ligands for CD94/NKG2 receptors on natural killer (NK) and T cells [88]. HLA-G is expressed in high amounts on trophoblastic cells and is thought to function in inducing maternal–fetal tolerance. The function of HLA-F, considered to be the progenitor of all MHC class I genes, is unknown and has a restricted pattern of expression in tonsil, spleen, and thymus. Also found are a number of pseudogenes (such as HLA-H, J, K, and L). There is little to support a significant role of this region in SLE, and it is not discussed further.

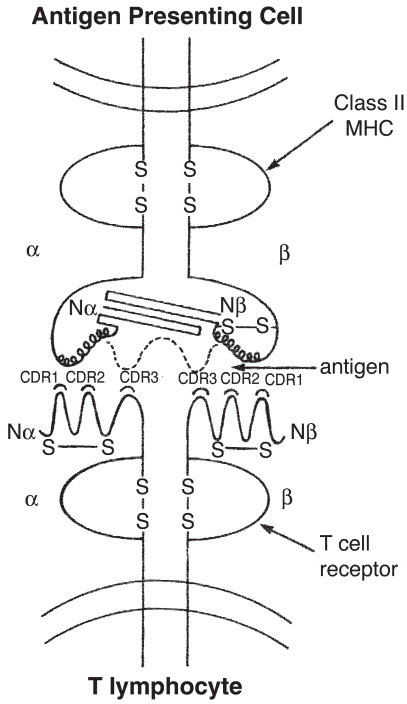


FIGURE 4 HLA class II heterodimer: antigen: T-cell α:β receptor complex (the trimolecular complex).

HLA Class II Molecules: Structure and Function (Fig. 4)

Unlike HLA class I molecules, which are expressed on the surface of all nucleated cells, HLA class II molecules have a more limited distribution, being present in varying amounts on the surface of antigen-presenting

cells such as macrophages, dendritic cells, and B cells (although other cells can be induced to express HLA class II molecules by interferon-γ). HLA class II molecules, which include HLA-DR, DQ, and DP, function in presenting to the α:β T-cell receptors on CD4-positive lymphocytes peptides, which are derived from proteins that have been internalized from outside the cell (exogenous antigens) and degraded within lysosomes to peptide fragments.

The HLA class II molecule is a glycosylated heterodimer composed of an α chain of 32 kDa in size and a β chain of 29 kDa. Each of these two polypeptides folds into four domains. The α1 and β1 domains project outermost from the cell surface and form the peptide-binding groove. These domains also form the contact point for interaction with the T-cell receptor, and it is in these domains that most of the polymorphism of the HLA class II molecule is found. This polymorphism is, for the most part, grouped into three "diversity" or "hypervariable" regions along the approximately 90 amino acid span of the domain, encompassing amino acids 9 to 13 (the first "diversity" region), 25 to 38 (the second), and 57 to 86 (the third). The next domains (α2 and β2) project more proximally outside the cell surface (membrane proximal domains). These are more conserved in amino acid sequence and share sequence homology with immunoglobulin and TCR membrane-proximal domains. They also contain the point of binding to the CD4 receptor, which in turn increases the affinity of interaction between the T cell and MHC class II:peptide complex and facilitates T-cell activation. Both α and β polypeptides also contain more highly conserved transmembrane and intracytoplasmic domains, which serve to anchor the HLA class II heterodimer into the cell surface. The HLA-DR molecule has now been crystallized and its fine molecule structure defined. Studies of the antigen-binding cleft of the HLA class II heterodimer show important differences from HLA class I molecules. Both types of MHC molecules are bordered by α helical loops, and the floor of the cleft consists of a series of β-pleated sheets. However, the class I molecule has rigid size requirements for what it binds, permitting peptides of only 9 to 10 amino acids in length. The class II cleft is less constrained, allowing peptides of 13–25 amino acids in length. This cleft, in turn, is composed of up to nine "pockets," into which specific amino acids of the peptide in the cleft protrude and are, to a greater or lesser extent, noncovalently bound. Deepest and most critical is the first pocket, P1, which anchors the peptide fragment and which has the most stringent binding requirements. The other pockets are shallower and more permissive in their binding. However, is noteworthy that these pockets are composed of amino acids that differ among different DR molecules, hence the amino acid polymorphism of HLA class II molecules critically affects which amino acids residues can be bound in the peptide-binding groove and hence which peptide fragments can be presented to the T-cell receptor.

Soon after MHC class II polypeptides are synthesized in and inserted into the endoplasmic reticulum of the cell, they come into association with another peptide, the invariant chain (I_i), which promotes the proper folding and assembly of the MHC class II heterodimer and protects the peptide-binding groove from premature occupancy by endogenous peptides. In addition, I_i is responsible for the delivery of the HLA class II α:β:I_i complex through the *trans*-Golgi network to the acidic endosomal–lysomal compartment, where it encounters peptide fragments of internalized exogenous proteins or internalized self-antigens. At that point I_i undergoes proteolytic cleavage, leaving only a peptide in the HLA class II peptide-binding groove called CLIP. Unless the CLIP peptide is removed, MHC class II peptide binding cannot occur. Removal of CLIP is facilitated by HLA-DM, which transiently binds to the HLA class II molecule in the endosomal–lysomal compartment, removes the CLIP peptide, and further acts as a peptide editor, selecting for high-stability MHC class II–peptide complexes, which are sorted to the cell surface for effective antigen presentation.

MHC Class II Genes and Susceptibility to SLE

Differences in the Frequencies of HLA-DR and DQ Genes in Different Ethnic Groups

Whereas certain HLA-DRB1 alleles occur in all populations (*DRB1*0701*, *DRB1*1301*, *DRB1*1302*), there are important differences in the frequencies of HLA class II alleles in different ethnic groups. Not only do frequencies of HLA class II alleles differ, but also the HLA-DQ alleles to which they are linked. For example, *HLA-DRB1*0405* is linked to *HLA-DQB1*0302* in Caucasians, whereas in Chinese and Japanese, linkage disequilibrium for *DRB1*0405* instead is found with the *HLA-DQB1*0401*. The *HLA-DRB1*1602*, *DQB1*0301* haplotype is well described in Native Americans, whereas in Africans the haplotype is more characteristically *HLA-DRB1*1602*, *DQB1*0502*. The different linkage disequilibrium seen in different ethnic groups gives one justification for examining disease associations thus in order to better evaluate the relative contributions of HLA-DR and DQ alleles to disease pathogenesis (Tables 3 and 4).

TABLE 3 Selected HLA-DRB1-DQA1-DQB1 Haplotypes in Different Ethnic Groups

HLA-DR specificity	DRB1 allele	DQ specificity	DQA1 allele	DQB1 allele	Ethnic group
DR2(DR15)	*1501*	DQ5	*0102*	*0602*	Caucasians (C)
DR2(DR15)	*1502*	DQ5	*0103*	*0601*	Orientals
DR2(DR15)	*1503*	DQ6	*0102*	*0602*	Africans
DR2(DR16)	*1601*	DQ5	*0102*	*0502*	Slavs
DR2(DR16)	*1602*	DQ5	*0102*	*0502*	Africans
DR2(DR16)	*1602*	DQ7	*0501*	*0301*	Native Americans
DR3(DR17)	*0301*	DQ2	*0501*	*0201*	C
DR3(DR18)	*0302*	DQ4	*0401*	*0402*	Africans
DR4	*0401*	DQ7,DQ8	*03*	*0301, 0302*	C, Eskimos
DR4	*0402*	DQ8	*03*	*0302*	Ashkenazi Jews
DR4	*0405*	DQ8	*03*	*0302*	C, Africans
DR4	*0405*	DQ4	*03*	*0401*	Orientals
DR4	*0406*	DQ8	*03*	*0302*	Orientals
DR4	*0407*	DQ8	*03*	*0302*	Native Americans
DR6(DR13)	*1301*	DQ6	*0103*	*0603*	All populations
DR6(DR13)	*1302*	DQ6	*0102*	*0604*	All populations
DR6(DR13)	*1302*	DQ6	*0102*	*0605*	Africans
DR6(DR13)	*1304*	DQ7	*0501*	*0301*	Africans
DR6(DR14)	*1401*	DQ5	*0104*	*0503*	All populations
DR6(DR14)	*1402*	DQ7	*0501*	*0301*	Native Americans
DR6(DR14)	*1406*	DQ7	*0501*	*0301*	Native American
DR7	*0701*	DQ2,9	*0201*	*0202, 0303*	All populations
DR8	*0801*	DQ4	*0401*	*0402*	C
DR8	*0802*	DQ4	*0401*	*0402*	Native Americans
DR8	*0803*	DQ6	*0103*	*0601*	Orientals
DR8	*0804*	DQ7	*0401,0501*	*0301*	Africans
DR9	*0901*	DQ2,9	*03*	*0201,0303*	Orientals, Native Americans

[a] From data in Reveille [1].

HLA-DR and DQ Genes and SLE Predisposition (Table 5)

The earliest attempts at defining HLA associations with SLE were with class I antigens, where either weak correlations with HLA-B8 were seen [89–91] or no associations were seen at all [92]. Over 25 years have passed since an association of HLA-DR2 and DR3 with SLE has been recognized, initially in Caucasians in the United States [93, 94]. HLA-DR3 has been associated with SLE in nearly every other Caucasian population group studied, including those from the United Kingdom [95, 96], Australia [97], Spain [98], Italy [99], Scandinavia [100–102], Switzerland [103, 104], Austria [105], Germany [106], and Iceland [107], as well as from other studies from the United States [108, 109]. The only exceptions come from studies of southern and eastern Europe, where Slavic genetic admixture predominates and significant associations of HLA-DR2 and DR3 were not seen [110, 111]. In a multicenter study from Europe, composed of 534 patients [112], HLA-DR3 (*DRB1*0301*) emerged as the only significant association with SLE *per se*.

However, the association of HLA-DR2 with SLE has remained consistent in other Caucasian groups [93, 113, 114] (where a more specific association with lupus nephritis was seen in one group [115]) as well as in Orientals [140–143]. With the advent of DNA typing, where numerous HLA-DR2 alleles are recognized, the DR2-allele *DRB1*1501* (linked to *HLA-DQA1*0102* and *DQB1*0602*) has been shown to constitute this association in Caucasians [96, 106]. Tsuchiya *et al.* [116] recruited 91 Caucasian SLE family samples recruited in southern California and analyzed them for the association with HLA-DRB1, TNF promoter positions at –1031, –863, –857, and –308, and TNFR2-

TABLE 4 HLA-DR,DQ Associations with SLE In Different Ethnic Groups

Ethnic Group	Number of patients	Typing method	HLA-DR association	C4 association	ref.
C					
North Americans					
Bethesda, MD	41	Serologic	DR2, DR3	n.t.[a]	93
New York, NY	24	Serologic	DR3	n.t.	94
Toronto, ON	30	Serologic	DR2	n.t.	114
Baltimore, MD	113	Serologic	DR2, DR3	*C4A*Q0*	113, 134
Boston, MA	62	Serologic	DR2, DR3	*C4A*Q0*	193
San Francisco, CA	78	Serologic/RFLP	DR2	n.t.	115
Birmingham, AL	60	RFLP	DR3	*C4A*Q0*	108
Houston, TX	127	Oligotyping	*DRB1*0301*	*C4A*Q0*	109
Europeans					
Zurich, Switzerland	40	Serologic	DR3	n.t.	103
Vienna, Austria	28	Serologic	DR3	n.t.	105
London, England	67	Serologic	DR3	*C4A*Q0*	95, 96
Rome, Italy	44	Serologic	DR3	n.t.	99
Madrid, Spain	62	Serologic	DR3	*C4B*Q0*	98
Germany (multicenter)	300	Serologic	DR2, DR3	*C4A*Q0*	106
Sofia, Bulgaria	59	Oligotyping	None	None	110
Ioanina, Greece	57	Oligotyping	None	n.t.	111
Trondheim, Norway	51	Oligotyping	*DRB1*0301*	n.t.	101
Geneva, Switzerland	52	Oligotyping	*DRB1*0301*	n.t.	104
Reyjavik, Iceland	64	Oligotyping	*DRB1*0301*	*C4A*Q0*	107
Europe (multicenter)	534	Oligotyping	*DRB1*0301*	n.t.	112
Australians					
Victoria	53	Serologic	DR3	n.t.	287
Canberra, NSW	46	RFLP	DR3	n.t.	97
Perth	62	Serologic	DR3	*C4A*Q0*	192
Orientals					
Tokyo, Japan	85	Serologic	DR2	*C4A*Q0*	117, 118
Tokyo, Japan	53	Oligotyping	*DRB1*1501*	n.t.	119
Hong Kong, China	100	Serologic	DR2	*C4A*Q0*	120
Kuala Lumpur, Malaysia	87	RFLP	DR15	*C4A*Q0*	123
Taipei, Taiwan	105	Oligotyping	*DRB1*1501*	*C4A*Q0*	124
Seoul, Korea	60	Oligotyping	*DRB1*1501*	*C4A*Q0*	128
Singapore	51	Serologic	DR2	*C4A*Q0*	121
Delhi, India	58	Serologic	DR4	n.t.	129
Mexican/Mexican Americans					
Houston, TX	70	Oligotyping	*DRB1*08, 0301*	*C4B*Q0*	139
Mexico City	81	Oligotyping	*DRB1*0301*	n.t.	138
African Americans					
Chicago, IL	25	Serologic	DR2, DR3	n.t.	111
Washington, DC	25	Serologic	DR2, DR3	n.t.	130
Baltimore, MD	37	Serologic	None	*C4A*Q0*	113, 134
French West Indies, Boston	xx	Serologic	*DRB1*1503*	*C4A*Q0*	136
Birmingham/Houston	130	Oligotyping	*DRB1*1503*	*C4A*Q0*	109

[a] Not tested.

196M/R polymorphisms. Significant transmission was observed for *HLA-DRB1*1501*, but not for *HLA-DRB1*0301* nor for the TNF haplotype that codes for –308A.

The HLA-DR2/*DRB1*1501* association has also been seen in Oriental groups, including Japanese [117–119], Chinese [120–126] and Koreans [127, 128]. This *HLA-DRB1*1501* association in Orientals is particularly noteworthy, as the most frequent HLA-DR2 allele in these groups is *DRB1*1502*, which is linked to different HLA-DQ alleles (*DQA1*0103*, *DQB1*0601*) [119]. Zhang *et al.* [126] reported an increased frequency of DR2 and DR3 alleles in northern Chinese Han SLE patients. A study of SLE patients from northern India described an association with HLA-DR4 [129], despite a nonsignificant increase in HLA-DR3 alleles.

TABLE 5 Hla Class II Alleles and Autoantibody Subsets of SLE

Autoantibody	Ethnic group	Technique	Association
Anti-dsDNA	Caucasians (C)	Farr	DR2, DR3
	Chinese		
Anti-Ro/La	C	Immunodiffusion	DR3
	C	Immunodiffusion	*DQA1*0501*, *DQB1*0201*
	C	ELISA	DQ1/DQ2
	C	ELISA	*DQA1*0501*, *DQB1*0201*
	C	Immunoblot, ELISA	*DQA1*0501*
	Chinese	Immunodiffusion	*DQA1*0102*
	Japanese	Immunodiffusion	*DRB1*0803*, *DQA1*0103*, *DQB1*0601*
Anti-U1-RNP	C	Immunodiffusion	None
	C	Immunodiffusion	DR4, DQ3
	Japanese	Immunodiffusion	DR4, DQ3
	Japanese	ELISA	*DRB1*0401*, *DQB1*0302*
	AA	Immunodiffusion	*DQA1*0101*, *DQB1*0501*
	C	Immunoblot	DR2, DR4
	Mexican-Americans	Immunodiffusion	*DQB1*0302*
Anti-Sm	C	Immunodiffusion	None
	C	Immunodiffusion	DR7
	AA	Immunodiffusion	*DQA1*0102*, *DQB1*0602*
	Japanese	Immunodiffusion	DQ3
Anticardiolipin	C	ELISA	DR4, DR7, DR53
	Mexicans	ELISA	DR7
Lupus anticoagulant	C	TTI	*DQB1*0301*, *DQB1*06*
Antiribosomal P	C, Mexican, African-American	ELISA	*DRB1*15*, *DQA1*0102*, *DQB1*0602*

Initial studies in African-Americans using serologic or restriction fragment length polymorphisms (RFLP) typing yielded either weak associations with HLA-DR2 and DR3 [130, 131] or no associations at all [132–134]. With the utilizing of oligotyping, the association of HLA-DR2 with SLE in African-Americans appears to reside with *HLA-DRB1*1503*, a uniquely African DR2 allele that differs from *DRB1*1501* by a single amino acid substitution at position 30 of the DRB1 outermost domain [108, 135, 136] and is also linked to *HLA-DQA1*0102* and *DQB1*0602*.

No immunogenetic studies have been conducted in Native Americans with SLE. However, Hispanics, especially those from Mexico and Central America, are composed of varying degrees of European and Native American ancestry. Reveille *et al*. [137] examined 52 Mexican-Americans, finding a similar, although weaker, association with HLA-DR3 (*DRB1*0301*) that has been described in other Caucasian groups. A similar observation was made in a group of patients from Mexico City [138]. However, another study of Mexican Mestizos residing in Texas has suggested a stronger association with HLA-DR8 (*DRB1*0802*) [139].

Some studies have focused on additional contributions to SLE pathogenesis of the *other* set of HLA class II alleles that an individual possesses. Reinharz *et al*. [104] reported the combination of HLA-DR2 (*DRB1*1501*) and DR3 (*DRB1*0301*), whose presence has been reported by others to produce the highest risk for anti-Ro autoantibodies (see later) [140–142] to occur rarely in SLE patients. Skarsvag *et al*. [101] found patients who were homozygous for MHC class II genes to have a higher likelihood of developing SLE, with *HLA-DRB1*0301*, *DQA1*0501*, *DQB1*0201* homozygosity having the highest risk, followed by non-*DRB1*0301*, *DQA1*0501*, *DQB1*0201* homozygotes. However, in another European study of 356 SLE patients by Hartung *et al*. [105], homozygous and heterozygous combinations of HLA class II antigens appeared with frequencies expected from observed gene frequencies. Among Japanese SLE patients, a combination of HLA-DR2 and DR4 has been reported as providing the highest risk for SLE [119].

HLA DR and DQ Genes and Autoantibody Subsets of SLE (Table 6)

SLE is both a clinically and a serologically heterogeneous disorder, such that many of the MHC associations are better explained when considering autoantibody subsets. Just as in the consideration of the HLA studies themselves, analyzing associations with autoantibody subsets should take into consideration the techniques

TABLE 6 Non-MHC Genes Implicated in Predisposition to SLE

Gene	Function	Location	Group
TNFR2	Cell surface receptor for TNF	1p36.3	EA
Glutathione-*S*-transferase	Protects against oxidative stress	1q12	C
FcγRIIa	Preferentially binds Fc portion of IgG2	1q22	C, AA, EA, H
FcγRIIIa	Preferentially binds Fc portion of IgG1 and IgG3	1q22	C, EA
FAS ligand	Mediates apoptosis	1q24	H, C, EA
E-Selectin	Leukocyte–endothelium interactions	1q23–25	C
T-cell receptor ζ chain	Anchor site for TCR on CD3	1q23–24	C
IL-10	Multifunctional cytokine, promotes apoptosis	1q31–32	C, H, EA
PARP	Involved in DNA repair and apoptosis	1q42	C, H, AA
HRES-1	Endogenous retroviral sequence	1q42	C
Igκ	Immunoglobulin light chain	2p12,	C
IL-1-Ra	Endogenous anti-inflammatory agent	2q12	C
CTLA-4	Maintains peripheral T-cell tolerance	2q33	C, EA
Prolactin	Influences T-cell function	6p22.2	C
IFNGR2	Interferon-γ receptor 2	6q23–24	EA
ERa	Estrogen receptor α	6q25.1	EA
IL-6	Multifunctional Th2 cytokine	7p21	C
PAI-1	Plasminogen activator inhibitor-1	7q21.3	EA
TCRβ	T-cell receptor β chain gene	7q35	C
Mannose-binding protein	Activates complement independent of antibody	10q11.2	C, A
ETS1	Transcription factor activation	11q23	C, AA
VDR	Vitamin D receptor	12q12–14	EA
TCRα	T-cell receptor α chain gene	14q11–12	C
ERb	Estrogen receptor β	14q23–24	EA
IgH	Immunoglobulin heavy chain gene	14q32.33	C, AA, EA
CYP1A1	Activate drugs to reactive metabolites	15q22	C
IL-4-R	B-cell activation, Th2 response	16p12.1	EA
MCP-1	Recruitment of monocytes to sites of injury/infection	17q11.2	C
β2-Glycoprotein	Required cofactor for the production of aPL	17q23	C
ACE	Angiotensin-converting enzyme gene	17q23	EA, C, AA
Bcl-2	Prolongs survival of lymphocyte	18q22	C, H
IFNGR1	Interferon-γ receptor 1	21q22.1	EA
Igλ	Immunoglobulin λ light chain gene	22q11.2	C

utilized in the autoantibody determinations, which differ significantly in the sensitivity and specificity. For example, in most of the "classical" studies, autoantibody determinations were carried out by immunodiffusion. By the mid-1980s, many studies began utilizing ELISAs, which improved the sensitivity of autoantibody detection 10- to 100-fold. Subsequently, immunoblot analyses allowed the detection of immune responses against constituents of the autoantigen.

Anti-dsDNA The anti-dsDNA response, which is associated with disease activity in SLE, particularly with glomerulonephritis, has been associated with both HLA-DR2 (*DRB1*1501*) [113, 143] and DR3 (*DRB1*0301*) [144] and their respective linked HLA-DQ alleles (*DQA1*0102*, *DQB1*0602* and *DQA1*0501*, *DQB1*0201*) [106, 145]. Azizah *et al*. [122] found an association of *DQA1*0301* with antibodies to dsDNA in Malaysian Chinese.

Anti-Ro and La Anti-Ro and La, associated with sicca symptoms, photosensitive skin rashes, vasculitis, and later onset disease, were initially associated in Caucasians with HLA-DR2 and DR3 [113, 134, 146].

Subsequent studies, also using serologic typing, implicated HLA-DQ molecules, specifically the broad specificities DQ1 and DQ2. In fact, the presence of both these alleles concomitantly gave the highest risk for and antibody levels of the anti-Ro and La responses [140, 141, 147], although more recent studies have implicated their respective linked DQ alleles, particularly those DQA1 alleles encoding a glutamine at position 34 and DQB1 alleles encoding a leucine at position 26 [148, 149]. The development of ELISA to anti-Ro and La allowed quantitation of the actual levels of the autoantibodies [150]. With the introduction of immunoblotting and the recognition of the 60- and 52-kDa isoforms of anti-Ro, it was found that both isoforms were most highly associated with *HLA-DRB1*0301* and its respective linked DQ alleles, *DQA1*0501* and *DQB1*0201* [151, 152], as well as with the linked gene *C4A*Q0* gene deletion [145]. Studies from two different European centers have implicated the HLA-B8-*C4A*Q0-DRB1*0301-DQA1*0501-DQB1*0201* haplotype in susceptibility to the anti-Ro/La autoantibody response in Caucasians [153, 154]. In addition, Logar *et al*. [155] examined 65 anti-La-negative Slovenian SLE patients and found the antibody response to anti-Ro without anti-La to be associated with *HLA-DQB1*0202*, a DQ2 allele closely related to *DQB1*0201* that is found on DR7-bearing haplotypes. Azizah *et al*. [122] found an association of DQA1*0102 with anti-Ro/La antibodies in Malaysian Chinese with anti-Ro, and in Japanese, the only association reported has been with the HLA class II haplotype *DRB1*08032/DQA1*0103/DQB1*0601* and *DRB1*08032* allele [156].

Anti-RNP and Sm Anti-U1-RNP has been associated with Raynaud's phenomenon and myositis [113]. The presence of high titers of anti-U1-RNP was initially linked to a disorder characterized by Raynaud's phenomenon, myositis, sclerodactyly, and lung disease designated "mixed connective tissue disease" [157]. Subsequent studies, however, have revealed that many of these patients evolved clinical patterns more compatible with SLE or systemic sclerosis [158]. These autoantibodies are particularly enriched in African-Americans [159]. Initial associations in both Japanese and Caucasians were with HLA-DR4 and DQw3 (the serologic group comprising *HLA-DQB1*0301*, **0302*, and **0303*) [101, 107, 160]. Subsequent studies using oligotyping and DNA sequencing have implicated both HLA-DRB1 and DQB1 alleles, specifically shared epitopes between HLA-DR2 and DR4 [161] and *HLA-DQB1*0302*, linked to HLA-DR4, as well as other specific DQB1 alleles sharing specific epitopes with *DQB1*0302* in both Caucasians [162] and Japanese [163].

Anticardiolipin Antibodies (aCL)/Lupus Anticoagulant These autoantibody systems have their clinical relevance in their association with venous and arterial thrombosis, recurrent fetal loss, livedo reticularis, and valvular heart disease and occur in up to 40% of SLE patients [164]. Savi *et al*. [165] reported an association with HLA-DR7 in northern Italians with SLE. McNeil *et al*. [166], in a study of Australian Caucasians, could not confirm this finding, instead showing an increased frequency of HLA-DR4 and an HLA-DR antigen whose gene is linked to HLA-DR4, DR7 and DR9 alleles, DR53 (whose gene is designated *HLA-DRB4*0101*). Hartung *et al*. [167], in a multicenter study of 412 German SLE patients, confirmed the HLA-DR4 and the DR53 (*DRB4*0101)* association, here with IgM aCL. Galeazzi *et al*. [112] studied 577 European SLE patients and showed positive associations with *HLA-DRB1*04*, *DRB1*0402*, *DRB1*0403*, *DRB1*07*, *DRB3*0301*, *DQA1*0201*, *DQA1*0301*, and *DQB1*0302* and negative associations with *DQA1*0501* and *DRB3*0202*. None of these studies, however, utilized DNA typing, and HLA-DQ alleles, other than the broad serologic specificities HLA-DQ1–3, were not examined. Arnett *et al*. [168] examined 20 patients with the lupus anticoagulant (of whom only nine met criteria for SLE, the other having 1°APS or other CTD) and reported an association with *HLA-DQB1*0301* (DQ7). Panzer *et al*. [169] examined 27 patients with the lupus anticoagulant and found instead an association with *HLA-DQB1*06* (DQ6). Both of these authors [168, 169] postulated that the actual association with the lupus anticoagulant was with the TRAELDT sequence in the third diversity region of the HLA-DQB1-outermost domain, which is shared by both alleles. Goldstein *et al*. [170] confirmed this in 91 Canadian SLE patients, of whom 19 had either IgG aCL or a positive lupus anticoagulant, and 16 with 1°APS, as well as confirming earlier serologic associations with HLA-DR53 (*DRB4*0101*) and HLA-DR7. Granados *et al*. [171] further confirmed the association of IgG and IgM aCL with HLA-DR7 in 80 Mexican SLE patients and found this association to persist when clinical symptoms of the aPL syndrome were examined regardless of the presence of aCL. Arnett *et al*. [172] examined 262 patients with primary antiphospholipid antibody syndrome (APS), SLE, or another connective tissue disease and found the *HLA-DQB1*0302* (DQ8) allele, typically carried on HLA-DR4 haplotypes, to be associated with anti-β2GPI when compared with both anti-β2GPI-negative SLE patients and ethnically matched normal controls, especially in Mexican-Americans and, to a lesser extent, in whites. Galeazzi *et al*. [173] studied 577 European SLE patients for IgG and IgM anti-β2GPI and found a positive association with *DRB1*0402*,

*DRB1*0403*, *DQB1*0302*, and *DRB1*0402* (RR = 4.6). Hashimoto *et al*. [174] studied 145 Japanese patients with SLE and found a significant association with *DRB1*0901* as compared to those without β2GPI-dependent aCL (41.4% vs 15.5%, $P < 0.005$, RR = 3.8), although the corrected *P* value was not significant. In two families multiplex for SLE and clinical/serologic manifestations of the aPL syndrome, affected family members shared an HLA-DR4, DQ3 (DQ7) haplotype [175, 176].

Antiribosomal P This autoantibody system, which occurs specifically in patients with SLE, is associated with neuropsychiatric disease, particularly with psychosis and/or depression [177–179], as well as with lupus hepatitis and renal disease [180, 181]. Arnett *et al*. [182] determined the frequency of anti-ribosomal P autoantibodies in a large multiethnic cohort of 394 SLE patients, finding a frequency of 13–20%. The HLA-DR2, DQ6-bearing haplotypes, specifically those bearing HLA-DQB1*0602, were seen most frequently in all ethnic groups.

Other Autoantibodies HRES-1 is a human T lymphotrophic virus (HTLV)-related human endogenous retrovirus, which encodes a 28-kDa nuclear autoantigen that is capable of eliciting cross-reactive antibodies with retroviral gag proteins. Perl *et al*. [183] and Magistrelli *et al*. [184] examined 153 patients with CTD, including 96 with SLE, and found that 52% of the latter had antibodies to HRES-1, especially those with anti-U1 RNP autoantibodies and those who were negative for anti-Ro and La. A significant association was seen with specific HLA-DQB1 alleles, especially *DQB1*0402*. Autoantibodies to the epidermolysis bullosa acquisita antigen (EBA) occur in SLE patients with bullous LE lesions. Gammon *et al*. [185] found them to be closely associated with HLA-DR2 alleles. Galeazzi *et al*. [186] studied antiganglioside antibodies (aGM1) in 448 European SLE patients in eight centers from six European countries. Associations were seen with various neuropsychiatric manifestations or SLE, and aGM1-IgG were associated with *HLA-DQB1*0404*, whereas aGM1-IgM were associated with *HLA-DQB1*0605*. No associations were found between aGM1 and anti-dsDNA, aCL, anti-β2GP1, or ANCA.

HLA-DPB1 Alleles in SLE

Few studies have addressed a possible role for HLA-DP alleles in the pathogenesis of SLE. Those that have found only associations with autoantibody subsets have been largely weak and inconsistent. Galeazzi *et al*. [187] examined 17 HLA-DPB1 alleles in 42 Caucasian SLE patients and found *HLA-DPB1*0301* and **1401*, which only differ in sequence by one amino acid, to be increased in frequency compared to controls, especially in those SLE patients with anti-Sm and RNP and anti-cardiolipin autoantibodies. Using the same technology, Reveille *et al*. [188] studied 68 Caucasian SLE patients, finding only a weak correlation of HLA-DPB1 alleles sharing the ILEE sequence around positions 65–70 with the presence of the lupus anticoagulant. Hsu *et al*. [189] examined 21 Caucasian connective tissue disease patients (including those with SLE, MCTD and systemic sclerosis) with anti-U1-70-kDa RNP autoantibodies and 27 without and found a weak association with *HLA-DPB1*0401*, the most common DPB1 allele in most populations. Thus it is unlikely that the HLA-DP locus is a significant factor in the pathogenesis of SLE.

Other MHC Class II Genes and SLE

Given the role of HLA-DM alleles in MHC class II:peptide loading, it would not be surprising to find an association with MHC class II-associated diseases. To date, two independent studies in Chinese and Japanese SLE patients have shown no convincing associations of HLA-DM alleles [190, 191].

Frequencies of the MHC class I transporter genes *TAP1* and *TAP2* have been studied in Caucasians and Japanese SLE patients. Savage *et al*. [121] examined 51 Chinese SLE patients and 103 controls, finding no association.

Extended Haplotypes and SLE Susceptibility

The subject of MHC class III genes and susceptibility to SLE is covered in Chapter 5. However, the HLA-A1, B8, C4A*Q0, DR3 haplotype explains the HLA-DR3 association in nearly every Caucasian population studied [106, 107, 193–196]. The sole exception is in Spain, where the predisposition to SLE, especially to renal involvement therein, has been associated with the HLA-B18, DR3 haplotype. The association of TNF genes with SLE, covered in Chapter 5, has been suggested to be independent of HLA-DR3 [195]. It is entirely possible that genes present on a given haplotype may actually function as an interrelated cassette of complementarily functioning genes, i.e., HLA-DM, DO, DQA1, DQB1, DRB1, DRA1, C4, C2, TNF, and so on, though this possibility has not been proven and needs further work.

HLA Genes and Outcome in SLE

Since the earliest HLA studies in SLE, it has been suggested that HLA genes affect the clinical presentation and prognosis of SLE. Rigby *et al*. [197] studied HLA class I antigens in 44 Australian Caucasian SLE patients classified as mild or severe on the basis of renal

biopsy changes and the degree of proteinuria, finding that the presence of HLA-A1 and B8 (associated with DR3) was associated with more severe disease and HLA-A2 and B7 with milder disease. Bell *et al*. [198] could not confirm this, however, in a study of 62 Canadian Caucasian SLE patients, finding only an association of HLA-B8 and DR3 with later-onset disease and female sex. Hochberg *et al*. [134] confirmed this association with later-onset disease in Caucasians. In a retrospective outcome study of 94 Caucasians with SLE, Gulko *et al*. [199] found HLA-DQ7 (*DQB1*0301*) to be independently associated with a higher likelihood of death. More recently, Alarcon *et al*. [200] in a prospective outcome study of 229 SLE patients of approximately equal proportions of Hispanics, African-Americans, and Caucasians, found that the *absence* of HLA-DR3 (*DRB1*0301*) was associated with greater disease activity. In another study of damage from SLE, 72 Hispanics, 104 African-Americans, and 82 Caucasians were included [201]. Greater damage was independently associated with different HLA-DRB1 and DQB1 genes (i.e., *DRB1*0301*, *DQB1*0201*, *DRB1*08*).

Non-MHC Genes in Predisposition to SLE (Table 4, Fig. 3)

Genes Located on Chromosome 1

Although not arranged as compactly as the MHC on chromosome 6p, genes on chromosome 1, the largest chromosome in the human genome, contain a number of immunologically relevant genes whose importance to the pathogenesis of SLE is only now being realized (Fig. 2).

TNF Receptor II (TNFR2)

A number of studies reported associations of HLA-DRB1, TNF-α promoter and TNF receptor II (TNFR2, TNFRSF1B) polymorphisms with systemic lupus erythematosus; however, the results have often been inconsistent. Komata *et al*. [202] genotyped 81 Japanese SLE patients and 207 healthy controls for exon 6 polymorphisms of the TNFR2 gene and found 37% of the SLE patients to have the 196R allele compared to 18.8% of the controls ($P = 0.001$). Lakahasi *et al*. [203], TDT, 91 Caucasian SLE family samples recruited in southern California for the association with HLA-DRB1, TNF promoter positions at –1031, –863, –857 and –308, and TNFR2-196M/R polymorphisms. No transmission distortion was observed for TNFR2 alleles.

Morita *et al*. [204] analyzed all 10 exons of the TNFR2 gene in 105 Japanese SLE patients and 99 controls and found an increased frequency for the 196R allele (20.5% vs 12.6%, $P = 0.03$). This finding has not been confirmed in other ethnic groups. Al-Ansari *et al*. [205] typed 128 Spanish and 74 United Kingdom Caucasoid SLE patients and found no differences, as did Sullivan *et al*. [206] in North American Caucasoid SLE patients, Lee *et al*. [207] in 139 Korean SLE patients and 137 healthy control subjects, and Takahasi *et al*. [203] in another cohort of 143 Japanese SLE patients.

BCMA (TNFRSF17), along with TACI, has been demonstrated to be a receptor for BLyS (TNFSF13B). Studies have indicated a substantial role of the BLyS signaling pathway for systemic lupus erythematosus. Kawasaki *et al*. [208] examined polymorphisms of human BCMA in Japanese SLE patients, finding only a nonsignificant increase in the frequency of the BCMA.03 allele ($P = 0.089$).

Glutathione-S-Transferase (GSTM1) Null Alleles

Data suggest a role for members of the glutathione-*S*-transferase supergene family in protection against oxidative stress. Ollier *et al*. [209] examined 90 British Caucasian patients for GSTM1 null homozygosity and found all patients with anti-Ro antibodies (without anti-La) were GSTM1 null, suggesting that the dysfunction of these genes may contribute either to the anti-Ro autoantibody response or to photosensitivity, with which anti-Ro is strongly associated. This could not be confirmed in another larger study by Tew *et al*. [210], who examined a prospectively followed cohort of Caucasian, African-American, and Mexican-American SLE patients, finding no association with SLE *per se*, anti-Ro autoantibodies, or clinical features.

The Fc γ Gene (FcγR) Family

FcγR receptors mediate the elimination of immune complexes and infectious agents by phagocytosis [211]. Abnormal handling of immune complexes is widely considered to be an important factor in the pathogenesis of SLE and has long been postulated that inefficient FcγR binding of immune complexes is pathogenic in SLE patients [212]. Three main classes of FcγR receptors have been defined in humans—FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16)—each containing multiple distinct genes and alternative splicing variants. Their genes have been mapped to chromosome 1q23. FcγRIIa has two codominantly expressed alleles, R131 and H131, which differ by two nucleotides, resulting in amino acid differences at positions 27 and 131, which differ substantially in their ability to bind IgG2 and IgG3, suggesting that they have significant functional differences. In fact, FcγRIIa-H131 is the only human FcγR that recognizes IgG2 efficiently. Salmon *et al*. [212] examined 103 African-American SLE patients with nephritis and 100 controls and demonstrated a skewed distribution of FcγRIIA alleles (although the same was

not seen in Caucasians examined) with only 12% of SLE patients versus 27% of controls being homozygous for FcγRIIa-H131 alleles. Moreover, patients with SLE who were homozygous for R131 alleles were at greater risk for invasive pneumococcal infections [213]. Duits *et al*. [214] examined 95 Caucasians and found the negative association with H131 homozygosity to cross ethnic boundaries. Botto *et al*. [215] found no association with SLE *per se*, although a correlation with nephritis persisted.

Manger *et al*. [216] examined 108 Caucasian SLE patients and 187 Caucasian controls, finding no significant skewing of the FcγRIIa polymorphism was observed in the SLE cohort. In patients with the genotype IIa-R/R131, significantly higher frequencies of proteinuria, hemolytic anemia, antinuclear RNP antibodies, and hypocomplementemia were found. The only clinical symptom observed more frequently in patients homozygous for IIa-H/H131 was livedo. Patients with the IIa-R/R131 genotype were significantly younger at disease onset and had an earlier incidence of arthritis, sicca syndrome, nephritis, lymphadenitis, hematologic abnormalities, immunologic abnormalities, lupus anticoagulant, cryoglobulinemia, and hypocomplementemia.

Norsworthy *et al*. [217] studied 195 Caucasian lupus patients for anti-C1q(CLR) antibodies and FcγRIIA genotypes. Fifty-six patients were anti-C1q antibody positive, and Ig subclass analysis indicated a predominance of IgG2 anti-C1q antibodies. Analysis of the SLE population as a whole revealed no significant difference in the allele frequencies of R131 and H131 compared with controls. There was, however, a significantly increased frequency of the R131 allele both in the anti-C1q-positive subgroup of patients and in the 71 patients with nephritis compared with controls.

Michel *et al*. [218] examined FcγRIIa genotypes in 80 French Canadian SLE patients and 183 controls and found that the proportion of SLE patients with the R/R131 genotype was significantly greater than controls (35% vs 18%, $P < 0.02$), although no association with nephritis was seen.

Dijstelbloem *et al*. [219] examined polymorphisms of FcγRIIa and -IIIa in 230 Caucasian patients with SLE and 154 controls. A strong trend toward skewing of FcγRIIa was found, with an enrichment of the homozygous FcγRIIa-R/R131 genotype in patients compared with controls. No correlation between this genotype and the development of lupus nephritis was seen.

Zuniga *et al*. [220] studied 67 Hispanic SLE patients and 53 disease-free control subjects. Patients were followed longitudinally for 3 years. In the nephritis group ($n = 46$), as well as the entire SLE cohort, there was a predominance of genotypes with low-binding alleles (FcγRIIa-R131 and FcγRIIIa-F176) at both loci. The frequency of individuals homozygous for high-binding alleles at either locus decreased as the burden of disease increased. There was no linkage disequilibrium between FcγRIIA and FcγRIIIA in Hispanics; however, in SLE patients, there was a clear overrepresentation of the FcγRIIa-R131:FcγRIIIa-F176 haplotype and a decrease in the frequency of the high-binding haplotype.

Song *et al*. [221] examined 73 Korean SLE patients and found an abnormal distribution of FcγRIIa alleles when compared with 64 controls: 11.0% of the SLE patients were homozygous for FcγRIIa-H131 compared with 34.4% of the controls. The allelic frequency of FcγRIIa-H131 was significantly lower in SLE patients than in controls, and it was also significantly lower in lupus patients with nephritis compared with the normal population, but was not significantly lower in lupus patients without nephritis. Clinically, the level of proteinuria was significantly higher in lupus nephritis patients who had R/R131 than in those who had H/H131 or R/H131.

Yun *et al*. [222] examined FcγRlla and FcγRIIIa polymorphisms and their association with clinical manifestations in 300 Korean lupus patients and 197 Korean disease-free controls. There was significant skewing in distribution of the three FcγRIIa genotypes between the SLE and the controls, but not in FcγRIIIa genotypes. The FcγRIIa-R allele was a significant predictor of lupus nephritis, as compared with SLE patients without nephritis, but proliferative nephritis (WHO classes III and IV) was less common in patients with FcγRlla-R/R131 and in FcγRIIa-R allele.

Yap *et al*. [223] investigated the polymorphism for FcγRIIA in 175 Chinese and 50 Malays SLE patients, as well as 108 and 50 ethnically matched healthy controls for the respective groups and found no significant differences between the two groups.

Sato *et al*. [224] studied the distribution of the FcγRIIa polymorphism in 90 Japanese patients with SLE, as well as the association between FcγRIIa polymorphism and the disease activity of SLE and the histopathological findings of lupus nephritis. The allelic frequency of H131 in patients with SLE was significantly lower than that of normal controls. No significant association was observed between FcγRIIa polymorphism and the clinical parameters for the activity of SLE. There was no association between FcγRIIa polymorphism and the histological findings in lupus nephritis.

Hatta *et al*. [225] determined FcγRIIA-131H/R, FcγRIIIA-176F/V, and FcγRIIIB-NA1/2 genotypes in 81 Japanese patients with SLE and 217 healthy individuals. Unlike in other populations, a significant difference was not observed in the frequencies of FcγRIIA and FcγRI-

IIA genotypes between patients with SLE and healthy individuals. However, a significant difference was detected in the frequencies of FcγRIIIB genotypes between SLE and healthy individuals. Among the patients with SLE, individuals with NA2/2 were significantly more likely to have lupus nephritis. Significant linkage disequilibrium was detected between FcγRIIIA and IIIB, but not between IIA and IIIA or between IIA and IIIB.

Seligman *et al*. [226] compared the frequency of low-affinity alleles of two FcγR polymorphisms (FcγRIIA and FcγRIIIA) among 235 patients with SLE and proven nephritis (nephritis patients) and among 352 SLE patients with no evidence of renal disease (non-nephritis control subjects). The ethnic distribution of patients in the study was 49% Caucasian, 20% Hispanic, 17% Asian/Pacific Islander, 12% African-American, and 2% from other ethnic groups. All patients were genotyped for the FcγRIIA-131R/H and FcγRIIIA-158V/F polymorphisms. Univariate and multivariate analyses demonstrated a striking association between the low-affinity FcγRIIIA-158F allele and the FF genotype and the risk of nephritis among Caucasians, but not among non-Caucasians. This association was even stronger among Caucasians with severe nephritis. In contrast, inheritance of the low-affinity FcγRIIA-131R allele (and RR genotype) was not associated with an increased risk of lupus nephritis among any of the ethnic groups examined. Smyth *et al*. [227], however, when examining 123 Caucasian SLE patients (81 from the United Kingdom and 42 from Greece), found no association with FcγRIIa alleles, nor did Villarreal *et al*. [228], who examined 125 Catalan SLE patients.

More recently, Karassa *et al*. [229] conducted a meta-analysis of 17 studies involving R/H 131 genotyping of 1404 patients with lupus nephritis and 1709 SLE patients without nephritis and found a dose–response relation between the R131 allele and risk for SLE, but no clear effect on susceptibility to nephritis.

FcγRIIB is unique in its ability to transmit inhibitory signals, and animal studies have demonstrated a role for FcγRIIB deficiency in the development of autoimmunity. Kyogoku *et al*. [230] screened for polymorphisms of the human FCγRIIB gene in 193 Japanese patients with SLE and 303 healthy individuals. A single nucleotide polymorphism in FCGR2B (c.695T > C), coding for a nonsynonymous substitution, Ile232Thr (I232T), within the transmembrane domain was increased significantly in SLE patients compared with healthy individuals.

FcγRIIIa is expressed on natural killer cells. Wu *et al*. [231] examined 200 "ethnically diverse" SLE patients and found an increase in homozygosity for a phenylalanine at position 176, especially in those with nephritis. Koene *et al*. [232] genotyped 70 Caucasian SLE patients for all known FcγR polymorphisms, finding the frequency of the FcγRIIIA-158F to be associated with SLE. Salmon *et al*. [233] examined 148 Korean SLE patients, including 79 with nephritis, and found an association with homozygosity for the FcγRIIIa allele-F176 compared with those without nephritis or with 97 controls. Similarly, Kyogoku *et al*. [230] found a significant association in 193 Japanese SLE patients of a FcγRIIIA-176F allele compared to 303 controls. However, Yap *et al*. [234] examined 183 Chinese and 55 Malays SLE patients, as well as 100 Chinese and 50 Malays healthy controls, and found no differences in the gene frequencies for FcγRIIIB-NA1 and FcγRIIIB-NA2.

These data suggest that Fcγ genes constitute the best candidate for a non-MHC gene in the pathogenesis of SLE. Clearly, this area will provide a fruitful area of investigation in the near future as the single nucleotide polymorphisms that best impact on the susceptibility to and outcome of SLE are better defined.

FAS *Ligand and* FAS

The APO1 (*fas*) antigen (48 kDa), defined by the mouse monoclonal antibody anti-APO1, is expressed on the cell surface of various normal and malignant cells, including activated human T and B lymphocytes and a variety of malignant human lymphoid cell lines. APO1 cDNA shows significant sequence similarity to members of the tumor necrosis factor/nerve growth factor receptor superfamily and is in fact the same as the *fas* antigen. Binding of the anti-APO1 antibody to the APO1 antigen induces apoptosis. Mutations in the human analogue of the *fas* gene have been associated with a lymphoproliferative disorder resembling those seen in the *lpr* mouse [235, 236]. The contribution of defective *fas* gene function to predisposition to SLE is controversial, as defects in activation-induced cell death or alterations in *fas* gene expression have not been documented in patients with SLE [237, 238].

Lee *et al*. [239] evaluated whether the polymorphism of *fas* promoter –670 is associated with susceptibility to SLE and clinical features thereof. The genotype distribution of the *fas* promoter –670 did not differ between patients with SLE and control subjects, nor were there any significant differences in age at onset, anti-dsDNA titer, C3, C4 level, renal involvement, number of ACR criteria met, disease activity, or damage. However, a significant association with the anti-RNP antibody was seen. Huang *et al*. [240, 241] could confirm neither an association with the –670 polymorphism nor another promoter polymorphism at position –1377 in 86 Australian Caucasian SLE patients, although homozygosity for the *MvaI*2* allele at the –670 promoter polymorphism was weakly associated with photosensitivity and oral ulcers. Horiuchi *et al*. [242] investigated the fre-

quency of a novel polymorphism at nucleotide 297 which was linked to another *fas* polymorphism at position 416 in 82 Japanese SLE patients and 132 ethnically matched controls, finding an increased frequency of a 297C/416G allele. Because this was a silent polymorphism at the amino acid level, if it is relevant to SLE at all it reflects linkage to another more disease-relevant but unidentified polymorphism. Finally, Cascino *et al*. [243] could find no differences of *fas* gene polymorphisms between patients and controls.

The *fas* ligand is a member of the T-cell receptor superfamily whose gene has been mapped to chromosome 1q23. Wu *et al*. [244] examined 75 SLE patients for mutations in the *fas* ligand gene and found one patient with a heterozygous mutation (an 84-bp deletion) whose expression resulted in decreased *fas* ligand activity, decreased activation-induced cell death, and increased T-cell activation after activation. However, Mehrian *et al*. [245] found a significant association of SLE with a *fas* ligand intragenic marker. Thus, whether either the *fas* gene or *fas* ligand mutations contribute significantly to the pathogenesis of SLE in most patients is controversial and needs further study. Kojima *et al*. [246] investigated the possible association of a *fas* ligand (FasL) gene mutation(s) or polymorphism(s) with SLE in 143 SLE patients, and by SSCP analysis did not identify any mutations or polymorphisms in the FasL mRNA transcripts or in any of the four exons or areas of the introns adjacent to the exons.

E-Selectin

E-selectin is expressed on cytokine-stimulated endothelial cells and plays an important role in leukocyte–endothelium interactions and inflammatory cell recruitment. The best-characterized polymorphism in the E-selectin molecule is A561C, which codes for Ser128Arg. El-Magadmi *et al*. [247] studied the prevalence of the A561C E-selectin gene polymorphism in Caucasian patients with SLE and controls from three different ethnic populations: the United Kingdom (113 and 148), Spain (145 and 179), and Turkey (93 and 96), respectively. The C allele occurred more frequently in United Kingdom and Spanish patients, but not in Turkish patients.

C-Reactive Protein (CRP)

Elevation of baseline CRP is associated with an increased risk of cardiac disease. This increase might reflect low-grade inflammation, but differences in CRP serum levels may also have a genetic component. To test this possibility, Szalai *et al*. [248] investigated whether a polymorphic GT repeat in the intron of the CRP gene contributes to variation in baseline CRP. The polymorphism was associated with differences in baseline CRP both in normal individuals and in patients with the inflammatory disease systemic lupus erythematosus, namely donors carrying two GT(16) alleles, two GT(21) alleles, or GT(16/21) heterozygotes had twofold lower serum CRP than those with other genotypes. The frequency of GT(16) and GT(21) was twofold higher in Caucasians than in African-Americans, but there was no difference in allele distribution between patients and controls. It is not yet known how this genetic polymorphism mediates its effect on CRP expression, and it probably is not a systemic lupus erythematosus susceptibility factor, although an impact on disease severity has not been evaluated.

T-Cell Receptor ζ (CD3-ζ)

Signaling molecules from the T-cell receptor ζ/Fc ε receptor γ (TCRζ/FcRγ) family play a critical role in the function of Fcγ receptors and the TCR and are located on human chromosome 1q22–23, where lupus susceptibility genes are located. Nambiar *et al*. [249] sequenced TCR ζ chain cDNA from 48 SLE patients, 18 disease controls, and 21 healthy volunteers and found several widely distributed missense mutations and silent polymorphisms in the coding region of the TCR ζ chain, which were more frequent in SLE patients than in patients with other rheumatic diseases or healthy controls ($P < 0.0001$). Several of the missense mutations were located in the three immunoreceptor tyrosine activation motifs or the GTP-binding domain, which could lead to functional alterations in the TCR ζ chain. A splice variant of the TCR ζ chain with a codon CAG (glutamine) insertion between exons IV and V was found in half of the SLE and control samples. Two larger spliced isoforms of the TCR ζ chain, with an insertion of 145 bases and 93 bases between exons I and II, were found only in SLE T cells.

Wu *et al*. [250], however, found no unique mutations or differences in the frequencies of splice isoforms of TCR ζ in Caucasian, African-American, Hispanic, Chinese, or Japanese SLE patients living in North America.

Complement Receptor 1 (CR1)

CR1, the receptor for complement fragments C3b and C4b, is a membrane glycoprotein important not only for the control of complement activation, but also (as expressed on erythrocytes) for the efficient removal of circulating immune complexes. Three types of polymorphisms are known to exist for CR1: one is recognized as the Knops blood group. A second determines the quantitative expression of CR1 on erythrocytes, where a *Hind*III RFLP has been found to correlate with high and low expression of erythrocyte CR1 [251, 252]. Early data suggested that a low erythrocyte copy

number might be a genetic risk factor for SLE [253, 254]. However, other studies have shown this to be an acquired defect in SLE patients [255–260]. Satoh *et al*. [261] examined the frequency of the *Hind*III RFLP in Japanese, Kumar *et al*. [262] in Indian, and Tebib *et al*. [263] in Mexican SLE patients and found no difference in frequency between patients and controls. This further suggested the low CR1 levels to be an acquired phenomenon.

A third type of polymorphism exists in the CR1 molecular structure. Four codominantly inherited allelic size variants have been identified, with molecular masses of 160 kDa (CR1-C), 190 kDa (CR1-A), 220 kDa (CRC-B), and 250 kDa (CR1-D). These size variants evolved through duplication or deletion of a highly homologous unit. Van Dyne *et al*. [264] found an increased frequency of CR1-C in Caucasians with SLE. This observation seemed relevant because Wong and Farrell [265] proposed that CR1-C might be less efficient in removing immune complexes because of having only one C3b-binding site. However, Cornillet *et al*. [266] found instead an association with CR1-B. Moulds *et al*. [267] examined structural polymorphisms of CR1 in 219 SLE patients of approximately equivalent proportions of Hispanics, African-Americans, and Caucasians and found no differences from healthy controls. Thus it is unlikely that CR1 size polymorphisms contribute to susceptibility to SLE. Thus to date there is no suggestion that polymorphisms of the gene encoding CR1 contribute to the pathogenesis of SLE.

Interleukin-10 (IL-10) and IL-10 Receptor (IL-10R)

IL-10 is an important multifunctional cytokine, which has been found to be important in a variety of rheumatic and neoplastic diseases. Relative to autoimmunity, IL-10 reduces MHC class II expression on macrophages [268], antagonizes human T-cell clonal expansion [269], and inhibits the development of antigen-specific cytotoxic T cells [270]. Il-10 also, under certain conditions, can inhibit apoptosis in human T cells [271]. Most relevant to autoimmunity, IL-10 can serve as a potent activator of B cells [272] and, in patients with rheumatoid arthritis and SLE, promotes the secretion of rheumatoid factor [273] and other autoantibodies [274]. The gene for IL-10 has been mapped to chromosome 1q31–q32, near the genes for CR1 and CR2 [275]. Eskdale *et al*. [276] studied 56 Scottish SLE patients for the frequency of two novel dinucleotide repeats in the IL-10 promoter region (IL10.G and IL10.R) and suggested an association with IL10.G alleles, which correlated even better with the presence of renal disease and specific autoantibody production. More recently, Mehrian *et al*. [245] found a synergistic effect between IL-10 and bcl-2 polymorphisms in susceptibility to SLE in Mexican-Americans.

D'Alfonso *et al*. [277] determined whether seven candidate genes, including tumor necrosis factor receptor II, bcl-2, CTLA-4, interleukin-10, CD19, Fcγ receptor type IIA (CD32), and the IL-1 receptor antagonist, may contribute to susceptibility to SLE in Italians: A significant increase was found in SLE patients in the frequency of the 140-bp allele of the IL10.G microsatellite located in the promoter region of the IL-10 gene. Conversely, no significant association was detected for the remaining six candidate genes, even when the patients were stratified according to the presence of different clinical and immunologic features according to the presence of the associated HLA-DR or IL-10 alleles.

Ou *et al*. [278] studied 100 SLE patients and 103 healthy controls for IL-10PR by polymerase chain reaction and electrophoretic analysis and found no difference in the distribution of IL-10PR alleles, genotypes, and SBA as compared to healthy controls. Forty-nine patients with nephritis also showed no difference in IL-10PR alleles, genotypes, and SBA, as compared to 51 SLE patients without nephritis, although a strong association between IL-10PR and severe progression of lupus nephritis was seen.

Rood *et al*. [279] identified an IL-10 promoter haplotype that was found more frequently in 42 SLE patients with neuropsychiatric involvement compared with 50 patients without this complication, although no association was seen with SLE *per se*.

Mok *et al*. [280] studied the association of interleukin-10 promoter polymorphisms in 88 Southern Chinese patients with SLE and 83 ethnically matched controls and found no significant difference in the allele or haplotype frequencies However, when clinical features were examined, the *A allele at the –597 position and the *T allele at the –824 position were significantly associated with lupus nephritis, and the haplotype –1087*A/–824*T/–597*A was also associated with renal involvement.

Alarcon-Riquelme *et al*. [281] found no significant difference in the frequencies of the IL-10 promoter microsatellite alleles of 330 Mexican patients with SLE compared to 368 controls from the same population, and two-point linkage analyses carried out using 13 Mexican, 13 Swedish, and 8 Icelandic families with two or more cases with SLE found no linkage between IL-10 and SLE. Similarly, Van der Linden *et al*. [54] determined IL-10 genotypes in 163 first-degree relatives of patients with systemic lupus, 50 first-degree relatives of patients with chronic cutaneous lupus, and 133 control persons. The frequency of –1082/–819/–592 haplotypes GCC, ACC, and ATA was similar among patients and compared with the controls. Likewise, Crawley *et al*.

[282], could find no association of IL-10 genotypes with SLE, renal disease, or autoantibodies.

This suggests that the impact of IL-10 genes on susceptibility to SLE *per se* is weak, if present at all, although an impact on disease severity may be operative.

The IL-10 receptor maps to chromosome 11q23.3. Nakashima *et al*. [283] examined IL-10R genotypes in 109 Japanese SLE patients and 102 controls and found no differences in genotype frequencies.

Poly(ADP-ribose) Polymerase

Tsao *et al*. [284] and Delrieu *et al*. [285] used a multiallelic, transmission disequilibrium test (TDT) and found a skewing of transmission of alleles of poly(ADP-ribose) polymerase, which encodes a zinc finger DNA-binding protein that is involved in DNA repair and apoptosis, to affected offspring in 124 families ($P = 0.00008$) and a lack of transmission to unaffected offspring. Unfortunately, these finding could not be confirmed in a study of 171 French Caucasians and 193 ethnically matched healthy controls [286] in a larger study of three cohorts from Minnesota, Oklahoma City, and San Francisco [287], or in another study from Germany [288].

HRES-1

HRES-1 is a single-copy human T-cell lymphotrophic virus type I (HTLV-I)-related endogenous retroviral sequence (ERS), which is transcriptionally active and contains open reading frames (ORFs) [184]. It is thought to have entered the genome of Old World primates. *HRES-1* encodes a 28,000 MW nuclear protein, which is expressed in a tissue- and cell type-specific manner. Its function is unknown. Magistrelli *et al*. [184] examined the frequency of alleles of a polymorphic *Hind*III site at position 653 of the long terminal repeat region in 60 Caucasian SLE patients and 100 controls and found that homozygosity for a 5.5-kb RFLP was 3.2-fold lower in frequency in patients compared to controls. The significance of this finding is unclear, especially as it relates to susceptibility to SLE, and needs further study.

Genes Implicated in Susceptibility to SLE on Other Chromosomes

Immunoglobulin Heavy and Light Chain Genes

Studies of immunoglobulin heavy chain genes (located on chromosome 14q32) and gene products (Gm protein allotypes) have yielded conflicting results. Earlier studies suggested a possible disease association in American and Australian Caucasian SLE patients [289, 290]. Fedrick *et al*. [291] examined 61 African-Americans and showed an association with the Gm phenotype 1,17;5,6,13. Nakao *et al*. [292] also found a Gm association in Japanese SLE patients. Hoffman *et al*. [293] examined 78 North American Caucasians with SLE, MCTD, and scleroderma and found an association with an immunoglobulin κ light chain phenotype Km [1] (encoded on chromosome 2). Genth *et al*. [294] found an association with the Gm (1,3:5,21) phenotype and the U1-RNP autoantibody response. Stenszky *et al*. [295] found no Gm association among 90 Hungarian SLE patients, although homozygosity for Gm and HLA-B8 predicted those at the highest risk for developing renal disease. Kumar *et al*. [296] found no disease association with a *Bst*EII immunoglobulin constant region RFLP in North American or Mexican SLE patients. Hartung *et al*. [297, 298] examined immunoglobulin heavy chain (Gm) and κ light chain (Km) allotypes in 323 central European SLE patients and found no association with SLE *per se*, autoantibodies, or clinical manifestations of the disease. Blasini *et al*. [299] examined constant region λ polymorphisms in 78 Venezuelan SLE patients and could likewise demonstrate no association.

It is difficult to exclude a contribution of immunoglobulin genes to the pathogenesis of SLE based on these data, however. The Gm and Km allotypes correspond to regions of the heavy and light chains of limited polymorphism, and RFLP studies give little information about the rich polymorphism that resides at the immunoglobulin genes. Just as for T-cell receptor genes, immunoglobulin genes undergo extensive rearrangement, and the role of these immune response genes in the pathogenesis of SLE remains to be determined. Queiroz *et al*. [300] examined 56 Brazilian SLE patients and 103 controls for *EcoRI* RFLP of the Ig λ V8 gene and found no occurrence of a 6.0-kb *Eco*RI RLFP in the patients compared to 10% in controls.

However, it has been hypothesized that deletions of certain Ig variable region genes may predispose to autoimmunity [301]. Olee *et al*. [301] and Huang *et al*. [302] found that approximately 20–25% of patients with RA or SLE have deletions of RFLP associated with certain autoreactive V genes, although these associations were felt to be "weak" [302].

Interleukin-1 Receptor Antagonist (IL-1-RA) Gene

IL-1RA is a powerful endogenous anti-inflammatory agent whose protein is 22–25 kDa in size and which competes with IL-1α and IL-1β for the occupancy of cell surface receptors, inhibiting the action of IL-1. The gene for IL-1RA lies on the long arm of chromosome 2, in the same gene complex as IL-1α and IL-1β [303].

Blakemore *et al*. [304] found a weak association of a five allelic variable length polymorphism in intron two of the IL-1RN gene (*IL1RN*2*) in 81 English SLE patients. This allele was particularly associated with photosensitivity and discoid rashes, leading to the conclusion that IL-1RA alleles were probably more important in the clinical expression of SLE than in actual predisposition. Tjernstrom *et al*. [305] investigated 81 patients from a defined area in southern Sweden and 10 consecutive Caucasian families with multiple cases of SLE. As control group, 189 healthy blood donors was used and an increased frequency of *IL1RN*2* was found in both the epidemiological cohort and the multicase families. Although alone *IL1RN*2* and MHC class II (*DRB1*0301*, *DQB1*0201*) separately increased the SLE risk moderately, the occurrence of these alleles together increased the risk for SLE sevenfold. An association between *IL1RN*1* and arthritis was also seen.

Cytotoxic T Lymphocyte Antigen 4 (CTLA-4)

Cytotoxic T lymphocyte associated antigen 4 (CTLA-4), a structural homologue of CD28, has been reported to be an important negative regulator of autoimmune diseases. The negatively signaling molecule CTLA-4 is involved in establishing and maintaining peripheral T-cell tolerance, which controls T-cell activation and reactivity. Its attenuating action helps to prevent an inappropriate initiation of T-cell responses to self-antigens and to terminate ongoing T-cell responses. CTLA-4 genotypes have been implicated in various autoimmune diseases (Hashimoto thyroiditis, Graves' disease, IDDM). Pullman *et al*. [306] tested if there was an association between CTLA-4 and SLE in 102 SLE patients and in 76 healthy controls and found that the distribution of CTLA-4 exon 1 genotypes in the SLE group was significantly different from that in the controls. The frequency of the G allele was significantly higher in SLE patients than in controls. These data were further confirmed by Ahmed *et al*. [307] in 113 Japanese SLE patients and 200 controls from the northern Kyushu area of Japan, where SLE patients had a higher frequency of the CTLA-4 49G allele and of the CTLA-4 (AT)n microsatellite repeat polymorphism.

These data have not been confirmed in other groups. Heward *et al*. [308] genotyped 126 SLE patients and 363 controls from the United Kingdom and found no differences in either allele or genotype frequencies. Matsushita *et al*. [309] screened 71 Japanese SLE patients and 150 controls and found no association with CTLA-4 polymorphisms, nor with polymorphisms of human CD28, CD80, and CD86 genes [310]. Liu *et al*. [311] examined CTLA-4 gene polymorphisms in the promoter and exon 1 in 81 Chinese SLE patients and 81 normal controls and found no statistically significant differences. Lee *et al*. [312] examined 80 Korean SLE patients and 86 healthy control subjects and no disease correlation was found between CTLA-4 exon 1 (+49) and promoter (–318) polymorphisms.

IL-4 Promoter and Receptor Polymorphisms

Interleukin-4 (IL-4) is a cytokine produced by T cells that regulates proliferation and differentiation of a variety of cells. It plays a major role in inducing activation and differentiation of B cells as well as in stimulating the Th2 response. The –590C/T polymorphism of the IL-4 gene is associated with increased IL-4 promoter activity. The interleukin-4 receptor is composed of two subunits: a 140-kDa α subunit, which binds interleukin-4 and transduces its growth-promoting and transcription-activating functions, and a γc subunit, common to several cytokine receptors, which amplifies signaling of the interleukin-4 receptor α. Polymorphisms at codons 50 and 551 of the IL-4R α chain affect IL-4R function; an isoleucine residue at position 50 enhances receptor responsiveness to IL-4 compared to a valine in this position. Similarly, an *arginine* at codon 551 impairs binding of a negative regulatory molecule, which leads to increased receptor activity relative to the alternative glutamine allele. Kanemitsu *et al*. [313] genotyped these polymorphisms in 50 Japanese SLE patients and 100 controls. SLE patients had a higher frequency of an isoleucine allele at codon 50 and an arginine allele at codon 551 in IL-4R than controls, although no differences were seen with the position –590 alleles of the IL-4 promoter.

Prolactin

Hyperprolactinemia is associated with systemic lupus erythematosus, but the mechanism is unknown. Prolactin is expressed in T lymphocytes and is under the control of an alternative promoter region. Altered local prolactin production by immune cells may contribute to disease progression by affecting T-cell function. Stevens *et al*. [314] characterized a G/T single nucleotide polymorphism (SNP) at position –1149 of this promoter and found a functionally significant polymorphism that altered prolactin promoter activity and mRNA levels in the lymphocytes.

Estrogen Receptor: α and β

The estrogen receptor (ESR) is a ligand-activated transcription factor composed of several domains important for hormone binding, DNA binding, and activation of transcription. Two isoforms of the human ESR, ESR-α and ESR-β, occur, each with distinct tissue and cell patterns of expression. Kassi *et al*. [315] analyzed the coding sequence of ERα and ERβ in 10 Greek SLE patients and 12 controls, finding splicing variants

and deletions in exons 2, 5, and 7 to occur in equivalent frequency in both groups. Chang *et al*. [316] and Liu *et al*. [317] examined *Pvu*II and *Xba*I RFLP of the ER gene (type not specified) in 245 biopsy-proven lupus nephritis patients (187 females, 58 males) and 172 normal controls (71 females, 101 males). Male SLE patients had an increased frequency of the PpXx genotype compared to male controls, although no difference was found in females. Also, skin rash and arthritis were seen more frequently in those with the PpXx genotype.

Interferon-γ Receptor 1 and 2

Interferon-γ has an important role in activating macrophages in host defenses. At least two steps are involved in the action of interferon-γ; the binding of IFN-γ to its receptor coded by chromosome 6 and the coupling of this binding event through a factor coded by chromosome 21 to trigger biologic action. The IFN-γ receptor is a complex consisting of IFN-γR1 and IFN-γR2. Tanaka *et al*. [318] found an increased frequency of a genetic polymorphism in the IFN-γ receptor 1 (IFNGRI) gene that causes an amino acid polymorphism (*Val14Met*) located at the COOH terminal of the signal peptide of IFNGRI in 110 Japanese SLE patients and 110 controls. In a subsequent study from this group, Nakashima *et al*. [319] found an amino acid polymorphism (*Gln64Arg*) within IFN-γR2 and discovered that the greatest risk of the development of SLE was detected in individuals who had the combination of IFNGR1 *Met14/Val14* genotype and IFNGR2 Gln64/Gln64 genotype.

Lee *et al*. [320] analyzed the gene frequency of eight different alleles in 99 controls and 136 patients with SLE. There were no statistically significant differences in allele frequencies between patients and controls, although allele 1 was associated with gastrointestinal lupus and allele 6 was associated with disease severity. Allele 2 appeared to be protective for arthritis. This suggests that genetic variation in interferon-γ expression might influence the disease course.

Interleukin 6

Linker-Israeli *et al*. [321] examined IL-6 genes in 57 SLE patients and 36 controls, finding allelic, higher molecular weight *Xba*I bands in 9/57 compared with one of 36 controls, suggesting that this variability may be related to the abnormal IL-6 expression seen by his group and others. This group further evaluated the association of alleles of regions having regulatory potential in the IL-6 gene with SLE in 146 Caucasian and African-American SLE patients and 139 controls, finding that the AT-rich minisatellite allele distribution pattern was significantly different in SLE as compared to controls [322]. In either race, short allele sizes (< or = 792 bp) were seen exclusively in SLE patients, whereas the 828-bp allele was overrepresented in controls. In contrast, there was no preferential association of SLE with G/C alleles in the 5′ region of the IL-6 gene. Furthermore, these results suggest that the 3′ minisatellite alleles have biological significance: (1) B lymphoblastoid cells of patients having one or two SLE-associated alleles secreted IL-6 in three- to four-fold higher levels than nonallelic cells; (2) higher percentages (approximately four-fold) of IL-6 positive monocytes were observed in individuals having SLE-associated IL-6 alleles; and (3) in lupus patients having SLE-associated minisatellite alleles, IL-6 mRNA stability was enhanced significantly.

However, Schotte *et al*. [323], examining 211 German SLE patients, found no difference in the frequency of the IL-6 –174G/C promoter polymorphism between patients and controls (where the G allele is associated with higher IL-6 expression), although there was a weak association with discoid skin lesions and antihistone antibodies. This attractive locus clearly needs further confirmation.

Plasminogen Activator Inhibitor-1 (PAI-1)

PAI1 cDNA encodes a protein containing 402 amino acids with a predicted nonglycosylated molecular mass of 45 kDa. The plasminogen activator inhibitor shows structural similarities to angiotensinogen, α_1-antitrypsin, and antithrombin III. Plasminogen activator inhibitor-2 is less similar to PAI1 than it is to the other proteins of this group. There are at least three immunologically distinct plasminogen activator inhibitors (PAIs): placental PAI, protease nexin, and endothelial cell-derived PAI. The last is also distinctive for its β mobility in agarose zone electrophoresis and its inhibition of both tissue-type PA and urokinase-type PA. Wang *et al*. [324] examined the frequency of a single base pair insertion/deletion polymorphism of the PAI-1 gene (4G/5G) in 118 southern Chinese patients with SLE and 103 controls that has been shown to alter PAI-1 gene expression. Although no differences were seen between patients and controls, lupus nephritis patients who had the 4G/4G polymorphism had significantly heavier proteinuria, higher activity indices, and a higher frequency of extensive necrotizing lesions on renal biopsy compared to patients with 4G/5G and 5G/5G, suggesting an impact of PAI-1 on the course of SLE.

T-Cell Receptor Genes

The α:β T-cell receptor molecule, expressed on $CD4^+$ and $CD8^+$ lymphocytes and to which both HLA class I and class II molecules present their processed peptides,

is a heterodimer whose respective α and β polypeptides are encoded on different chromosomes. The TCRα gene complex spans over 1000 kb and has been mapped to chromosome 14q11–12. The TCRβ complex spans 600 kb at chromosome 7q35. A number of studies have examined the frequencies of TCR in SLE patients and their families. Neither Wong *et al*. [325] nor Dunckley *et al*. [326], using RFLP analysis and Southern blotting, found no linkage of TCRα, -β, or -γ genes in five multiplex Caucasian families. Tebib *et al*. [327], working with the same group, subsequently described an association of a 1.3- and 3.0-kb *Pst*I TCRα constant region RFLP band pair in Caucasians with SLE, although the same was not seen in nine Mexican multiplex SLE families examined concomitantly or in another North American study of 41 unrelated SLE patients and 14 multiplex SLE families [327]. Frank *et al*. [328] described an association of a 9.8-kb *Bgl*II and a 1.75-kb *Kpn*I TCRβ RFLP with the anti-Ro autoantibody response in 76 SLE patients of mixed ethnicity.

There are technical problems of these studies of TCR polymorphism in that the RFLP analyses performed are usually done on genomic DNA. Much of the antigenic and MHC specificity results from the DNA rearrangement that the constant, diversity, joining genes and variable region genes, which exist at different segments on the chromosome, undergo. Any one of over 80 known Vβ genes can combine with one of two Dβ, one of 12 Jβ, and one of two Cβ segments. Similarly, any of over 100 Vα segments can combine with one of 50 Jα and the one Cα gene, which is not considered by RFLP studies of genomic DNA, and further studies are necessary to rule in or out a significant contribution of TCR genes to the pathogenesis of SLE.

Mannose-Binding Protein (MBP) Genes

Mannose binding protein is an acute-phase serum protein whose polypeptide chains form trimers that associate to give a structure analogous to that of C1q, thus being able to activate the classical and alternative complement component pathways independent of antibody [329]. MBP deficiency results in an opsonic defect that causes recurrent infections in children. The best-characterized hereditary defect in MBP is a point mutation at nucleotide 230 of exon 1, causing the substitution of aspartic acid for glycine [330]. This results in a significant reduction of serum MBL. Homozygosity for this mutation is considered the most common inherited immunodeficiency, and the gene frequency of this allele is 17% in the Caucasian British population [331]. Given the associations of complement deficiencies with SLE, it would be reasonable that MBP deficiency might contribute to predisposition to SLE. Davies *et al*. [332] examined 102 Caucasians with SLE from northwestern England, finding the allele in 41% (compared to 30% of controls), leading to the conclusion that this MBP allele might represent a "minor" risk factor for SLE. Further studies in 50 Caucasians from Spain [333] found an even higher frequency (52%), although the frequency of individuals homozygous for this mutation was not increased. This led to the conclusion that this "dysfunctional" MBP allele might affect the susceptibility to SLE in an additive manner with C4 null alleles [333]. Sullivan *et al*. [334] confirmed this association in 92 African-American SLE patients, finding two MBP structural polymorphisms associated with low serum levels of MBP to be increased in frequency and a MBP promoter haplotype associated with high serum MBP levels to be negatively associated with SLE.

Ip *et al*. [335] studied serum MBL levels and codon 54 mutations in 112 Chinese patients with SLE and 110 healthy controls and found a significant difference in the distribution of the two pairs of promoter polymorphisms, H/L and Y/X, between SLE patients and controls.

Garred *et al*. [336] determined MBL alleles and serum concentrations in 91 Danish patients with SLE and in 250 controls. Homozygotes for MBL had an increased risk of acquiring serious infections compared with patients who were heterozygous or homozygous for the normal. In a subsequent study, Garred *et al*. [337] conducted a meta-analysis of eight previously published studies, suggesting that the presence of MBL variant alleles confers a 1.6 times overall increased risk for SLE . Moreover, in 99 Danish SLE patients, MBL variant allele carriers had higher disease activity (SLEDAI index) in a 2-year follow-up period and had an increased risk of acquiring complicating infections in general and respiratory infections in particular.

Tsutsumi *et al*. [338] analyzed the MBL gene polymorphisms of Japanese SLE patients and controls and found that patients studied had a significantly higher frequency of having a homozygous codon 54 mutation compared to controls. In particular, patients with SLE or Sjogren's syndrome showed higher probabilities of being homozygous for this mutation. Among subjects with the same genotype, SLE patients tended to have higher serum MBL concentration than controls. Analysis of the promoter region suggested that SLE patients heterozygous for the codon 54 mutation have a higher probability of having a low producing haplotype for the gene without the codon 54 mutation.

Horiuchi *et al*. [339] examined the frequency of the polymorphism at codon 54 (substitution from Gly to Asp; G54D) in 105 healthy Japanese individuals and 95

SLE patients, as well as two polymorphisms at positions of –550 and –221 in the promoter region, and found no associations.

Acetylation Phenotype and Cytochrome P450 Oxidase Genes

It has long been recognized that patients with drug-induced LE were genetically low acetylators of aromatic amine and hydrazine compounds [340]. However, extensive acetylation phenotyping of SLE patients has failed to show this as a contributing factor to SLE [341, 342]. In several cases, however, xenobiotics (i.e., drugs and occupational agents) were identified as etiologic agents, and associations with certain polymorphic alleles of xenobiotic-metabolizing enzymes have been reported. Cytochrome P4501A1 (*CYP1A1*) and *N*-acetyltransferase 2 (NAT-2) are xenobiotic-metabolizing enzymes of phase 1 and phase 2 metabolism, respectively. *CYP1A1* may activate drugs and other chemicals to reactive metabolites. NAT-2 is the most important enzyme in the acetylation of aromatic amines, and thus may be responsible for the detoxification of many of these compounds. Two polymorphisms of the human *CYP1A1* gene, a point mutation in the 3′ flanking region of the gene (Msp1) and a mutation in exon 7 leading to an isoleucine–valine–exchange in the heme-binding region of the enzyme, have been described and may lead to higher basal and inducible enzyme activity. With respect to NAT-2, several alleles that combine for the two phenotype "fast" and "slow" acetylators have been described. Von Schmiedeberg *et al*. [343] analyzed the gene frequencies of the *CYP1A1* polymorphisms and the phenotypes of NAT-2 in 68 and 88 German SLE patients, respectively, compared to 184 controls and found a significant increase in the mutant Val allele when compared to controls. Regarding the NAT-2 phenotype, 80.2% of patients with SLE were slow acetylators compared to 55% slow acetylators in the healthy German population. This supported the notion that nonacetylated xenobiotics in slow acetylators may accumulate and are subsequently metabolized by other enzymes into reactive intermediates. Thus, an enhanced formation of reactive metabolites could alter self-proteins presented to the immune system, thus stimulating autoreactive T cells, which induce autoimmunity.

Sullivan *et al*. [344] found no association of the polymorphic *CYP17* allele with SLE, nor did Kortunay *et al*. [345] find any differences in the *CYP2C19* (an enzyme important in the metabolism of a number of drugs, including mephenytoin, diazepam, and omeprazole) genotypes between 37 Turkish SLE patients and 161 healthy controls.

ETS1

ETS1 encodes a set of nuclear phosphoproteins ranging in size from 42 to 52 kDa, which are transcription factors that activate transcription by interaction with purine-rich sequences present in the promoter/enhancer region of the T-cell antigen receptor, α subunit (TCRA) gene. Given the identification of chromosome 11q23 containing a potential susceptibility locus for SLE in a genome-wide scan [65], Sullivan *et al*. [346] examined 119 SLE patients and 130 controls for polymorphisms of the ES1 gene. None of the seven alleles examined were seen more frequently in patients vs controls, although marginal associations were seen of specific alleles and discoid LE and vasculitis. These findings need confirmation in a larger series of patients.

Vitamin D Receptor (VDR)

Previous studies have shown that polymorphisms of the vitamin D receptor can not only influence bone density, but also, indirectly, IL-2 gene transcription. Ozaki *et al*. [347] examined 58 Japanese SLE patients and 87 controls for VDR genotypes, finding the BB genotype to occur in 15.5% of patients compared to 5.7% of controls. Furthermore, the BB genotype was seen more frequently in patients with nephritic syndrome (61.5% vs 35.7). Given the relatively small number of patients studied and the smaller proportion that had the BB genotype, this finding needs to be confirmed in a larger cohort.

DNase I

Deoxyribonucleases are important enzymes involved in the clearance of DNA from apoptotic cells, and low serum deoxyribonuclease activity has been documented in human SLE patients [348]. Yasumoto *et al*. [349] reported that 2 of 20 Japanese SLE patients (and 0/100 controls) possessed a heterozygous *AWG* mutation in exon 2 of *DNASE1*, resulting in a premature stop codon. Although these patients had very low serum deoxyribonuclease activity, the authors also found that compared to normal controls, serum deoxyribonuclease activity was also low in SLE patients who did not carry any *DNASE1* mutations. In contrast, Tew *et al*. [350] sequenced a cohort of 18 North American SLE patients with lupus nephritis and known low serum deoxyribonuclease activity and found no mutations in *DNASE1*. Furthermore, transcript levels of *DNASE1* were not significantly different between SLE patients compared to age-, sex-, and race-matched controls. This and the fact that the majority of SLE patients (whose *DNASE1* was sequenced) did not possess mutations in *DNASE1*, yet have a low serum deoxyribonuclease phenotype, imply factors other than germ line mutations

may be more important in influencing serum deoxyribonuclease activity in SLE patients.

Monocyte Chemoattractant Protein-1 (MCP-1) Promoter

Monocyte chemotactic protein-1, a member of the small inducible gene (SIG) family, plays a role in the recruitment of monocytes to sites of injury and infection and is regulated by NF-κB. Aguilar *et al*. [351] examined a biallelic G/A polymorphism at position –2518 of the MCP-1 gene in 276 Spanish SLE patients and 194 controls and found no correlation with SLE *per se* or with nephritis. However, patients with cutaneous vasculitis were more likely to have the genotype A/G than other genotypes. This gene may represent a disease modifier in SLE, although it is premature to consider it a candidate gene in SLE susceptibility.

Angiotensin-Converting Enzyme Gene Polymorphisms

ACE takes part in the renin–angiotensin and kallikrein–kininogen systems by creating angiotensin II and inactivating bradykinin. ACE gene insertion/deletion polymorphism is associated with the level of circulating enzymes—subjects with the DD genotype have higher levels of circulating ACE than subjects with the II genotype and show an increased tendency toward impaired vascular function and structure. Patients with systemic lupus erythematosus suffer from differentially expressed vascular pathology.

Sato *et al*. [352] investigated the correlation between insertion (I)/deletion (D) polymorphism of the ACE gene and the disease activity in 93 newly diagnosed SLE patients. Individuals with II genotype showed a significant increase in SLE activity. Patients with the ACE II genotype showed a higher serum level of anti-dsDNA antibodies than those with the DD genotype. Moreover, patients with the II genotype also showed lower levels of serum CH50 than those with the DD genotype. Patients with the II or DI genotype had a significantly higher SLEDAI score than those with the DD genotype.

Tassiullas *et al*. [353] investigated the potential role of ACE polymorphisms in 216 SLE patients (121 Caucasians, 78 African-Americans, and 17 others) and 200 normal controls; 134 patients had evidence of renal disease. The frequency of genotype DD was increased in African-American normal controls compared to Caucasians and in African-American normal controls vs African-American lupus patients. Trend analysis of the genotype distribution across the three African-American groups (renal, nonrenal, controls) revealed a trend of an increased frequency of I and an decreased frequency of D as the likelihood of renal disease increases ($P = 0.008$). No association between any ACE genotype with parameters of renal disease and/or response to therapy was identified. African-American patients with lupus have a lower frequency of DD genotype as compared to African-American normal controls.

Conversely, Pullman *et al*. [354] determined ACE polymorphisms in 101 SLE patients and found that the frequency of the D allele was higher in the SLE group than in controls. Moreover, an association between the DD genotype and visceral damage was also observed. Prkacin *et al*. [355] found that the DD genotype was more common in 18 patients with systemic lupus erythematosus compared to 21 controls and that patients with the II genotype had a lower proteinuria and creatinine level and a shorter time to disease remission than those with the DD genotype.

Finally, in a study of 56 Israeli SLE patients and 48 controls, Molad *et al*. [356] found a similar distribution of the ACE genotype D/D, D/I, and I/I in the lupus group and controls and also failed to find any significant association among the ACE genotype and disease manifestations, SLEDAI, renal function, or cardiovascular and cerebrovascular morbidity. Likewise Kaufman *et al*. [357], analyzing 158 pedigrees (56 African-American, 90 European American, and 12 from other ethnic groups), was unable to detect significant linkage or genetic association between the ACE gene and SLE.

These contradictory data suggest that if this gene has any relevance to SLE, it appears to be marginal.

β2-glycoprotein

Apolipoprotein H (apoH, protein; APOH, gene) is a required cofactor for the production of antiphospholipid antibodies (APA). Kamboh *et al*. [358] examined 222 Caucasian women with SLE for four APOH polymorphisms (codons 88, 247, 306, and 316) and for plasma apoH concentrations by ELISA. Although none of the four APOH polymorphisms were significantly associated with variation in risk for SLE, the codons 306 and 316 polymorphisms showed significant, gene dosage effects on plasma apoH concentrations. No significant association was observed between anti-apoH status and APOH polymorphisms or plasma apoH levels; however, plasma apoH concentrations were significantly higher in patients positive for APA than in patients negative for APA. The frequency of the mutant allele (Ser316) was significantly lower in the APA-positive group than the APA-negative group, indicating that the Ser316 mutation is protective against the production of phospholipid–apoH-dependent APA.

Gushiken *et al*. [359] determined the frequency of mutations in the phospholipid-binding domain of β2-glycoprotein I (β2GPI) in 95 patients with SLE. In patients with SLE, 4 of 6 patients with exon 8 mutations

had thrombosis compared with 22 of 82 patients without the mutation; 5.6% were heterozygous for the mutation at exon 7 (codon 306) and 7.7% were heterozygous for the mutation at exon 8 (codon 316). No homozygous subjects were found for either mutation. No significant correlation between these mutations and the presence of aPL, LAC, or anti-β2GPI antibodies was found.

Bcl-2

The *bcl-2* protooncogene encodes a 24-kDa protein that is localized in the inner membrane of mitochondria, which promotes prolonged lymphocyte survival, by the prevention of apoptosis. Graninger [360] studied 24 Caucasian SLE patients, finding an increased concentration of *bcl-2* mRNA in 19 of the patients. The degree of overexpression of *bcl-2* was found to correlate with disease activity both here and in another study by Miret *et al*. [361], who observed increased levels of bcl-2 in SLE patients with active disease in relation to those with inactive SLE and controls. There was also a significant correlation between bcl-2 levels and SLEDAI values. Mehrian *et al*. [245] described an association of a *bcl-2* intragenic marker with SLE in 162 Mexican-American SLE patients and suggested synergism between the IL-10 locus and *bcl-2* genes in heightening disease susceptibility.

Johansson *et al*. [362] examined 378 Mexican SLE patients (n = 378) and 112 Swedish simplex families using a microsatellite marker and two single nucleotide polymorphisms located within the gene and were unable to detect association between *bcl-2* and SLE in either population. Clearly this gene, if indeed it has any relevance to SLE, needs to be confirmed in another cohort of patients.

CONCLUSION

Although family and twin studies clearly have established the importance of genetic factors in SLE, and studies of HLA class II and III genes since the mid 1970s have underscored their consistent associations with disease in nearly all ethnic groups, the contribution of non-MHC genes to SLE predisposition, clinical and autoantibody subsets, and course and prognosis are only being determined presently. Studies defining associations with non-MHC genes and linkage studies of multiplex families from several centers have raised as many new questions as they have answered. Inadequate power from small sample sizes and family collections, stemming undoubtedly from the clinical heterogeneity posed by SLE in no small part, has caused this. However, as the family collections continue, data are pooled and reanalyzed, and clinical subsets of SLE further considered, definitive candidate genes should emerge byond perhaps those three that have withstood time and reconfirmation in different groups of patients: the MHC, the FCγ genes, and whatever disease-relevant gene found in the region on chromosome ′q41–42. This will result in better insights into the immunologic perturbations that set the stage for SLE, which will in turn allow novel approaches to therapy. Similarly, as the contribution of genetics to prognosis in SLE is better understood, those at highest risk for a worse outcome can be targeted earlier with aggressive which may result in improvement in the quality and length of life of those affected.

References

1. Reveille, J. D. (1999). MHC class II and non-MHC genes in the pathogenesis of systemic lupus erythematosus. *In* Systemic Lupus Erythematosus (R. G. Lahita, ed.), p. 67. Academic Press San Diego.
2. Sequeira, J. H. (1903). Lupus erythematosus in two sisters. *Br. J. Dermatol* **15**, 171.
3. Siegel, M., Lee, S. L., Widelock, D., Gwon, N. V., and Kravitz, H. (1965). A comparative family study of rheumatoid arthritis and systemic lupus erythematosus. *N. Engl. J. Med*. **273**, 893–897.
4. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85–95.
5. Buckman, K. J., Moore, S. K., Ebbin, A. J., Cox, M. B., and Dubois, E. L. (1978). Familial systemic lupus eryhtmatosus. *Arch. Intern. Med*. **138**, 1674–1676.
6. Hochberg, M. C. (1987). The application of genetic epidemiology to systemic lupus erythematosus. *J. Rheumatol*. **14**, 867–869.
7. Lawrence, J. S., Martins, C. L., and Drake, G. L. (1987). A family survey of lupus erythematosus. 1. Heritability. *J. Rheumatol*. **14**, 913–921.
8. Koskenmies, S., Widen, E., Kere, J., and Julkunen, H. (2001). Familial systemic lupus erythematosus in Finland. *J. Rheumatol*. **28**, 758–760.
9. Michel, M., Johanet, C., Meyer, O., Frances, C., Wittke, F., Michel, C., Arfi, S., Tournier-Lasserve, E., and Piette, J. C. (2001). Group for Research on Auto-Immune Disorders (GRAID). Familial lupus erythematosus. Clinical and immunologic features of 125 multiplex families. *Medicine* (*Baltimore*) **80**, 153–158.
10. Lahita, R. G., Chiorazzi, N., Gifofsky, A., Winchester, R. J., and Kunkel, H. G. (1983). Familial systemic lupus erythematosus in males. *Arthritis Rheum*. **26**, 39–44.
11. Arnett, F. C., Bias, W. B., and Shulman, L. E., Studies in familial systemic lupus erythematosus. *Medicine* (*Baltimore*) **55**, 313–322.
12. Kaplan, D. (1984). The onset of disease in twins and siblings with systemic lupus eryythemastosus. *J. Rheumatol*. **11**, 648–652.
13. Reveille, J. D., Bias, W. B., Winkelstein, J. A., Provost, T. T., Dorsch, C. A., and Arnett, F. C. (1983). Familial

systemic lupus erythematosus: immunogenetic studies in eight families. *Medicine* (*Baltimore*) **62**, 21–35.
14. Rodnan, G. P., Maclachlan, M. J., and Creighton, A. S. (1960). Study of serum proteins and serologic reactions in relatives of patients with SLE. *Clin. Res.* **8**, 197. [Abstract]
15. Larsen, R. A. (1972). Family studies in systemic lupus erythematosus (SLE). Presence of rheumatoid factors in relatives and spouses. *J. Chronic Dis.* **25**, 191–203.
16. Holman, H., and Deicher, H. R. (1960). The appearance of hypergammaglobulinemia, positive serologic reactions for rheumatoid arthritis and complement fixation reactions with tissue constituents in the sera of relatives of patients with systemic lupus erythematosus. *Arthritis Rheum.* **3**, 244. [Abstract]
17. Dubois, E. L., Chandor, S., Friou, G. J., and Bischel, M. (1971). Progressive systemic sclerosis (PSS) and localized scleroderma (morphea) with positive LE cell test and unusual systemic manifestations compatible with systemic lupus erythematosus (SLE): presentation of 14 cases including one set of identical twins, one with scleroderma and the other with SLE. *Medicine* **50**, 199–222.
18. Hagberg, B., Leonhardt, T., and Skogh, M. (1961). Familial occurrence of collagen diseases. Progressive systemic sclerosis and systemic lupus erythematosus. *Acta Med. Scand.* **169**, 727.
19. Tuffanelli, D. L. (1969). Scleroderma, immunological and genetic disease in three families. *Dermatologica* **13**, 893.
20. Flores, R. H., Stevens, M. B., and Arnett, F. C. (1984). Familial occurrence of progressive systemic sclerosis and systemic lupus erythematosus. *J. Rheumatol.* **11**, 321–323.
21. Arnett, F. C., Reveille, J. D., Wilson, R. W., Provost, T. T., and Bias, W. B. (1984). Systemic lupus erythematosus: current state of the genetic hypothesis. *Semin. Arthritis Rheum.* **14**, 24–35.
22. Bias, W. B., Reveille, J. D., Beaty, T. L. H., Meyers, D. A., and Arnett, F. C. (1986). Evidence that autoimmunity in man is a mendelian–dominant trait. *Am. J. Hum. Genet.* **39**, 584–602.
23. Pollak, V. E. (1964). Antinuclear antibodies in families of patients with systemic lupus erythematosus. *N. Engl. J. Med.* **271**, 165–171.
24. Bywaters, E. G. L. (1961). Family studies of rheumatoid arthritis and lupus erythematosus in Great Britain. *In* "The Epidemiology of Chronic Rheumatism" (J. H. Kellgren, ed.), p. 255. Davis, Philadelphia.
25. Morteo, O. G., Franklin, E. C., McEwen, C., Phythyon, J., and Tanner, M. (1961). Clinical and laboratory studies of relatives with systemic lupus erythematosus. *Arthritis Rheum.* **4**, 356–361.
26. Widelock, D., Gilbert, G., Siegel, M., and Lee, S. (1961). Fluorescent antibody procedure for lupus erythematosus: comparative us of nucleated erythrocytes and calf thymus cells. *Am. J. Public Health* **51**, 829–835.
27. Fennel, P. H., Maclachlan, M. J., and Rodnan, G. (1962). The occurrence of antinuclear factors in the sera of relatives of patients with systemic rheumatic diseases. *Arthritis Rheum.* **5**, 296.
28. Holborow, J., and Johnson, G. D. (1964). Antinuclear factor in systemic lupus erythematosus: A consideration of immunofluorescent method of detecting antinuclear antibodies with results obtained in a family study. *Arthritis Rheum.* **7**, 119.
29. Pollak, V. E., Mandema, E., and Kark, R. M. (1960). Antinuclear factors in the serum of relatives of patients with systemic lupus erythematosus. *Lancet* **2**, 1061–1063.
30. Solheim, B., and Larsen, R. (1972). Presence of antinuclear factor in relatives and spouses of patients with systemic lupus erythematosus. *Acta. Med. Scand.* **543**, 43–47.
31. Isenberg, D. A., Shoenfeld, Y., Walport, M., Mackworth-Young, C., Dudeney, C., Todd-Pokropek, A., Brill, S., Weinberger, A., and Pinkas, J. (1985). Detection of cross-reactive anti-DNA antibody idiotypes in the serum of systemic lupus erythematosus patients and their relatives. *Arthritis Rheum.* **21**, 999–1007.
32. Lehman, T. J. A., Curd, J. G., Zvaifler, N. J., and Hanson, V. (1982). The association of antinuclear antibodies, anti-lymphocyte antibodies and C4 activation among the relatives of children with systemic lupus erythematosus: preferential activation of complement in sisters. *Arthritis Rheum.* **25**, 556–561.
33. Haug, B. L., Lee, J. S., and Sibley, J. T. (1984). Altered poly(ADP-ribose) metabolism in family members of patients with systemic lupus erythematosus. *J. Rheumatol.* **21**, 851–856.
34. Lowenstein, M. B., and Rothfield, N. F. (1977). Family study of systemic lupus erythematosus. *Arthritis Rheum.* **20**, 1293–1303.
35. Sato, E. I., Atra, E., Gabriel, A., and Masi, A. T. (1991). Systemic lupus erythematosus: A family study of 25 probands. *Clin. Exp. Rheumatol.* **9**, 455–461.
36. Miles, S., and Isenberg, D. (1993). A review of serological abnormalities in relatives of SLE patients. *Lupus* **2**, 145–150.
37. Arnett, F. C., Hamilton, R. G., Reveille, J. D., Bias, W. B., Harley, J. B., and Reichlin, M. (1989). Genetic studies of Ro (SS-A) and La (SS-B) autoantibodies in families with systemic lupus erythematosus and primary Sjogren's syndrome. *Arthritis Rheum.* **32**, 413–419.
38. van der Linden, M. W., Westendorp, R. G., Zidane, M., Meheus, L., and Huizinga, T. W. (2001). Autoantibodies within families of patients with systemic lupus erythematosus are not directed against the same nuclear antigens. *J. Rheumatol.* **28**, 284–287.
39. Radway-Bright, E. L., Ravirajan, C. T., and Isenberg, D. A. (2000). The prevalence of antibodies to anionic phospholipids in patients with the primary antiphospholipid syndrome, systemic lupus erythematosus and their relatives and spouses. *Rheumatology* (*Oxford*) **39**, 427–431.
40. DeHoratius, R. J., Pillarisetty, R., Messner, R. P., and Talal, N. (1975). Anti-nucleic acid antibodies in systemic lupus erythematosus patients and their families. Incidence and correlation with lymphocytotoxic antibodies. *J. Clin. Invest.* **56**, 1149–1154.
41. DeHoratius, R. J., and Messner, R. P. (1975). Lymphocytotoxic antibodies in family members of patients with

systemic lupus erythematosus. *J. Clin. Invest.* **55**, 1254–1258.
42. Cleland, L. G., Bell, D. A., Williams, M., and Saurino, B. C. (1978). Familial lupus. Family studies of HLA and serologic fidings. *Arthritis Rheum.* **21**, 183–191.
43. Folomeeva, D. O., Nassonova, V. A., Alekberova, A. S., Talal, N., and Williams, R. D., Jr. (1978). Comparative studies of antilymphocyte, antipolynucleotide, and antiviral antibodies among families of patients with systemic lupus erythematosus. *Arthritis Rheum.* **21**, 23–27.
44. Eroglu, G. E., and Kohler, P. F. (2002). Familial systemic lupus erythematosus: the role of genetic and environmental factors. *Ann. Rheum. Dis.* **61**, 29–31.
45. Malave, I., Papa, R., and Layrisse, Z. (1976). Lymphocytotoxic antibodies in SLE patients and their relatives. *Arthritis Rheum.* **19**, 700–704.
46. Hazelton, R. A. (1984). A study of lymphocytotoxins in families of patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **43**, 136–139.
47. Le Page, S. H., Williams, W., Parkhouse, D., Cambridge, G., MacKenzie, L., and Lydyard, P. M. (1989). Relation between lymphocytotoxic antibodies, anti-DNA antibodies and a common anti-DNA antibody idiotype PR4 in patients with systemic lupus erythematosus, their relatives and spouses. *Clin. Exp. Immunol.* **77**, 314–318.
48. Mohan, C., Liu, F., Xie, C., and Williams, R. C., Jr. (2001). Anti-subnucleosome reactivities in systemic lupus erythematosus (SLE) patients and their first-degree relatives. *Clin. Exp. Immunol.* **123**, 119–126.
49. Lippman, S. M., Arnett, F. C., Conley, C. L., Ness, P. M., Myers, D. A., and Bias, W. B. (1982). Genetic factors predisposing to autoimmune diseases. Autoimmune hemolytic anemia, chronic thrombocytopenia purpura, and systemic lupus erythematosus. *Am. J. Med.* **73**, 827–840.
50. Miller, K. B., and Schwartz, R. S. (1979). Familial abnormalities of suppressor-cell function in systemic lupus erythematosus. *N. Engl. J. Med.* **301**, 803–809.
51. Mendlovic, S., Segal, R., Shoenfeld, Y., and Mozes, E. (1990). Anti-DNA idiotype- and anti-idiotype-specific T cell responses in patients with systemic lupus erythematosus and their first-degree relatives. *Clin. Exp. Immunol.* **82**, 504–508.
52. Tomana, M., Schrohenloher, R. E., Reveille, J. D., Arnett, F. C., and Koopman, W. J. (1992). Abnormal galactosylation of serum IgG in patients with systemic lupus erythematosus and members of families with high frequency of autoimmune diseases. *Rheumatol. Int.* **12**, 191–194.
53. Chui, D., Sellakumar, G., Green, R., Sutton-Smith, M., McQuistan, T., Marek, K., Morris, H., Dell, A., and Marth, J. (2001). Genetic modeling of protein glycosylation in vivo induces autoimmune disease. *Proc. Natl. Acad. Sci. USA* **98**, 1142–1147.
54. van der Linden, M. W., Westendorp, R. G., Sturk, A., Bergman, W., and Huizinga, T. W. (2000). High interleukin-10 production in first-degree relatives of patients with generalized but not cutaneous lupus erythematosus. *J. Invest. Med.* **48**, 327–334.
55. Grondal, G., Kristjansdottir, H., Gunnlaugsdottir, B., Arnason, A., Lundberg, I., Klareskog, L., and Steinsson, K. (1999). Increased number of interleukin-10-producing cells in systemic lupus erythematosus patients and their first-degree relatives and spouses in Icelandic multicase families. *Arthritis Rheum.* **42**, 1649–1654.
56. Block, S. R., Winfield, J. B., Lockshin, M. D., D'Angelo, W. A., and Christian, C. L. (1975). Studies of twins with systemic lupus erythematosus. A review of the literature and presentation of 12 additional sets. *Am. J. Med.* **59**, 533–552.
57. Block, S. R., Lockshin, M. D., Winfield, J. B., Weksler, M. E., Imamura, M., Winchester, R. J., Mellors, R. C., and Christian, C. L. (1976). Immunologic observations on 9 sets of twins either concordant or discordant for SLE. *Arthritis Rheum.* **19**, 545–554.
58. Reichlin, M., Harley, J. B., and Lockshin, M. D. (1992). Serologic studies of monozygotic twins with systemic lupus erythematosus. *Arthritis Rheum.* **35**, 457–464.
59. Deapen, D., Escalante, A., Weinrib, L., Horwitz, D., Bachman, B., Roy-Burman, P., Walker, A., and Mack, T. M. (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 311–318.
60. Jarvinen, P., Kaprio, J., Makitalo, R., Koskenvuo, M., and Aho, K. (1992). Systemic lupus erythematosus and related systemic diseases in a nationwide twin cohort: an increased prevalence of disease in MZ twins and concordance of disease features. *J. Intern. Med.* **231**, 67–72.
61. Greenan, D. M., Parfitt, A., Manolios, N., Huang, Q., Hyland, V., Dunckley, H., Doran, T., Gatenby, P., and Badcock, C. (1997). Family and twin studies in systemic lupus erythematosus. *Dis. Mark.* **13**, 93–98.
62. Tsao, B. P., Cantor, R. M., Kalunian, K. C., Chen, C. J., Badsha, H., Singh, R., Wallace, D. J., Kitridou, R. C., Chen, S. L., Shen, N., Song, Y. W., Isenberg, D. A., Yu, C. L., Hahn, B. H., and Rotter, J. I. (1997). Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J. Clin. Invest.* **99**, 725–731.
63. Morel, L., Croker, B. P., Blenman, K. R., Mohan, C., Huang, G., Gilkeson, G., and Wakeland, E. K. (2000). Genetic reconstitution of systemic lupus erythematosus immunopathology with polycongenic murine strains. *Proc. Natl. Acad. Sci. USA* **97**, 6670–6675.
64. Shai, R., Quismorio, F. P., Jr., Li, L., Kwon, O. J., Morrison, J., Wallace, D. J., Neuwelt, C. M., Brautbar, C., Gauderman, W. J., and Jacob, C. O. (1999). Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum. Mol. Genet* **8**, 639–644.
65. Gaffney, P. M., Kearns, G. M., Shark, K. B., Ortmann, W. A., Selby, S. A., Malmgren, M. L., Rohlf, K. E., Ockenden, T. C., Messner, R. P., King, R. A., Rich, S. S., and Behrens, T. W. (1998). A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc. Natl. Acad. Sci. USA* **95**, 14875–14879.
66. Gaffney, P. M., Ortmann, W. A., Selby, S. A., Shark, K. B., Ockenden, T. C., Rohlf, K. E., Walgrave, N. L., Boyum,

W. P., Malmgren, M. L., Miller, M. E., Kearns, G. M., Messner, R. P., King, R. A., Rich, S. S., and Behrens, T. W. (2000). Genome screening in human systemic lupus erythematosus: Results from a second Minnesota cohort and combined analyses of 187 sib-pair families. *Am. J. Hum. Genet.* **66**, 547–556.

67. Graham, R. R., Langefeld, C. D., Gaffney, P. M., Ortmann, W. A., Selby, S. A., Baechler, E. C., Shark, K. B., Ockenden, T. C., Rohlf, K. E., Moser, K. L., Brown, W. M., Gabriel, S. E., Messner, R. P., King, R. A., Horak, P., Elder, J. T., Stuart, P. E., Rich, S. S., and Behrens, T. W. (2001). Genetic linkage and transmission disequilibrium of marker haplotypes at chromosome 1q41 in human systemic lupus erythematosus. *Arthritis Res.* **3**, 299–305.
68. Gray-McGuire, C., Moser, K. L., Gaffney, P. M., Kelly, J., Yu, H., Olson, J. M., Jedrey, C. M., Jacobs, K. B., Kimberly, R. P., Neas, B. R., Rich, S. S., Behrens, T. W., and Harley, J. B. (2000). Genome scan of human systemic lupus erythematosus by regression modeling: evidence of linkage and epistasis at 4p16-15.2. *Am. J. Hum. Genet.* **67**, 1460–1469.
69. Moser, K. L., Neas, B. R., Salmon, J. E., Yu, H., Gray-McGuire, C., Asundi, N., Bruner, G. R., Fox, J., Kelly, J., Henshall, S., Bacino, D., Dietz, M., Hogue, R., Koelsch, G., Nightingale, L., Shaver, T., Abdou, N. I., Albert, D. A., Carson, C., Petri, M., Treadwell, E. L., James, J. A., and Harley, J. B. (1998). Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc. Natl. Acad. Sci. USA* **95**, 14869–14874.
70. Moser, K. L., Gray-McGuire, C., Kelly, J., Asundi, N., Yu, H., Bruner, G. R., Mange, M., Hogue, R., Neas, B. R., and Harley, J. B. (1999). Confirmation of genetic linkage between human systemic lupus erythematosus and chromosome 1q41. *Arthritis Rheum.* **42**, 1902–1907.
71. Nath, S. K., Kelly, J. A., Namjou, B., Lam, T., Bruner, G. R., Scofield, R. H., Aston, C. E., and Harley, J. B. (2001). Evidence for a susceptibility gene, SLEV1, on chromosome 17p13 in families with vitiligo-related systemic lupus erythematosus. *Am. J. Hum. Genet.* **69**, 1401–1406.
72. Rao, S., Olson, J. M., Moser, K. L., Gray-McGuire, C., Bruner, G. R., Kelly, J., and Harley, J. B. (2001). Linkage analysis of human systemic lupus erythematosus-related traits: A principal component approach. *Arthritis Rheum.* **44**, 2807–2818.
73. Johanneson, B., Steinsson, K., Lindqvist, A. K., Kristjansdottir, H., Grondal, G., Sandino, S., Tjernstrom, F., Sturfelt, G., Granados-Arriola, J., Alcocer-Varela, J., Lundberg, I., Jonasson, I., Truedsson, L., Svenungsson, E., Klareskog, L., Alarcon-Segovia, D., Gyllensten, U. B., and Alarcon-Riquelme, M. E. (1999). A comparison of genome-scans performed in multicase families with systemic lupus erythematosus from different population groups. *J. Autoimmun.* **13**, 137–41.
74. Lindqvist, A. K., Steinsson, K., Johanneson, B., Kristjansdottir, H., Arnasson, A., Grondal, G., Jonasson, I., Magnusson, V., Sturfelt, G., Truedsson, L., Svenungsson, E., Lundberg, I., Terwilliger, J. D., Gyllensten, U. B., and Alarcon-Riquelme, M. E. (2000). A susceptibility locus for human systemic lupus erythematosus (hSLE1) on chromosome 2q. *J. Autoimmun.* **14**, 169–178.
75. Magnusson, V., Lindqvist, A. K., Castillejo-Lopez, C., Kristjansdottir, H., Steinsson, K., Grondal, G., Sturfelt, G., Truedsson, L., Svenungsson, E., Lundberg, I., Gunnarsson, I., Bolstad, A. I., Haga, H. J., Jonsson, R., Klareskog, L., Alcocer-Varela, J., Alarcon-Segovia, D., Terwilliger, J. D., Gyllensten, U. B., and Alarcon-Riquelme, M. E. (2000). Fine mapping of the SLEB2 locus involved in susceptibility to systemic lupus erythematosus. *Genomics* **70**, 307–314.
76. Kelly, A. P., Monaco, J. J., Cho, S., and Trowsdale, J. (1991). A new human HLA class II-related locus, DM. *Nature* **353**, 571–573.
77. Kropshofer, H., Vogt, A. B., Moldenhauer, G., Hammer, J., Blum, J. S., and Hammerling, G. (1996). Editing of the HLA-DR-peptide repertoire by HLA-DM. *EMBO J.* **15**, 6144–6154.
78. Carrington, M., Yeager, M., and Mann, D. (1993). Characterization of HLA-DMB polymorphism. *Immunogenetics* **38**, 446.
79. Sanderson, F., Powis, S. H., Kelly, A. P., and Trowsdale, J. (1994). Limited polymorphism in HLA-DM does not involve the peptide binding groove. *Immunogenetics* **39**, 56.
80. Kim, T. G., Carrington, M., Choi, H. B., Kim, H. Y., and Han, H. (1996). Three HLA-DMB variants in Korean patients with autoimmune diseases. *Hum. Immunol.* **46**, 58–60.
81. Spies, T., Bresnahan, M., Bahram, S., Arnold, D., Blanck, G., Mellins, E., Pious, D., and DeMars, R. (1990). A gene in the human major histocompatibility complex class II region controlling the class I antigen presentation pathway. *Nature* **348**, 744–747.
82. Trowsdale, J., Hanson, I., Mockridge, I., Beck, S., Townsend, A., and Kelly, A. (1990). Sequences encoded in the class II region of the MHC related to the 'ABC' super family of transporters. *Nature* **348**, 741–744.
83. Deverson, E. V., Gow, I. R., Coadwell, W. J., Monaco, J. J., Butcher, G. W., and Howard, J. D. (1990). MHC class II region encoding proteins related to the multidrug resistance family of transmembrane transporters. *Nature* **348**, 738–741.
84. Powis, S. H., Mockridge, I., Kelly, A., Kerr, L.-A., Glynne, R., Gileadi, U., Beck, S., and Trowsdale, J. (1992). Polymorphism in a second ABC transporter gene located within the class II region of the human major histocompatibility complex. *Proc. Natl. Acad. Sci. USA* **89**, 1463–1467.
85. Glynne, R., Powis, S. H., Beck, S., Kelly, A., Kerr, L.-A., and Trowsdale, J. (1991). A proteasome-related gene between the two ABC transporter loci in the class II region of the human MHC. *Nature* **353**, 357–360.
86. Brocke, P., Garbi, N., Momburg, F., and Hammerling, G. J. (2002). HLA-DM, HLA-DO and tapasin: Functional similarities and differences. *Curr. Opin. Immunol.* **14**, 22–9.
87. Garcia, P., Llano, M., de Heredia, A. B., Willberg, C. B., Caparros, E., Aparicio, P., Braud, V. M., and Lopez-Botet,

M. (2002). Human T cell receptor-mediated recognition of HLA-E. Eur. *J. Immunol.* **32**, 936–944.
88. Shiina, T., Tamiya, G., Oka, A., Takishima, N., Yamagata, T., Kikkawa, E., Iwata, K., Tomizawa, M., Okuaki, N., Kuwano, Y., Watanabe, K., Fukuzumi, Y., Itakura, S., Sugawara, C., Ono, A., Yamazaki, M., Tashiro, H., Ando, A., Ikemura, T., Soeda, E., Kimura, M., Bahram, S., and Inoko, H. (1999). Molecular dynamics of MHC genesis unraveled by sequence analysis of the 1,796,938-bp HLA class I region. *Proc. Natl. Acad. Sci. USA* **96**, 13282–13287.
89. Grumet, F. C., Coukell, A., Bodmer, J. G., Bodmer, W. F., and McDevitt, H. O. (1971). Histocompatibility (HL-A) antigens associated with systemic lupus erythematosus. A possible genetic predisposition to disease. *N. Engl. J. Med.* **285**, 193–196.
90. Goldberg, M. A., Arnett, F. C., Bias, W. B., and Shulman, L. E. (1976). Histocompatibility antigens in systemic lupus erythematosus. *Arthritis Rheum.* **19**, 129–132.
91. Waters, H., Konrad, P., and Walford, R. L. (1971). The distribution of HL-A histocompatibility factors and genes in patients with systemic lupus erythematosus. *Tissue Antigens* **1**, 68–73.
92. Nies, K. M., Brown, J. C., Dubois, E. G., Quismorio, F. G., Friou, G. J., and Terasaki, P. I. (1974). Histocompatibility (HLA) antigens and lymphocytotoxic antibodies in systemic lupus erythematosus (SLE). *Arthritis Rheum.* **17**, 397–402.
93. Reinertsen, J. L., Klippel, J. H., Johnson, A. H., Steinberg, A. D., Decker, J. L., and Mann, D. L. (1978). B-lymphocyete alloantigens associated with systemic lupus erythematosus. *N. Engl. J. Med.* **299**, 515–518.
94. Gibofsky, A. M., Winchester, R. J., Patarroyo, M., Fotino, M., and Kunkel, H. G. (1978). Disease association of the Ia-like human alloantigens: Contrasting patterns in rheumatoid arthritis and systemic lupus erythematosus. *J. Exp. Med.* **148**, 1728–1732.
95. Black, C. M., Welsh, K. I., Fielder, A., Hughes, G. R. V., and Batchelor, J. R. (1982). HLA antigens and Bf allotypes in SLE: evidence for the association being with specific haplotypes. *Tissue Antigens* **19**, 115–120.
96. So, A. K. L., Fielder, A. H. L., Warner, C. A., Isenberg, D. A., Batchelor, J. R., and Walport, M. J. (1990). DNA polymorphism of major histocompatibility complex class II and class III genes in systemic lupus erythematosus. *Tissue Antigens* **35**, 144–147.
97. Dunckley, H., Gatenby, P. A., and Serjeantson, S. W. (1986). DNA typing of HLA-DR antigens in systemic lupus erythematosus. *Immunogenetics* **24**, 158–162.
98. Gomez-Reino, J. J., Martinez-Laso, J., Vicario, J. L., Paz-Artal, E., Aragon, A., Martin-Villa, J. M., DeJuan, M. D., Perez-Aciego, P., and Arnaiz-Villena, A. (1991). Immunogenetics of systemic lupus erythematosus in Spanish patients: differential HLA markers. *Immunobiolics* **182**, 465–471.
99. Lulli, P., Sebastiani, G. D., Trabace, S., Passiu, G., Cappellacci, S., Porzio, F., Morellini, M., Cutrupi, F., and Galeazzi, M. (1991). HLA antigens in Italian patients with systemic lupus erythematosus: evidence for the association of DQw2 with the autoantibody response to extractable nuclear antigens. *Clin. Exp. Rheumatol.* **9**, 475–479.
100. Cowland, J. B., Andersen, V., Halberg, P., and Morling, N. (1994). DNA polymorphism of HLA class II genes in systemic lupus erythematosus. *Tissue Antigens* **43**, 34–37.
101. Skarsvag, S., Hansen, K. E., Holst, A., and Moen, T. (1992). Distribution of HLA Class II alleles smong Scandinavian patients with systemic lupus erythematosus (SLE): an increased risk of SLE among non DRB1*03, DQA1*0501, DQB1*0201 Class II homozygotes. *Tissue Antigens* **40**, 128–133.
102. Ruuska, P., Hameenkorpi, R., Forsberg, S., Julkunen, H., Makitalo, R., Ilonen, J., and Tiilikainen, A. (1992). Differences in HLA antigens between patients with mixed connective tissue disease and systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 52–55.
103. Celada, A., Barras, C., Benzonana, G., and Jeannet, M. (1980). Increased frequency of HLA-DRw3 in systemic lupus erythematosus. *Tissue Antigens* **15**, 283–288.
104. Reinharz, D., Tiercy, J. M., Mach, B., and Jeannet, M. (1991). Absence of DRw15/3 and of DRw15/7 heterozygotes in Caucasian patients with systemic lupus erythematosus. *Tissue Antigens* **37**, 10–15.
105. Scherak, O., Smolen, J. S., and Mayr, W. R. (1979). Prevalence of HLA-DRw2 not increased in systemic lupus erythematosus. *N. Engl. J. Med.* **301**, 612.
106. Hartung, K., Baur, M. P., Coldewey, R., Fricke, M., Kalden, J. R., Lakomek, H. J., Peter, H. H., Schendel, D., Schneider, P. M., Seuchter, S. A., Stangel, W., and Deicher, H. R. G. (1992). Major histocompatibility complex haplotypes and complement C4 alleles in systemic lupus erythematosus results of a multicenter study. *J. Clin. Invest.* **90**, 1346–1351.
107. Steinsson, K., Jonsdottir, S., Arason, G. J., Kristjansdottir, H., Fossdal, R., Skaftadottir, I., and Arnason, A. (1998). A study of the association of HLA DR, DQ, and complement C4 alleles with systemic lupus erythematosus in Iceland. *Ann. Rheum. Dis.* **57**, 503–505.
108. Reveille, J. D., Anderson, K. L., Schrohenloher, R. E., Acton, R. T., and Barger, B. O. (1991). Restriction Fragment Length Polymorphism analysis of HLA-DR, DQ, DP and C4 alleles in Caucasians with systemic lupus erythematosus. *J. Rheumatol.* **18**, 14–18.
109. Barron, K. S., Silverman, E. D., Gonzales, J., Arnett, F. C., and Reveille, J. D. (1993). Childhood-onset systemic lupus erythematosus (SLE): A clinical, serologic and immunogenetic analysis. *Arthritis Rheum.* **36**, 348–354.
110. Marintchev, L. M., Naumova, E. J., Rashkov, R. K., Arnett, F. C., and Reveille, J. D. (1995). HLA-class II alleles and autoantibodies in Bulgarians with systemic lupus erythematosus. *Tissue Antigens* **46**, 422–425.
111. Reveille, J. D., Arnett, F. C., Olsen, M. L., Sakkas, L. P., Papasteriades, C., and Moutsopoulos, H. M. (1995). HLA-class II alleles and C4 null genes in Greeks with systemic lupus erythematosus. *Tissue Antigens* **46**, 417–421.
112. Galeazzi, M., Sebastiani, G. D., Morozzi, G., Carcassi, C., Ferrara, G. B., Scorza, R., Cervera, R., de Ramon

Garrido, E., Fernandez-Nebro, A., Houssiau, F., Jedryka-Goral, A., Passiu, G., Papasteriades, C., Piette, J. C., Smolen, J., Porciello, G., and Marcolongo, R. (2002). European Concertee Action on the immunogenetics of SLE. HLA class II DNA typing in a large series of European patients with systemic lupus erythematosus: Correlations with clinical and autoantibody subsets. *Medicine* (*Baltimore*) **81**, 169–178.

113. Ahearn, J. M., Provost, T. T., Dorsch, C. A., Stevens, M. B., Bias, W. B., and Arnett, F. C. (1982). Interrelationships of HLA-DR, MB and MT phenotypes, autoantibody expression and clinical features in systemic lupus erythematosus. *Arthritis Rheum*. **25**, 1031–1040.
114. Gladman, D. D., Terasaki, P. I., Park, M. S., Iwaki, Y., Louie, S., Quismorio, F. P., Barnett, E. V., and Liebling, M. R. (1972). Increased frequency of HLA-DRw2 in SLE. *Lancet* **2**, 902–905.
115. Fronek, Z., Timmerman, L. A., Alper, C. A., Hahn, B. H., Kalunian, K., Peterlin, B. M., and McDevitt, H. O. (1990). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus. *Arthritis Rheum*. **33**, 1542–1552.
116. Tsuchiya, N., Kawasaki, A., Tsao, B. P., Komata, T., Grossman, J. M., and Tokunaga, K. (2001). Analysis of the association of HLA-DRB1, TNFalpha promoter and TNFR2 (TNFRSF1B) polymorphisms with SLE using transmission disequilibrium test. *Genes Immun*. **2**, 317–322.
117. Hashimoto, H., Tsuda, H., Matsumoto, T., Nasu, H., Takasaki, Y., Shokawa, Y., Hirose, S., Terasaki, P. I., and Iwaki, Y. (1985). HLA antigens associated with systemic lupus erythematosus in Japan. *J. Rheumatol*. **12**, 919–923.
118. Hirose, S., Ogawa, S., Nishimura, H., Hashimoto, H., and Shirai, T. (1988). Association of HLA-DR2/DR4 heterozygosity with systemic lupus erythematosus in Japanese patients. *J. Rheumatol*. **15**, 1489–1492.
119. Hashimoto, M., Kinoshita, T., Yamasaki, M., Tanaka, H., Imanishi, H., Ihara, H., Ichikawa, T., and Fukunishi, T. (1994). Gene frequencies and haplotypic associations within the HLA region in 916 unrelated Japanese individuals. *Tissue Antigens* **44**, 166–173.
120. Hawkins, B. R., Wong, K. L., Wong, R. W. S., Chan, K. H., Dunckley, H., and Serjeantson, S. W. (1987). Strong association between the major histocompatibility complex and systemic lupus eryhtematosus in Southern Chinese. *J. Rheumatol*. **14**, 1128–1131.
121. Savage, D. A., Ng, S. C., Howe, H. S., Ngai, J. L. F., Darke, C., and Hui, K. M. (1995). HLA and TAP associations in Chinese systemic lupus erythematosus patients. *Tissue Antigens* **46**, 213–216.
122. Azizah, M. R., Ainoi, S. S., Kuak, S. H., Kong, N. C., Normaznah, Y., and Rahim, M. N. (2001). The association of the HLA class II antigens with clinical and autoantibody expression in Malaysian Chinese patients with systemic lupus erythematosus. *Asian Pac J. Allergy Immunol*. **19**, 93–100.
123. Doherty, D. G., Ireland, R., Demaine, A. G., Wang, F., Veerapan, K., Welsh, K. I., and Vergani, D. (1992). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in southern chinese. *Arthritis Rheum*. **35**, 641–646.
124. Lu, L. Y., Ding, W. Z., Fici, D., Deulofeut, R., Cheng, H. H., Cheu, C. C., Sung, P. K., Schur, P. H., and Fraser, P. A. (1997). Molecular analysis of major histocompatibility complex allelic associations with systemic lupus erythematosus in Taiwan. *Arthritis Rheum*. **40**, 1138–1145.
125. Huang, J. L., Shaw, C. K., Lee, A., Lee, T. D., Chou, Y. H., and Kuo, M. L. (2001). HLA-DRB1 antigens in Taiwanese patients with juvenile-onset systemic lupus erythematosus. *Rheumatol. Int*. **21**, 103–105.
126. Zhang, J., Ai, R., and Chow, F. (1997). The polymorphisms of HLA-DR and TNF B loci in northern Chinese Han nationality and susceptibility to systemic lupus erythematosus. *Chin. Med. Sci. J*. **12**, 107–110.
127. Dong, R. P., Kimura, A., Hashimoto, H., Akizuki, M., Nishimura, Y., and Sasazuki, T. (1993). Difference in HLA-linked genetic background between mixed connective tissue disease and systemic lupus erythematosus. *Tissue Antigens* **41**, 20–25.
128. Hong, G. H., Kim, H. Y., Takeuchi, F., Nakano, K., Yamada, H., Matsuta, K., Han, H., Tokunaga, K., Ito, K., and Park, K. S. (1994). Association of complement C4 and HLA-DR alleles with systemic lupus erythematosus in Koreans. *J. Rheumatol*. **21**, 442–447.
129. Mehra, N. K., Pande, I., Taneja, V., Uppal, S. S., Saxena, S. P., Kumar, A., and Malaviya, A. N. (1993). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in northern India. *Lupus* **2**, 313–314.
130. Alarif, L. I., Ruppert, G. B., Wilson, R. Jr., and Barth, W. F. (1983). HLA-DR antigens in blacks with rheumatoid arthritis and systemic lupus erythematosus. *J. Rheumatol*. **10**, 297–300.
131. Kachru, R. B., Sequeira, W., Mittal, K. K., Siegel, M. E., and Telischi, M. (1984). A significant increase of HLA-DR3 and DR2 in systemic lupus erythematosus among blacks. *J. Rheumatol*. **11**, 471–474.
132. Reveille, J. D., Schrohenloher, R. E., Acton, R. T., and Barger, B. O. (1989). DNA analysis of HLA-DR and DQ genes in American Blacks with systemic lupus erythematosus. *Arthritis Rheum*. **32**, 1243–1251.
133. Monplaisir, N., Valette, I., Pierre-Louis, S., Yoyo, M., Sobesky, G., Verpre, F. C., Quist, D., Arfi, S., Gervaise, G., Gabriel, J. M., Artax, H., and Raffoux, C. (1988). Study of HLA antigens in systemic lupus erythematosus in the French West Indies. *Tissue Antigens* **31**, 238–242.
134. Hochberg, M. C., Boyd, R. E., Ahearn, J. M., Arnett, F. C., Bias, W. B., Provost, T. T., and Stevens, M. B. (1985). Systemic lupus erythematosus: a review of clinico-laboratory features and immunogenetic markers in 150 patients, with emphasis on demographic subsets. *Medicine* **64**, 285–295.
135. Reveille, J. D., Barger, B. O., and Hodge, T. W. (1991). HLA-DR2-DRB1 allele frequencies in DR-positive black Americans with and without systemic lupus erythematosus. *Tissue Antigens* **38**, 178–180.
136. Fraser, P. A., Lu, L. Y., Ding, W. Z., Najundaswamy, S. N., Chen, D. F., Uko, G., and Tonks, S. (2000). HLA-

B44031;DRB1*1503 and other sub-Saharan African major histocompatibility complex haplotypes in African Americans and Afro-Caribbeans carry C4A gene deletions: Implications for ethnicity-specific lupus susceptibility genes. *Arthritis Rheum*. **43**, 2378–2379.
137. Reveille, J. D., Moulds, J. M., and Arnett, F. C. (1995). Major histocompatibility complex class II and C4 alleles in Mexican Americans with systemic lupus erythematosus. *Tissue Antigens* **45**, 91–97.
138. Vargas-Alarcon, G., Salgado, N., Granados, J., Gomez-Casado, E., Martinez-Laso, J., Alcocer-Varela, J., Arnaiz-Villena, A., and Alarcon-Segovia, D. (2001). Class II allele and haplotype frequencies in Mexican systemic lupus erythematosus patients: the relevance of considering homologous chromosomes in determining susceptibility. *Hum. Immunol*. **62**, 814–820.
139. Reveille, J. D., Moulds, J. M., Ahn, C., Friedman, A. W., Baethge, B., Roseman, J., Straaton, K. V., and Alarcon, G. S. (1998). Systemic lupus erythematosus in three ethnic groups. I. The effects of HLA class II, C4, and CR1 alleles, socioeconomic factors, and ethnicity at disease onset. *Arthritis Rheum*. **41**, 1161–1172.
140. Arnett, F. C., Bias, W. B., and Reveille, J. D. (1989). Genetic studies in Sjogren's syndrome and systemic lupus erythematosus. *J. Autoimmun*. **2**, 403–413.
141. Hamilton, R. G., Harley, J. B., Bias, W. B., Roebber, M., Reichlin, M., Hochberg, M. C., and Arnett, F. C. (1988). Two Ro (SS-A) autoantibody responses in systemic lupus erythematosus: correlation of HLA-DR/DQ specificities with quantitative expression of Ro (SS-A) autoantibody. *Arthritis Rheum*. **31**, 496–505.
142. Alexander, E. L., Arnett, F. C., Provost, T. T., and Stevens, M. B. (1983). Sjogren's syndrome: Association of anti-Ro(SS-A) antibodies with vasculitis, hematologic abnormalities, and serologic hyperreactivity. *Ann. Intern. Med*. **98**, 155–159.
143. Alvarellos, A., Ahearn, J. M., Provost, T. T., Dorsch, C. A., Stevens, M. B., Bias, W. B., and Arnett, F. C. (1983). Relationships of HLA-DR and MT antigens to autoantibody expression in SLE. *Arthritis Rheum*. **26**, 1533–1535.
144. Griffing, W. L., Moore, S. B., Luthra, H. S., McKenna, C. H., and Fathman, C. G. (1980). Associations of antibodies to native DNA with HLA-DRw3: A possible major histocompatibility linked human immune response gene. *J. Exp. Medline* **152**, 3195–3205.
145. Podrebarac, T. A., Boisert, D. M., and Goldstein, R. (1998). Clinical correlates, serum autoantibodies and the role of the major histocompatibility complex in French Canadian and non-French Canadian Caucasians with SLE. *Lupus* **7**, 183–191.
146. Bell, D. A., and Maddison, P. J. (1980). Serologic subsets in systemic lupus erythematosus: An examination of autoantibodies in relationship to clinical features of disease and HLA antigens. *Arthritis Rheum*. **23**, 1268–1272.
147. Fukisaku, A., Frank, M. B., Neas, B., Reichlin, M., and Harley, J. B. (1990). HLA-DQ gene complementation and other histocompatibility relationships in man with the Ro/SSA autoantibody response of systemic lupus erythematosus. *J. Clin. Invest*. **86**, 606–611.
148. Reveille, J. D., MacLeod, M. J., Whittington, K., and Arnett, F. C. (1991). Specific amino acid residues in the second hypervariable region of HLA-DQA1 and DQB1 chain genes promote the Ro (SS-A)/La (SS-B) autoantibody responses. *J. Immunol*. **146**, 3871–3876.
149. Scofield, R. H., and Harley, J. B. (1994). Association of anti-Ro/SS-A autoantibodies with glutamine in position 34 of DQA1 and leucine in position 26 of DQB1. *Arthritis Rheum*. **37**, 961–962.
150. Harley, J. B., Yamagata, H., and Reichlin, M. (1984). Anti-La/SS-B antibody is present in some normal sera and it coincident with anti-Ro/SS-A precipitins in systemic lupus erythematosus. *J. Rheumatol*. **11**, 309–314.
151. Ehrfeld, H., Hartung, K., Renz, M., Coldewey, R., Deicher, H., Fricke, M., Kalden, J.R., Lakomek, J., Peter, H. H., Schendel, D., and Seelig, H. P. (1992). MHC associations of autoantibodies against recombinant Ro and La proteins in systemic lupus erythematosus. *Rheumatol Int*. **5**, 1–5.
152. Buyon, J. P., Slade, S. G., Reveille, J. D., Hamel, J. C., and Chan, E. K. L. (1994). Autoantibody responses to the "native" 52-kDa SS-A/Ro protein in neonatal lupus syndromes, systemic lupus erythematosus, and Sjogren's syndrome. *J. Immunol*. **152**, 3675–3684.
153. Hartung, K., Ehrfeld, H., Lakomek, H. J., Coldewey, R., Lang, B., Krapf, F., Muller, R., Schendel, D., Deicher, H., and Seelig, H. P. (1992). Results of a multicenter study. The genetic basis of Ro and La antibody formation in systemic lupus erythematosus. *Rheumatol. Int*. **11**, 243–249.
154. Martin-Villa, J. M., Martinez-Laso, J., Moreno-Pelayo, M. A., Castro-Panete, M. J., Martinez-Quiles, N., Alvarez, M., de Juan, M. D., Gomez-Reino, J. J., and Arnaiz-Villena, A. (1998). Differential contribution of HLA-DR, DQ, and TAP2 alleles to systemic lupus erythematosus susceptibility in Spanish patients: role of TAP2*01 alleles in Ro autoantibody production. *Ann. Rheum. Dis*. **57**, 214–219.
155. Logar, D., Vidan-Jeras, B., Dolzan, V., Bozic, B., and Kveder, T. (2002). The contribution of HLA-DQB1 coding and QBP promoter alleles to anti-Ro alone autoantibody response in systemic lupus erythematosus. *Rheumatology (Oxford)* **41**, 305–311.
156. Miyagawa, S., Shinohara, K., Nakajima, M., Kidoguchi, K., Fujita, T., Fukumoto, T., Yoshioka, A., Dohi, K., and Shirai, T. (1998). Polymorphisms of HLA class II genes and autoimmune responses to Ro/SS-A-La/SS-B among Japanese subjects. *Arthritis Rheum*. **41**, 927–934.
157. Sharp, G. C., Irvin, W. S., Tan, E. M., Gould, R. G., and Holman, H. R. (1972). Mixed connective tissue disease-an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med*. **52**, 148–159.
158. Nimelstein, S. H., Brody, S., McShane, D., and Holman, H. R. (1980). Mixed connective tissue disease: a subsequent evaluation of the original 25 patients. *Medicine* **59**, 239–248.

159. Arnett, F. C., Hamilton, R. G., Roebber, M. G., Harley, J. B., and Reichlin, M. (1988). Increased frequencies of Sm and nRNP autoantibodies in American blacks compared to whites with systemic lupus erythematosus. *J. Rheumatol.* **1538**, 1773–1776.
160. Smolen, J. S., Klippel, J. H., Penner, E., Reichlin, M., Steinberg, A. D., Chused, T. M., Scherak, O., Grainger, W., Hartter, E., Zielinski, C. C., Wolf, A., Davey, R. J., Mann, D. L., and Mayr, W. R. (1987). HLA-DR antigens in systemic lupus erythematosus: Association with specificity of autoantibody responses to nuclear antigens. *Ann. Rheum. Dis.* **46**, 457–462.
161. Kaneoka, H., Hsu, K. C., Takeda, Y., Sharp, G. C., and Hoffman, R. W. (1992). Molecular genetic analysis of HLA-DR and HLA-DQ genes among anti-U1-70 kd autoantibody positive connective tissue disease patients. *Arthritis Rheum.* **35**, 83–94.
162. Olsen, M. L., Arnett, F. C., and Reveille, J. D. (1993). Contrasting molecular patterns of MHC class II alleles associated with the Anti-Sm and Anti-RNP autoantibodies in systemic lupus erythematosus. *Arthritis Rheum.* **36**, 94–104.
163. Kuwana, M., Okana, Y., Kaburaki, J., Tsuji, K., and Inoko, H. (1995). Major histocompatibility complex class II gene associations with anti-U1 small nuclear ribonucleoprotein antibody. *Arthritis Rheum.* **38**, 396–405.
164. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann. Intern. Med.* **112**, 682–698.
165. Savi, M., Ferraccioli, G. F., Neri, T. M., Zanelli, P., Dall'Aglio, P. P., Tincani, A., Balestriere, G., Carella, G., and Cattaneo, R. (1988). HLA-DR antigens and anticardiolipin antibodies in Northern Italian systemic lupus erythematosus patients. *Arthritis Rheum.* **31**, 1568–1570.
166. McNeil, H. P., Gavaghan, T. P., Krilis, S. A., Geczy, A. F., and Chesterman, C. N. (1990). HLA-DR antigens and anticardiolipin antibodies. *Clin. Exp. Rheumatol.* **8**, 425–427.
167. Hartung, K., Coldewey, R., Corvetta, A., Deicher, H., Kalden, J. R., Krapf, F., Lang, B., Lakomek, H. J., Liedvogel, B., Peter, H. H., Schendel, D., Specker, Ch, Stangel, W., and Members of the SLE study group. (1992). MHC gene products and anticardiolipin antibodies in systemic lupus erythematosus results of a multicenter study. *Autoimmunity* **13**, 95–99.
168. Arnett, F. C., Olsen, M. L., Anderson, K. L., and Reveille, J. D. (1991). Molecular analysis of major histocompatibility complex alleles associated with the lupus anticoagulant. *J. Clin. Invest.* **87**, 1490–1495.
169. Panzer, S., Pabinger, I., Gschwandtner, M. E., Mayr, W. R., and Hutter, D. (1997). Lupus anticoagulants: strong association with the major histocompatibility complex class II and platelet antibodies. *Br. J. Haematol.* **98**, 342–345.
170. Goldstein, R., Moulds, J. M., Smith, C. D., and Sengar, D. P. (1996). MHC studies of the primary antiphospholipid syndrome and of antiphospholipid antibodies in systemic lupus erythematosus. *J. Rheumatol.* **23**, 1173–1179.
171. Granados, J., Vargas-Alarcon, G., Drenkard, C., Andrade, F., Melin-Aldana, H., Alcocer-Varela, J., and Alarcon-Segovia, D. (1997). Relationship of anticardiolipin antibodies and antiphospholipid syndrome to HLA-DR7 in Mexican patients with systemic lupus erythematosus (SLE). *Lupus* **6**, 57–62.
172. Arnett, F. C., Thiagarajan, P., Ahn, C., and Reveille, J. D. (1999). Associations of anti-beta2-glycoprotein I autoantibodies with HLA class II alleles in three ethnic groups. *Arthritis Rheum.* **42**, 268–274.
173. Galeazzi, M., Sebastiani, G. D., Tincani, A., Piette, J. C., Allegri, F., Morozzi, G., Bellisai, F., Scorza, R., Ferrara, G. B., Carcassi, C., Font, J., Passiu, G., Smolen, J., Papasteriades, C., Houssiau, F., Nebro, A. F., Ramon Garrido, E. D., Jedryka-Goral, A., and Marcolongo, R. (2000). HLA class II alleles associations of anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus. *Lupus* **9**, 47–55.
174. Hashimoto, H., Yamanaka, K., Tokano, Y., Iida, N., Takasaki, Y., Kabasawa, K., Nishimura, HLA-DRB1 alleles and beta 2 glycoprotein I-dependent anticardiolipin antibodies in Japanese patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **16**, 423–427.
175. May, K. P., West, S. G., Moulds, J., and Kotzin, B. L. (1993). Different manifestations of the antiphospholipid antibody syndrome in a family with systemic lupus erythematosus. *Arthritis Rheum.* **36**, 528–532.
176. Dagenias, P., Urowitz, M. B., Gladman, D. D., and Norman, C. S. (1992). A family study of the antiphospholipid syndrome associated with other autoimmune diseases. *J. Rheumatol.* **19**, 1393–1396.
177. Bonfa, E., Golombeck, S. J., Kaufman, C. D., Skelly, S., Weissbach, H., Brot, N., and Elkon, K. B. (1987). Association between lupus psychosis and anti-ribosomal P proteins. *N. Engl. J. Med.* **317**, 265–271.
178. Schneebaum, A. B., Singleton, J. D., West, S. G., Blodgett, J. K., Allen, L. G., Cheronis, J. C., and Kotzin, B. L. (1991). Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am. J. Med.* **90**, 54–62.
179. Nojima, Y., Minota, S., Yamada, A., Takaku, F., Aotsuka, S., and Yokohari, R. (1992). Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 1053–1055.
180. Hulsey, M., Goldstein, R., Scully, L., Surbeck, W., and Reichlin, M. (1995). Antiribosomal P antibodies in systemic lupus erythematosus: A case-control study correlating hepatic and renal disease. *Clin. Immunol. Immunopathol.* **74**, 252–256.
181. Arnett, F. C., and Reichlin, M. D. (1995). Lupus Hepatitis: An under-recognized disease feature associated with autoantibodies to ribosomal P. *Am. J. Med.* **99**, 465–472.
182. Arnett, F. C., Reveille, J. D., Moutsopoulos, H. M., Georgescu, L., and Elkon, K. B. (1996). Ribosomal P autoantibodies in systemic lupus erythematosus: Fre-

quencies in different ethnic groups and clinical and immunogenetic associations. *Arthritis Rheum*. **39**, 1833–1839.

183. Perl, A., Colombo, E., Dai, H., Agarwal, R., Mark, K. A., Banki, K., Poiesz, B. J., Phillips, P. E., Hoch, S. O., Reveille, J. D., and Arnett, F. C. (1995). Antibody reactivity to the HRES-1 endogenous retroviral element identifies a subset of patients with systemic lupus erythematosus and overlap syndromes: Correlation with antinuclear antibodies and HLA class II alleles. *Arthritis Rheum*. **38**, 1660–1671.
184. Magistrelli, C., Samoilova, E., Agarwal, R. K., Banki, K., Ferrante, P., Vladutiu, A., Phillips, P. E., and Perl, A. (1999). Polymorphic genotypes of the HRES-1 human endogenous retrovirus locus correlate with systemic lupus erythematosus and autoreactivity. *Immunogenetics* **49**, 829–834.
185. Gammon, W. R., Heise, E. R., Burke, W. A., Fine, J. D., and Woodley, D. T. (1988). Increased frequency of HLA-DR2 in patients with autoantibodies to epidermolysis bullosa acquisita antigen: Evidence that the expression of autoimmunity to type VII collagen is HLA class II allele associated. *J. Invest. Dermatol*. **91**, 228–232.
186. Galeazzi, M., Annunziata, P., Sebastiani, G. D., Bellisai, F., Campanella, V., Ferrara, G. B., Font, J., Houssiau, F., Passiu, G., De Ramon Garrido, E., Fernandez-Nebro, A., Bracci, L., Scorza, R., Puddu, P., Jedryka-Goral, A., Smolen, J., Tincani, A., Carcassi, C., Morozzi, G., and Marcolongo, R. (2000). Anti-ganglioside antibodies in a large cohort of European patients with systemic lupus erythematosus: Clinical, serological, and HLA class II gene associations. *J. Rheumatol*. **27**, 135–141.
187. Galeazzi, M., Sebastiani, G. D., Passiu, G., Angelini, G., Delfino, L., Asherson, R. A., Khamashta, M. A., and Hughes, G. R. V. (1992). HLA-DP genotyping in patients with systemic lupus erythematosus: Correlations with autoantibody subsets. *J. Rheumatol*. **19**, 42–46.
188. Reveille, J. D., Brady, J., MacLeod-St. Clair, M., and Durban, E. (1992). HLA-DPB1 alleles and autoantibody subsets in systemic lupus erythematosus, Sjogren's syndrome and progressive systemic sclerosis: A question of disease relevance. *Tissue Antigens* **40**, 45–48.
189. Hsu, K. C., Hill, D. L., and Hoffman, R. W. (1992). HLA-DPB1*0401 is associated with the presence of autoantibodies reactive with the U1-70 kD polypeptide antigen of U1-small nuclear ribonucleoprotein among connective tissue disease patients. *Tissue Antigens* **39**, 272–275.
190. Takeuchi, F., Nabeta, H., Hong, G. H., Kuwata, S., Tanimoto, K., and Ito, K. (1998). Polymorphisms of DMA and DMB genes in Japanese systemic lupus erythematosus. *Br. J. Rheumatol*. **37**, 95–97.
191. Yen, J. H., Chen, C. J., Tsai, W. C., Tsai, J. J., Ou, T. T., and Liu, H. W. (1999). HLA-DMA and HLA-DMB genotyping in patients with systemic lupus erythematosus. *J. Rheumatol*. **26**, 1930–1933.
192. Christiansen, F. T., Zhang, W. J., Griffiths, M., Mallal, S. A., and Dawkins, R. L. (1991). Major histocompatibility complex (MHC) complement deficiency, ancestral haplotypes and systemic lupus erythematosus (SLE): C4 deficiency explains some but not all of the influence of the MHC. *J. Rheumatol*. **18**, 1350–1358.
193. Schur, P. H., Marcus-Bagley, D., Awdeh, Z., Yunis, E. J., and Alper, C. A. (1990). The effect of ethnicity on major histocompatibility complex complement allotypes and extended haplotypes in patients with systemic lupus erythematosus. *Arthritis Rheum*. **33**, 985–992.
194. Steinsson, K., Arnason, A., Erlendsson, K., Fossdal, R., Skaftadottir, I., Jonsdottir, S., Fjalarson, M., and Thorsteinsson, J. (1995). A study for the major histocompatibility complex in Caucasian family with multiple cases of systemic lupus erythematosus: Association with the C4AQ0 phenotype. *J. Rheumatol*. **22**, 1862–1866.
195. van der Linden, M. W., van der Slik, A. R., Zanelli, E., Giphart, M. J., Pieterman, E., Schreuder, G. M., Westendorp, R. G., and Huizinga, T. W. (2001). Six microsatellite markers on the short arm of chromosome 6 in relation to HLA-DR3 and TNF-308A in systemic lupus erythematosus. *Genes Immun*. **2**, 373–380.
196. Truedsson, L., Sturfelt, G., Johansen, P., Nived, O., and Thuresson, B. (1995). Sharing of MHC haplotypes among patients with systemic lupus erythematosus from unrelated Caucasian multicase families: Disease association with the extended haplotype [HLA-B8, SC01, DR17]. *J. Rheumatol*. **22**, 1852–1861.
197. Rigby, R. J., Dawkins, R. L., Wetherall, J. D., and Hawkins, B. R. (1978). HLA in systemic lupus erythematosus: Influence on severity. *Tissue Antigens* **12**, 25–31.
198. Bell, D. A., Rigby, R., Stiller, C. R., Clark, W. F., Harth, M., and Ebers, G. (1984). HLA Antigens in systemic lupus erythematosus: Relationship to disease severity, age at onset, and sex. *J. Rheumatol*. **11**, 475–479.
199. Gulko, P. S., Reveille, J. D., Koopman, W. J., Burgard, S. L., Bartolucci, A. A., and Alarcon, G. S. (1993). Anticardiolipin antibodies in systemic lupus erythematosus: Clinical correlates, HLA associations, and impact on survival. *J. Rheumatol*. **20**, 1684–1693.
200. Alarcon, G. S., Roseman, J., Bartolucci, A. A., Friedman, A. W., Moulds, J. M., Goel, N., Straaton, K. V., and Reveille, J. D. (1998). Systemic lupus erythematosus in three ethnic groups. I. Features predictive of disease activity early in its course. *Arthritis Rheum*.
201. Alarcon, G. S., McGwin, G., Jr., Bartolucci, A. A., Roseman, J., Lisse, J., Fessler, B. J., Bastian, H. M., Friedman, A. W., and Reveille, J. D. (2002). Systemic lupus erythematosus in three ethnic groups. IX. Differences in damage accrual. *Arthritis Rheum*. **44**, 2797–2806.
202. Komata, T., Tsuchiya, N., Matsushita, M., Hagiwara, K., and Tokunaga, K. (1999). Association of tumor necrosis factor receptor 2 (TNFR2) polymorphism with susceptibility to systemic lupus erythematosus. *Tissue Antigens* **53**, 527–533.
203. Takahashi, M., Hashimoto, H., Akizuki, M., Sasazuki, T., Nishikimi, N., Ouchi, H., Kobayashi, Y., Numano, F., and Kimura, A. (2002). Lack of association between the Met196Arg polymorphism in the TNFR2 gene and autoimmune diseases accompanied by vasculitis including SLE in Japanese. *Tissue Antigens* **57**, 66–69.

204. Morita, C., Horiuchi, T., Tsukamoto, H., Hatta, N., Kikuchi, Y., Arinobu, Y., Otsuka, T., Sawabe, T., Harashima, S., Nagasawa, K., and Niho, Y. (2001). Association of tumor necrosis factor receptor type II polymorphism 196R with systemic lupus erythematosus in the Japanese: Molecular and functional analysis. *Arthritis Rheum*. **44**, 2819–2827.
205. Al-Ansari, A. S., Ollier, W. E., Villarreal, J., Ordi, J., Teh, L. S., and Hajeer, A. H. (2000). Tumor necrosis factor receptor II (TNFRII) exon 6 polymorphism in systemic lupus erythematosus.*Tissue Antigens* **55**, 97–99.
206. Sullivan, K. E., Piliero, L. M., Goldman, D., and Petri, M. A. (2000). A TNFR2 3' flanking region polymorphism in systemic lupus erythematosus. *Genes Immun*. **1**, 225–227.
207. Lee, E. B., Yoo, J. E., Lee, Y. J., Choi, Y. J., Park, K. S., and Song, Y. W. (2001). Tumor necrosis factor receptor 2 polymorphism in systemic lupus erythematosus: No association with disease. *Hum. Immunol*. **62**, 1148–1152.
208. Kawasaki, A., Tsuchiya, N., Fukazawa, T., Hashimoto, H., and Tokunaga, K. (2001). Presence of four major haplotypes in human BCMA gene: Lack of association with systemic lupus erythematosus and rheumatoid arthritis. *Genes Immun*. **2**, 276–279.
209. Ollier, W., Davies, E., Snowden, N., Alldersea, J., Fryer, A., Jones, P., and Strange, R. (1996). Association of homozygosity for glutathione-S-transferase GSTM1 null alleles with the R0+/La–autoantibody profile in patients with systemic lupus erythematosus. *Arthritis Rheum*. **39**, 1763–1764.
210. Tew, M. B., Ahn, C. W., Friedman, A. W., Reveille, J. D., Tan, F. K., Alarcón, G. S., Bastian, H. M., Fessler, B. J., McGwin, G., and Lisse, J. R. (2001). Systemic lupus erythematosus from three ethnic groups: Glutathione S-Transferase null alleles lack association with disease manifestations. *Arthritis Rheum*. **44**, 981–983.
211. Salmon, J. E., Kimberly, R. P., Gibofsky, A., and Fotino, M. (1984). Defective mononuclear phagocyte function in systemic lupus erythematosus: Dissociation of Fc receptor-ligand binding and internalization. *J. Immunol*. **133**, 2525–2531.
212. Salmon, J. E., Millard, S., Schacter, L. A., Arnett, F. C., Ginzler, E. M., Gourley, M. F., Ramsey-Goldman, R., Peterson, M. G. E., and Kimberly, R. P. (1996). Fc gamma RIIA alleles are heritable risk factors for lupus nephritis in African-Americans. *J. Clin. Invest*. **97**, 1348–1354.
213. Yee, A. M., Ng, S. C., Sobel, R. E., and Salmon, J. E. (1997). Fc gammaRIIA polymorphism as a risk factor for invasive pneumococcal infections in systemic lupus erythematosus. *Arthritis Rheum*. **40**, 1180–1182.
214. Duits, A. J., Bootsma, H., Derksen, R. H. W. M., Spronk, P. E., Kater, L., Kallenberg, C. G. M., Capel, P. J. A., Westerdaal, N. A. C., Spierenburg, G., Gmelig-meyling, F. H. J., and van de Winkel, J. G. J. (1995). Skewed distribution of IgG Fc receptor IIa (CD32) polymorphism is associated with renal disease in systemic lupus erythematosus patients. *Arthritis Rheum*. **39**, 1832–1836.
215. Botto, M., Theodoridis, E., Thompson, E. M., Beynon, H. L., Briggs, D., Isenberg, D. A., Walport, M. J., and Davies, K. A. (1996). Fc gamma RIIa polymorphism in systemic lupus eryhematosus (SLE): No association with disease. *Clin. Exp. Immunol*. **104**, 264–268.
216. Manger, K., Repp, R., Spriewald, B. M., Rascu, A., Geiger, A., Wassmuth, R., Westerdaal, N. A., Wentz, B., Manger, B., Kalden, J. R., and van de Winkel, J. G. (1998). Fcgamma receptor IIa polymorphism in Caucasian patients with systemic lupus erythematosus: Association with clinical symptoms. *Arthritis Rheum*. **41**, 1181–1189.
217. Norsworthy, P., Theodoridis, E., Botto, M., Athanassiou, P., Beynon, H., Gordon, C., Isenberg, D., Walport, M. J., and Davies, K. A. (1999). Overrepresentation of the Fcgamma receptor type IIA R131/R131 genotype in caucasoid systemic lupus erythematosus patients with autoantibodies to C1q and glomerulonephritis. *Arthritis Rheum*. **42**, 1828–1832.
218. Michel, M., Piette, J. C., Roullet, E., Duron, F., Frances, C., Nahum, L., Pelletier, N., Crassard, I., Nunez, S., Michel, C., Bach, J., and Tournier-Lasserve, E. (2000). The R131 low-affinity allele of the Fc gamma RIIA receptor is associated with systemic lupus erythematosus but not with other autoimmune diseases in French Caucasians. *Am. J. Med*. **108**, 580–583.
219. Dijstelbloem, H. M., Bijl, M., Fijnheer, R., Scheepers, R. H., Oost, W. W., Jansen, M. D., Sluiter, W. J., Limburg, P. C., Derksen, R. H., van de Winkel, J. G., and Kallenberg, C. G. (2000). Fcgamma receptor polymorphisms in systemic lupus erythematosus: Association with disease and in vivo clearance of immune complexes. *Arthritis Rheum*. **43**, 2793–2800.
220. Zuniga, R., Ng, S., Peterson, M. G., Reveille, J. D., Baethge, B. A., Alarcon, G. S., and Salmon, J. E. (2001). Low-binding alleles of Fcgamma receptor types IIA and IIIA are inherited independently and are associated with systemic lupus erythematosus in Hispanic patients. *Arthritis Rheum*. **44**, 361–367.
221. Song, Y. W., Han, C. W., Kang, S. W., Baek, H. J., Lee, E. B., Shin, C. H., Hahn, B. H., and Tsao, B. P. (1998). Abnormal distribution of Fc gamma receptor type IIa polymorphisms in Korean patients with systemic lupus erythematosus. *Arthritis Rheum*. **41**, 421–426.
222. Yun, H. R., Koh, H. K., Kim, S. S., Chung, W. T., Kim, D. W., Hong, K. P., Song, G. G., Chang, H. K., Choe, J. Y., Bae, S. C., Salmon, J. E., Yoo, D. H., Kim, T. Y., and Kim, S. Y. (2000). FcgammaRIIa/IIIa polymorphism and its association with clinical manifestations in Korean lupus patients. *Lupus* **10**, 466–472.
223. Yap, S. N., Phipps, M. E., Manivasagar, M., Tan, S. Y., and Bosco, J. J. (1999). Human Fc gamma receptor IIA (FcgammaRIIA) genotyping and association with systemic lupus erythematosus (SLE) in Chinese and Malays in Malaysia. *Lupus* **8**, 305–310.
224. Sato, H., Iwano, M., Akai, Y., Nishino, T., Fujimoto, T., Shiiki, H., and Dohi, K. (2001). FcgammaRIIa polymorphism in Japanese patients with systemic lupus erythematosus. *Lupus* **10**, 97–101.
225. Hatta, Y., Tsuchiya, N., Ohashi, J., Matsushita, M., Fujiwara, K., Hagiwara, K., Juji, T., and Tokunaga, K. (1999). Human Fc gamma receptor (Fc gamma R) genes

form a clustered gene family on chromosome 1q21-24: Association of Fc gamma receptor IIIB, but not of Fc gamma receptor IIA and IIIA polymorphisms with systemic lupus erythematosus in Japanese. *Genes Immun*. **1**, 53–60.
226. Seligman, V. A., Suarez, C., Lum, R., Inda, S. E., Lin, D., Li, H., Olson, J. L., Seldin, M. F., and Criswell, L. A. (2001). The Fcgamma receptor IIIA-158F allele is a major risk factor for the development of lupus nephritis among Caucasians but not non-Caucasians. *Arthritis Rheum*. **44**, 618–625.
227. Smyth, L. J., Snowden, N., Carthy, D., Papasteriades, C., Hajeer, A., and Ollier, W. E. (1997). Fc gamma RIIa polymorphism in systemic lupus erythematosus. *Ann*. *Rheum*. *Dis*. **56**, 744–746.
228. Villarreal, J., Crosdale, D., Ollier, W., Hajeer, A., Thomson, W., Ordi, J., Balada, E., Villardell, M., Teh, L. S., and Poulton, K. (2001). Mannose binding lectin and FcgammaRIIa (CD32) polymorphism in Spanish systemic lupus erythematosus patients. *Rheumatology (Oxford)* **40**, 1009–1012.
229. Karrassa, F. B., Trikalinos, T. A., and Ioannidis, J. P. A. (2002). Role of the Fcγ receptor IIa polymorphism in susceptibility to systemic lpus erythematosus and lupus nephritis. *Arthritis Rheum*. **46**, 1563–1571.
230. Kyogoku, C., Dijstelbloem, H. M., Tsuchiya, N., Hatta, Y., Kato, H., Yamaguchi, A., Fukazawa, T., Jansen, M. D., Hashimoto, H., Van De Winkel, J. G., Kallenberg, C. G., and Tokunaga, K. (2002). Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: Contribution of FCGR2B to genetic susceptibility. *Arthritis Rheum*. **46**, 1242–1254.
231. Wu, J., Edberg, J. C., Redecha, P. B., Bansal, V., Guyre, P. M., Coleman, K., Salmon, J. E., and Kimberly, R. P. (1997). A novel polymorphism of Fc gamma RIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J*. *Clin*. *Invest*. **100**, 1059–1070.
232. Koene, H. R., Kleijer, M., Swaak, A. J., Sullivan, K. E., Bijl, M., Petri, M. A., Kallenberg, C. G., Roos, D., von dem Borne, A. E., and de Haas, M. (1998). The Fc gamma-RIIIA-158F allele is a risk factor for systemic lupus erythematosus. *Arthritis Rheum*. **41**, 1813–1818.
233. Salmon, J. E., Ng, S., Yoo, D. H., Kim, T. H., Kim, S. Y., and Song, G. G. (1999). Altered distribution of Fcgamma receptor IIIA alleles in a cohort of Korean patients with lupus nephritis. *Arthritis Rheum*. **42**, 818–819.
234. Yap, S. N., Phipps, M. E., Manivasagar, M., and Bosco, J. J. (1999). Fc gamma receptor IIIB-NA gene frequencies in patients with systemic lupus erythematosus and healthy individuals of Malay and Chinese ethnicity. *Immunol*. *Lett*. **68**, 295–300.
235. Fisher, G. H., Rosenberg, F. J., Straus, S. E., Dale, J. K., Middleton, L. A., Lin, A. Y., Strober, W., Lenardo, M. J., and Puck, J. M. (1995). Dominant interfering fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* **81**, 935–946.
236. Rieux-Laucat, F., Le Deist, F., Hivroz, C., Roberts, A. G., Debatin, K. M., Fischer, A., and deVillartay, J. P. (1995). Mutations in fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* **268**, 1347–1349.
237. Elkon, K. B. (1994). Apoptosis in SLE: Too little or too much. *Clin*. *Exp*. *Rheumatol*. **12**, 553–559.
238. Mountz, J. D., Wu, J., Cheng, J., and Zhou, T. (1994). Defective apoptosis in autoimmune disease. *Arthritis Rheum*. **37**, 1415–1420.
239. Lee, Y. H., Kim, Y. R., Ji, J. D., Sohn, J., and Song, G. G. (2001). Fas promoter –670 polymorphism is associated with development of anti-RNP antibodies in systemic lupus erythematosus. *J*. *Rheumatol*. **28**, 2008–2011.
240. Huang, Q. R., Danis, V., Lassere, M., Edmonds, J., and Manolios, N. (1999). Evaluation of a new Apo-1/Fas promoter polymorphism in rheumatoid arthritis and systemic lupus erythematosus patients. *Rheumatology (Oxford)* **38**, 645–651.
241. Huang, Q. R., and Manolios, N. (2000). Investigation of the –1377 polymorphism on the Apo-1/Fas promoter in systemic lupus erythematosus patients using allele-specific amplification. *Pathology* **32**, 126–130.
242. Horiuchi, T., Nishizaka, H., Yasunaga, S., Higuchi, M., Tsukamoto, H., Hayashi, K., and Nagasawa, K. (1999). Association of Fas/APO-1 gene polymorphism with systemic lupus erythematosus in Japanese. *Rheumatology (Oxford)* **38**, 516–520.
243. Cascino, I., Ballerini, C., Audino, S., Rombola, G., Massacesi, L., Colombo, G., Scorza Smeraldi, R., d'Alfonso, S., Momigliano Richiardi, P., Tosi, R., and Ruberti, G. (1998). Fas gene polymorphisms are not associated with systemic lupus erythematosus, multiple sclerosis and HIV infection. *Dis*. *Mark*. **13**, 221–225.
244. Wu, J., Wilson, J., He, J., Xiang, L., Schur, P. H., and Mountz, J. D. (1996). Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. *J*. *Clin*. *Invest*. **98**, 1107–1113.
245. Mehrian, R., Quismorio, Jr., F. P., Strassmann, G., Stimmler, M. M., Horwitz, D. A., Kitridou, R. C., Gauderman, W. J., Morrison, J., Brautbar, C., and Jacob, C. O. (1998). Synergistic effect between *IL-10* and *bcl-2* genotypes in determining susceptibility to systemic lupus erythematosus. *Arthritis Rheum*. **41**, 596–602.
246. Kojima, T., Horiuchi, T., Nishizaka, H., Sawabe, T., Higuchi, M., Harashima, S. I., Yoshizawa, S., Tsukamoto, H., Nagasawa, K., and Niho, Y. (2000). Analysis of fas ligand gene mutation in patients with systemic lupus erythematosus. *Arthritis Rheum*. **43**, 135–139.
247. El-Magadmi, M., Alansari, A., Teh, L. S., Ordi, J., Gul, A., Inanc, M., Bruce, I., and Hajeer, A. (2001). Association of the A561C E-selectin polymorphism with systemic lupus erythematosus in 2 independent populations. *J*. *Rheumatol*. **28**, 2650–2652.
248. Szalai, A. J., McCrory, M. A., Cooper, G. S., Wu, J., and Kimberly, R. P. (2002). Association between baseline levels of C-reactive protein (CRP) and a dinucleotide repeat polymorphism in the intron of the CRP gene. *Genes Immun*. **3**, 14–19.
249. Nambiar, M. P., Enyedy, E. J., Warke, V. G., Krishnan, S., Dennis, G., Wong, H. K., Kammer, G. M., and Tsokos, G. C. (2001). T cell signaling abnormalities in systemic

lupus erythematosus are associated with increased mutations/polymorphisms and splice variants of T cell receptor zeta chain messenger RNA. *Arthritis Rheum*. **44**, 1336–1350.

250. Wu, J., Edberg, J. C., Gibson, A. W., Tsao, B., and Kimberly, R. P. (1999). Single-nucleotide polymorphisms of T cell receptor zeta chain in patients with systemic lupus erythematosus. *Arthritis Rheum*. **42**, 2601–2605.
251. Wilson, J. G., Murphy, E. E., Wong, W. W., Klickstein, L. B., Weis, J. H., and Fearon, D. T. (1986). Identification of a restriction fragment polymorphism by a CR1 cDNA that correlates with the number of CR1 on erythrocytes. *J. Exp. Medline* **164**, 50–59.
252. Wong, W. W., Kennedy, C. A., Bonaccio, E. T., Wilson, J. G., Klickstein, L. B., Weis, J. H., and Fearon, D. T. (1986). Analysis of multiple restriction fragment length polymorphisms of the gene for the human complement receptor type 1: Duplication of genomic sequences occurs in association with a high molecular mass receptor allotype. *J. Exp. Medline* **164**, 1531–1546.
253. Wilson, J. G., Wong, W. W., Schur, P. H., and Fearon, D. T. (1982). Mode of inheritance of decreased C3b receptors on erythrocytes of patients with systemic lupus erythematosus. *N. Engl. J. Med*. **307**, 981–986.
254. Wilson, J. G., Ratnoff, W. D., Schur, P. H., and Fearon, D. T. (1986). Decreased expression of the C3b/C4b receptor (CR1) and the C3d receptor (CR2) on B lymphocytes and of CR1 on neutrophils of patients with systemic lupus erythematosus. *Arthritis Rheum*. **29**, 739–747.
255. Ross, G. D., Yount, W. J., Walport, M. J., Winfield, J. B., Parker, C. J., Fuller, C. R., Taylor, R. P., Myones, B. L., and Lachmann, P. J. (1985). Disease-associated loss of erythrocyte complement receptors (CR1, C3b receptors) in patients with systemic lupus erythematosus and other diseases involving autoantibodies and/or complement activation. *J. Immunol*. **135**, 2005–2013.
256. Iida, K., Mornaghi, R., and Nussenzweig, V. (1982). Complement receptor (CR1) deficiency in erythrocytes from patients with systemic lupus erythematosus. *J. Exp. Med*. **155**, 1427–1438.
257. Moldenhauer, F., David, J., Fielder, A. H. L., Lachmann, P. J., and Walport, M. J. (1987). Inherited deficiency of erythrocyte complement receptor type 1 does not cause susceptibility to systemic lupus erythematosus. *Arthritis Rheum*. **30**, 961–966.
258. Uko, G., Dawkins, R. L., Kay, P., Christiansen, F. T., and Hollingsworth, P. N. (1985). CR1 deficiency in SLE: acquired or genetic? *Clin. Exp. Immunogenet*. **62**, 329–336.
259. Walport, M. J., Ross, G. D., Mackworth-Young, C., Watson, J. V., Hogg, N., and Lachmann, P. J. (1985). Family studies of erythrocyte complement receptor type 1 levels: Reduced levels in patients with SLE are acquired, not inherited. *Clin. Exp. Immunol*. **59**, 547–554.
260. Holme, E., Fyfe, A., Zoma, A., Veitch, J., Hunter, J., and Whaley, K. (1986). Decreased C3b receptors (CR1) on erythrocytes from patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **63**, 41–48.
261. Satoh, H., Yokota, E., Tokiyama, K., Kawaguchi, T., and Niho, Y. (1990). Distribution of the *Hind* III restriction fragment length polymorphism among patients with systemic lupus erythematosus with different concentrations of CR1. *Ann. Rheum. Dis*. 765–768.
262. Kumar, A., Kumar, A., Sinha, S., Khanderkar, P. S., Banerjee, K., and Srivastava, L. M. (1995). *Hind* III genomic polymorphism of the C3b receptor (CR1) in patients with SLE: Low erythrocyte CR1 expression is an acquired phenomenon. *Immuno Cell. Biol*. **73**, 457–462.
263. Tebib, J. G., Martinez, C., Granados, J., Alarcon-Segovia, D., and Schur, P. H. (1989). The frequency of complement receptor type 1 (CR1) gene polymorphisms in nine families with multiple cases of systemic lupus erythematosus. *Arthritis Rheum*. **32**, 1465–1469.
264. Van Dyne, S., Holers, V. M., Lublin, D. M., and Atkinson, J. P. (1987). The polymorphism of the C3b/C4b receptor in the normal population and in patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **68**, 570–579.
265. Wong, W. W., and Farrell, S. A. (1991). Proposed structure of the F′ allotype of human CR1. Loss of a C3b binding site may be associated with altered function. *J. Immunol*. **146**, 656–662.
266. Cornillet, P., Gredy, P., Pennaforte, J. L., Meyer, O., and Kazatchkine, M. D. (1992). Increased frequency of the long (S) allotype of CR1 (the C3b/C4b receptor, CD35) in patients with systemic lupus erythematosus. *Clin. Exp. Immunol*. **89**, 22–25.
267. Moulds, J. M., Reveille, J. D., and Arnett, F. C. (1996). Structural polymorphisms of complement receptor 1 (CR1) in systemic lupus erythematosus (SLE) patients and normal controls of three ethnic groups. *Clin. Exp. Immunol*. **105**, 302–305.
268. Malefyt, R. D., Haanen, J., Spits, H., Roncarolo, M. G., Tevelde, A., Figor, C., Johnson, K., Kastelein, R., Yssel, H., and DeVries, J. E. (1991). Interleukin-10 (IL-10) and viral-IL-10 strongly reduce antigen specific T-cell proliferation by diminishing the antigen presentation capacity of monocytes via down-regulation of class-II MHC expression. *J. Exp. Med*. **174**, 915–924.
269. Taga, K., Mostowski, H., and Tosato, G. (1993). Human interleukin-10 can directly inhibit T-cell growth. *Blood* **81**, 2964–2971.
270. Bejarano, M. T., Malefyt, R. D., Abrams, J. S., Sigler, M., Bachetta, R., DeVries, J. E., and Roncarolo, M. G. (1992). Interleukin-10 inhibits allo-specific proliferative and cytotoxic T-cell responses generated in mixed lymphocyte cultures. *Int. Immunol*. **4**, 1389–1397.
271. Taga, K., Cherney, B., and Tosato, G. (1993). IL-10 inhibits apoptotic cell-death in human T-cell starved of IL-2. *Int. Immunol*. **5**, 1599–1608.
272. Rousset, F., Garcia, E., Defrance, T., Perone, C., Vezzio, N., Hsu, D. H., Kastein, R., Moore, K. W., and Banchereau, J. (1992). IL-10 is a potent growth and differentiation factor for human B-cells. *Proc. Natl. Acad. Sci. USA* **89**, 1890–1893.
273. Perez, L., Orte, J., and Brieva, J. A. (1995). Terminal differentiation of spontaneous rheumatoid factor-secreting B-cells from rheumatoid arthritis patients depends on

endogenous interleukin-10. *Arthritis Rheum*. **38**, 1771–1776.
274. Llorente, L., Zou, W., Levy, Y., Richaud-Patin, Y., Wijdennes, J., Alcocer-Varela, J., Morel-Fourrier, B., Brouet, J. C., Alarcon-Segovia, D., Galanaud, P., and Emilie, D. (1995). Role of interleukin-10 in the B-lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med*. **181**, 839–844.
275. Eskdale, J., Kube, D., Tesch, H., and Gallagher, G. (1997). Mapping of the human IL10 gene and further characterization of the 5′ flanking sequence. *Immunogenetics* **46**, 120–128.
276. Eskdale, J., Wordsworth, P., Bowman, S., Field, M., and Gallagher, G. (1997). Association between polymorphisms at the human IL-10 locus and systemic lupus erythematosus. *Tissue Antigens* **49**, 635–639.
277. D'Alfonso, S., Rampi, M., Bocchio, D., Colombo, G., Scorza-Smeraldi, R., and Momigliano-Richardi, P. (2000). Systemic lupus erythematosus candidate genes in the Italian population: Evidence for a significant association with interleukin-10. *Arthritis Rheum*. **43**, 120–128.
278. Ou, T. T., Tsai, W. C., Chen, C. J., Chang, J. G., Yen, J. H., Wang, W. S., Lin, C. H., Tsai, J. J., and Liu, H. W. (1998). Genetic analysis of interleukin-10 promoter region in patients with systemic lupus erythematosus in Taiwan. *Kaohsiung J. Med. Sci*. **14**, 599–606.
279. Rood, M. J., Keijsers, V., van der Linden, M. W., Tong, T. Q., Borggreve, S. E., Verweij, C. L., Breedveld, F. C., and Huizinga, T. W. (1999). Neuropsychiatric systemic lupus erythematosus is associated with imbalance in interleukin 10 promoter haplotypes. *Ann. Rheum. Dis*. **58**, 85–89.
280. Mok, C. C., Lanchbury, J. S., Chan, D. W., and Lau, C. S. (1998). Interleukin-10 promoter polymorphisms in southern Chinese patients with systemic lupus erythematosus. *Arthritis Rheum*. **41**, 1090–1095.
281. Alarcon-Riquelme, M. E., Lindqvist, A. K., Jonasson, I., Johanneson, B., Sandino, S., Alcocer-Varela, J., Granados, J., Kristjansdottir, H., Grondal, G., Svenungsson, E., Lundberg, I., Steinsson, K., Klareskog, L., Sturfelt, G., Truedsson, L., Alarcon-Segovia, D., and Gyllensten, U. B. (1999). Genetic analysis of the contribution of IL10 to systemic lupus erythematosus. *J. Rheumatol*. **26**, 2148–2152.
282. Crawley, E., Woo, P., and Isenberg, D. A. (1999). Single nucleotide polymorphic haplotypes of the interleukin-10 5′ flanking region are not associated with renal disease or serology in Caucasian patients with systemic lupus erythematosus. *Arthritis Rheum*. **42**, 2017–2018.
283. Nakashima, H., Akahoshi, M., Tanaka, Y., Yamaoka, K., Ogami, E., Nagano, S., Arinobu, Y., Niiro, H., Otsuka, T., and Niho, Y. (1999). Polymorphisms within the interleukin-10 receptor cDNA gene (IL10R) in Japanese patients with systemic lupus erythematosus. *Rheumatology (Oxford)* **38**, 1142–1144.
284. Tsao, B. P., Cantor, R. M., Grossman, J. M., Shen, N., Teophilov, N. T., Wallace, D. J., Arnett, F. C., Hartung, K., Goldstein, R., Kalunian, K. C., Hahn, B. H., and Rotter, J. I. (1999). PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus. *J. Clin. Invest*. **103**, 1135–1140.
285. Delrieu, O., Michel, M., Frances, C., Meyer, O., Michel, C., Wittke, F., Crassard, I., Bach, J. F., Tournier-Lasserve, E., and Piette, J. C. (1999). Poly(ADP-ribose) polymerase alleles in French Caucasians are associated neither with lupus nor with primary antiphospholipid syndrome. *Arthritis Rheum*. **42**, 2194–2197.
286. Tan, F. K., Reveille, J. D., Arnett, F. C., Stivers, D. N., and Tsao, B. P. (2000). Poly(ADP)-ribose polymerase and susceptibility to systemic lupus erythematosus and primary antiphospholipid syndrome: Comment on the article by Delrieu *et al*. *Arthritis Rheum*. **43**, 1421–1423.
287. Criswell, L. A., Moser, K. L., Gaffney, P. M., Inda, S., Ortmann, W. A., Lin, D., Chen, J. J., Li, H., Gray-McGuire, C., Neas, B. R., Rich, S. S., Harley, J. B., Behrens, T. W., and Seldin, M. F. (2000). PARP alleles and SLE: Failure to confirm association with disease susceptibility. *J. Clin. Invest*. **105**, 1501–1502.
288. Boorboor, P., Drescher, B. E., Hartung, K., Sachse, C., Tsao, B. P., Schneider, P. M., Kalden, J. R., Lakomek, H. J., Peter, H. H., Schmidt, R. E., and Witte, T. (2000). Poly(ADP-ribose) polymerase polymorphisms are not a genetic risk factor for systemic lupus erythematosus in German Caucasians. *J. Rheumatol*. **27**, 2061.
289. Whittingham, S., Matthews, J. D., Schanfield, M. S., Tait, B. D., and Mackay, I. R. (1983). HLA and Gm genes in systemic lupus erythematosus. *Tissue Antigens* **21**, 50–51.
290. Schur, P. H., Pandey, J. P., and Fedrick, J. A. (1985). Gm allotypes in white patients with systemic lupus erythematosus. *Arthritis Rheum*. **28**, 828–830.
291. Fedrick, J. A., Pandey, J. P., Chen, Z., Fudenberg, H. H., Ainsworth, S. K., and Dobson, R. L. (1985). Gm allotypes in blacks with systemic lupus erythematosus. *Arthritis Rheum*. **28**, 828–830.
292. Nakao, Y., Matsumoto, H., Miyazaki, T., Nishitani, H., Takatsuki, K., Kasukawa, R., Nakayama, S., Izumi, S., Fujita, T., and Tsuji, K. (1980). IgG heavy chain allotypes (Gm) in autoimmune diseases. *Clin. Exp. Immunol*. **42**, 20–26.
293. Hoffman, R. W., Sharp, G. C., Irvin, W. S., Anderson, S. K., Hewett, J. E., and Pandey, J. P. (1991). Association of immunoglobulin Km and Gm allotypes with specific antinuclear antibodies and disease susceptibility among connective tissue disease patients. *Arthritis Rheum*. **34**, 453–458.
294. Genth, E., Zarnowski, H., Mierau, R., Wohltmann, D., and Hartl, O. W. (1987). HLA-DR4 and Gm (1,3:5,21) are associated with U1-nRNP antobody positive connective tissue disease. *Ann. Rheum. Dis*. **46**, 189–196.
295. Stenszky, V., Kozma, L., Szegedi, G., and Farid, N. R. (1986). Interplay of immunoglobulin G heavy chain markers (Gm) and HLA in predisposing to systemic lupus erythematosus. *J. Immunogenet*. **13**, 11–17.
296. Kumar, A., Martinez-Tarquino, C., Maria-Forte, A., Kumar, P., Alarcon-Segovia, D., Granados, J., Pandey, J. P., Buxbaum, J., and Schur, P. H. (1991). Immunoglob-

ulin heavy chain constant-region gene polymorphism in systemic lupus erythematosus. *Arthritis Rheum*. **34**, 1553–1556.

297. Hartung, K., Coldewey, R., Rother, E., Pirner, K., Specker, C., Peter, H. H., Kalden, J. R., Lakomek, H. J., Deicher, H., and De Lange, G. G. (1991). Members of the SLE Study Group.: Immunoglobulin allotypes in systemic lupus erythematosus: Results of a Central European multicenter study. *Exp. Clin. Immunogenet.* **8**, 11–15.
298. Hartung, K., Coldewey, R., Rother, E., Pirner, K., Specker, C., Schendel, D., Stangel, W., Stannat-KieBling, S., and De Lange, G. G. (1991). Members of the SLE study group: Immunoglobulin allotypes are not associated with HLA-antigens, autoantibodies and clinical symptoms in systemic lupus erythematosus. *Rheumatol. Int*. **11**, 179–182.
299. Blasini, A. M., Delgado, M. B., Valdivieso, C., Guevara, P., Ramirez, J. L., Stekman, I. L., Rodriguez, M. A., and Williams, R. C., Jr. (1996). Restriction fragment length polymorphisms of constant region genes of immunoglobulin lambda chains in Venezuelan patients with systemic lupus erythematosus. *Lupus* **5**, 300–302.
300. Queiroz, R. G., Tamia-Ferreira, M. C., Carvalho, I. F., Petean, F. C., and Passos, G. A. (2001). Association between EcoRI fragment-length polymorphism of the immunoglobulin lambda variable 8 (IGLV8) gene family with rheumatoid arthritis and systemic lupus erythematosus. *Braz. J. Med. Biol. Res*. **34**, 525–528.
301. Olee, T., Yang, P. M., Siminovitch, K. A., Olsen, N. J., Hillson, J., Wu, J., Kozin, F., Carson, D. A., and Chen, P. P. (1991). Molecular basis of an autoantibody-associated restriction fragment length polymorphism that confers susceptibility to autoimmune diseases. *J. Clin. Invest*. **88**, 193–203.
302. Huang, D., Siminovitch, K. A., Liu, X. Y., Olee, T., Olsen, N. J., Berry, C., Carson, D. A., and Chen, P. P. (1992). Population and family studies of three disease-related polymorphic genes in systemic lupus erythematosus. *J. Clin. Invest*. **95**, 1766–1772.
303. Nicklin, M. J. H., Weith, A., and Duff, G. W. (1994). A physical map of the region encompassing the human interleukin-1 alpha, interleukin-1 beta, and interleukin-1 receptor antagonist genes. *Genomics* **19**, 382–384.
304. Blakemore, A. I. F., Tarlow, J. K., Cork, M. J., Gordon, C., Emery, P., and Duff, G. W. (1994). Interleukin-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. *Arthritis Rheum*. **37**, 1380–1385.
305. Tjernstrom, F., Hellmer, G., Nived, O., Truedsson, L., and Sturfelt, G. (1999). Synergetic effect between interleukin-1 receptor antagonist allele (IL1RN*2) and MHC class II (DR17,DQ2) in determining susceptibility to systemic lupus erythematosus. *Lupus* **8**, 103–108.
306. Pullmann, R., Jr., Lukac, J., Skerenova, M., Rovensky, J., Hybenova, J., Melus, V., Celec, S., (1999). Pullmann, R., and Hyrdel, R. (1999). Cytotoxic T lymphocyte antigen 4 (CTLA-4) dimorphism in patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol*. **17**, 725–729.
307. Ahmed, S., Ihara, K., Kanemitsu, S., Nakashima, H., Otsuka, T., Tsuzaka, K., Takeuchi, T., and Hara, T. (2001). Association of CTLA-4 but not CD28 gene polymorphisms with systemic lupus erythematosus in the Japanese population. *Rheumatology (Oxford)* **40**, 662–667.
308. Heward, J., Gordon, C., Allahabadia, A., Barnett, A. H., Franklyn, J. A., and Gough, S. (1999). Che A-G polymorphism in exon 1 of the CTLA-4 gene is not associated with systemic lupus erythematosus. *Ann. Rheum. Dis*. **58**, 193–195.
309. Matsushita, M., Tsuchiya, N., Shiota, M., Komata, T., Matsuta, K., Zama, K., Oka, T., Juji, T., Yamane, A., and Tokunaga, K. (1999). Lack of a strong association of CTLA-4 exon 1 polymorphism with the susceptibility to rheumatoid arthritis and systemic lupus erythematosus in Japanese: An association study using a novel variation screening method. *Tissue Antigens* **54**, 578–584.
310. Matsushita, M., Tsuchiya, N., Oka, T., Yamane, A., and Tokunaga, K. (2000). New polymorphisms of human CD80 and CD86: Lack of association with rheumatoid arthritis and systemic lupus erythematosus. *Genes Immun*. **1**, 428–434.
311. Liu, M. F., Wang, C. R., Lin, L. C., and Wu, C. R. (2001). CTLA-4 gene polymorphism in promoter and exon-1 regions in Chinese patients with systemic lupus erythematosus. *Lupus* **10**, 647–649.
312. Lee, Y. H., Kim, Y. R., Ji, J. D., Sohn, J., and Song, G. G. (2001). Polymorphisms of the CTLA-4 exon 1 and promoter gene in systemic lupus erythematosus. *Lupus* **10**, 601–605.
313. Kanemitsu, S., Takabayashi, A., Sasaki, Y., Kuromaru, R., Ihara, K., Kaku, Y., Sakai, K., and Hara. (1999). Association of interleukin-4 receptor and interleukin-4 promoter gene polymorphisms with systemic lupus erythematosus. *Arthritis Rheum*. **42**, 1298–1300.
314. Stevens, A., Ray, D., Alansari, A., Hajeer, A., Thomson, W., Donn, R., Ollier, W. E., Worthington, J., and Davis, J. R. (2001). Characterization of a prolactin gene polymorphism and its associations with systemic lupus erythematosus. *Arthritis Rheum*. **44**, 2358–2366.
315. Kassi, E. N., Vlachoyiannopoulos, P. G., Moutsopoulos, H. M., Sekeris, C. E., and Moutsatsou, P. (2001). Molecular analysis of estrogen receptor alpha and beta in lupus patients. *Eur. J. Clin. Invest*. **31**, 86–93.
316. Chang, M., Lubahn, D. B., and Hoffman, R. W. (2001). DNA microsatellite markers for estrogen receptor-beta are not associated with systemic lupus erythematosus. *J. Rheumatol*. **28**, 924–925.
317. Liu, Z. H., Cheng, Z. H., Gong, R. J., Liu, H., Liu, D., and Li, L. S. (2002). Sex differences in estrogen receptor gene polymorphism and its association with lupus nephritis in Chinese. *Nephron* **90**, 174–180.
318. Tanaka, Y., Nakashima, H., Hisano, C., Kohsaka, T., Nemoto, Y., Niiro, H., Otsuka, T., Otsuka, T., Imamura, T., and Niho, Y. (1999). Association of the interferon-gamma receptor variant (Val14Met) with systemic lupus erythematosus. *Immunogenetics* **49**, 266–271.
319. Nakashima, H., Inoue, H., Akahoshi, M., Tanaka, Y., Yamaoka, K., Ogami, E., Nagano, S., Arinobu, Y., Niiro,

H., Otsuka, T., and Niho, Y. (1999). The combination of polymorphisms within interferon-gamma receptor 1 and receptor 2 associated with the risk of systemic lupus erythematosus. *FEBS Lett*. **453**, 187–190.
320. Lee, J. Y., Goldman, D., Piliero, L. M., Petri, M., and Sullivan, K. E. (2001). Interferon-gamma polymorphisms in systemic lupus erythematosus. *Genes Immun*. **2**, 254–257.
321. Linker-Israeli, M., Wallace, D. J., Prehn, J. L., Nand, R., Li, L., and Klinenberg, J. R. (1996). A greater varibility in the 3′ flanking region of the IL-6 gene in patients with systemic lupus erythematosus (SLE). *Autoimmunity* **23**, 199–209.
322. Linker-Israeli, M., Wallace, D. J., Prehn, J., Michael, D., Honda, M., Taylor, K. D., Paul-Labrador, M., Fischel-Ghodsian, N., Fraser, P. A., and Klinenberg, J. R. (1999). Association of IL-6 gene alleles with systemic lupus erythematosus (SLE) and with elevated IL-6 expression. *Genes Immun*. **1**, 45–52.
323. Schotte, H., Schluter, B., Rust, S., Assmann, G., Domschke, W., and Gaubitz, M. (2001). Interleukin-6 promoter polymorphism (–174 G/C) in Caucasian German patients with systemic lupus erythematosus. *Rheumatology (Oxford)* **40**, 393–400.
324. Wang, A. Y., Poon, P., Lai, F. M., Yu, L., Choi, P. C., Lui, S. F., and Li, P. K. (2001). Plasminogen activator inhibitor-1 gene polymorphism 4G/4G genotype and lupus nephritis in Chinese patients. *Kidney Int*. **59**, 1520–1528.
325. Wong, D. W., Bentwich, Z., Martinez-Tarquino, C., Seidman, J. G., Duby, A. D., Quertermous, T., and Schur, P. H. (1988). Nonlinkage of the T-cell receptor alpha, beta, and gamma genes to systemic lupus erythematosus in multiplex families. *Arthritis Rheum*. **31**, 1371–1376.
326. Dunckley, H., Gatenby, P. A., and Serjeantson, S. W. (1998). T-cell receptor and HLA-class II RFLP's in systemic lupus erythematosus. *Immunogenetics* **27**, 392–395.
327. Tebib, J. G., Alcocer-Verela, J., Alarcon-Segovia, D., and Schur, P. H. (1990). Association between a T-cell receptor restriction fragment length polymorphism and systemic lupus erythematosus. *J. Clin. Invest*. **86**, 1961–1967.
328. Frank, M. B., McArthur, R., Harley, J. B., and Fujisaku, A. (1990). Anti-Ro (SSA) autoantibodies are associated with T-cell receptor beta genes in systemic lupus erythematosus patients. *J. Clin. Invest*. **85**, 33–39.
329. Lu, J. H., Thiel, S., Wiedmann, H., Timpl, R., and Reid, K. B. (1990). Binding of the pentamer/hexamer forms of mannan-binding protein to zymosan activates the proenzyme C1r2C1s2 complex of the classical pathway of complement without involvement of C1q. *J. Immunol*. **144**, 2287–2294.
330. Sumiya, M., and Super, M. (1991). Molecular basis of opsonin defect in immunodeficient children. *Lancet* **337**, 1569–1570.
331. Lipscombe, R. J., Sumiya, M., and Hill, A. V. S. (1992). High frequencies in African and non-African populations of independent mutations in the mannose binding protein gene. *Hum. Mol. Genet*. **1**, 709–715.
332. Davies, E. J., Snowden, N., Hillarby, M. C., Carthy, D., Grennan, D. M., Thompson, W., and Ollier, W. E. R. (1995). Mannose-binding protein gene polymorphism in systemic lupus erythematosus. *Arthritis Rheum*. **38**, 110–114.
333. Davies, E. J., Teh, L-S., Ordi-Ros, J., Snowden, N., Hillarby, M. C., Hajeer, A., Donn, R., Perez-Pemen, P., Vilardell-Tarres, M., and Ollier, W. E. R. (1997). A dysfunctional allele of the mannose binding protein gene associates with systemic lupus erythematosus in a Spanish population. *J. Rheumatol*. **24**, 485–488.
334. Sullivan, K. E., Wooten, C., Goldman, D., and Petri, M. (1996). Mannose-binding protein genetic polymorphisms in black patients with systemic lupus erythematosus. *Arthritis Rheum*. **39**, 2046–2051.
335. Ip, W. K., Chan, S. Y., Lau, C. S., and Lau, Y. L. (1998). Association of systemic lupus erythematosus with promoter polymorphisms of the mannose-binding lectin gene. *Arthritis Rheum*. **41**, 1663–1668.
336. Garred, P., Madsen, H. O., Halberg, P., Petersen, J., Kronborg, G., Svejgaard, A., Andersen, V., and Jacobsen, S. (1999). Mannose-binding lectin polymorphisms and susceptibility to infection in systemic lupus erythematosus. *Arthritis Rheum*. **42**, 2145–2152.
337. Garred, P., Voss, A., Madsen, H. O., and Junker, P. (2001). Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. *Genes Immun*. **2**, 442–450.
338. Tsutsumi, A., Sasaki, K., Wakamiya, N., Ichikawa, K., Atsumi, T., Ohtani, K., Suzuki, Y., Koike, T., and Sumida, T. (2001). Mannose-binding lectin gene: Polymorphisms in Japanese patients with systemic lupus erythematosus, rheumatoid arthritis and Sjogren's syndrome. *Genes Immun*. **2**, 99–104.
339. Horiuchi, T., Tsukamoto, H., Morita, C., Sawabe, T., Harashima, S., Nakashima, H., Miyahara, H., Hashimura, C., and Kondo, M. (2000). Mannose binding lectin (MBL) gene mutation is not a risk factor for systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Japanese. *Genes Immun*. **1**, 464–466.
340. Reidenberg, M. M. (1983). Aromatic amines and the pathogenesis of lupus erythematosus. *Am. J. Med*. **75**, 1037–1042.
341. Morris, R. J., Freed, C. R., and Kohler, P. F. (1979). Drug acetylation phenotype unrelated to development of spontaneous systemic lupus erythematosus. *Arthritis Rheum*. **22**, 777–780.
342. Reidenberg, M. M., Drayer, D. E., Lorenzo, B., Strom, B. L., West, S. L., Snyder, E. S., Freundlich, B., and Stolley, P. D. (1993). Acetylation phenotypes and environmental chemical exposure of people with idiopathic systemic lupus erythematosus. *Arthritis Rheum*. **36**, 971–973.
343. von Schmiedeberg, S., Fritsche, E., Ronnau, A. C., Specker, C., Golka, K., Richter-Hintz, D., Schuppe, H. C., Lehmann, P., Ruzicka, T., Esser, C., Abel, J., Gleichmann, E. (1999). Polymorphisms of the xenobiotic-metabolizing enzymes CYP1A1 and NAT-2 in systemic sclerosis and lupus erythematosus. *Adv. Exp. Med. Biol*. **455**, 147–152.

344. Sullivan, K. E., Mannery, T., and Petri, M. A. (1998). The polymorphic CYP17 allele is not found with increased frequency in systemic lupus erythematosus. *Arthritis Rheum*. **41**, 940–941.
345. Kortunay, S., Bozkurt, A., Bathum, L., Basci, N. E., Calguneri, M., Brosen, K., and Kayaalp, S. O. (1999). CYP2C19 genotype does not represent a genetic predisposition in idiopathic systemic lupus erythematosus. *Ann. Rheum. Dis*. **58**, 182–185.
346. Sullivan, K. E., Piliero, L. M., Dharia, T., Goldman, D., and Petri, M. A. (2002). 3′ polymorphisms of ETS1 are associated with different clinical phenotypes in SLE. *Hum. Mutat*. **16**, 49–53.
347. Ozaki, Y., Nomura, S., Nagahama, M., Yoshimura, C., Kagawa, H., and Fukuhara, S. (2000). Vitamin-D receptor genotype and renal disorder in Japanese patients with systemic lupus erythematosus. *Nephron* **85**, 86–91.
348. Chitrabamrung, S., Rubin, R. L., and Tan, E. M. (1981). Serum deoxyribonuclease I and clinical activity in systemic lupus erythematosus. *Rheumatol. Int*. **1**, 55–60.
349. Yasutomo, K., Horiuchi, T., Kagami, S., Tsukamoto, H., Hashimura, C., Urushihara, M., and Kuroda, Y. (2001). Mutation of DNASE1 in people with systemic lupus erythematosus. *Nature Genet*. **28**, 313–314.
350. Tew, M. B., Johnson, R. W., Reveille, J. D., and Tan, F. K. (2001). A molecular analysis of the low serum deoxyribonuclease activity in lupus patients. *Arthritis Rheum*. **44**, 2446–2447.
351. Aguilar, F., Gonzalez-Escribano, M. F., Sanchez-Roman, J., and Nunez-Roldan, A. (2001). MCP-1 promoter polymorphism in Spanish patients with systemic lupus erythematosus. *Tissue Antigens* **58**, 335–338.
352. Sato, H., Akai, Y., Iwano, M., Kurumatani, N., Kurioka, H., Kubo, A., Yamaguchi, T., Fujimoto, T., and Dohi, K. (1998). Association of an insertion polymorphism of angiotensin-converting enzyme gene with the activity of systemic lupus erythematosus. *Lupus* **7**, 530–534.
353. Tassiulas, I. O., Aksentijevich, I., Salmon, J. E., Kim, Y., Yarboro, C. H., Vaughan, E. M., Davis, J. C., Scott, D. L., Austin, H. A., Klippel, J. H., Balow, J. E., Gourley, M. F., and Boumpas, D. T. (1998). Angiotensin I converting enzyme gene polymorphisms in systemic lupus erythematosus: Decreased prevalence of DD genotype in African American patients. *Clin. Nephrol*. **50**, 8–13.
354. Pullmann, R., Jr., Lukac, J., Skerenova, M., Rovensky, J., Hybenova, J., Melus, V., Celec, S., Pullmann, R., and Hyrdel, R. (1999). Association between systemic lupus erythematosus and insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene. *Clin. Exp. Rheumatol*. **17**, 593–596.
355. Prkacin, I., Novak, B., Sertic, J., and Mrzljak, A. (2001). Angiotensin-converting enzyme gene polymorphism in patients with systemic lupus. *Acta. Med. Croatica*. **55**, 73–76.
356. Molad, Y., Gal, E., Magal, N., Sulkes, J., Mukamel, M., Weinberger, A., Lalazari, S., and Shohat, M. (2000). Renal outcome and vascular morbidity in systemic lupus erythematosus (SLE): Lack of association with the angiotensin-converting enzyme gene polymorphism. *Semin. Arthritis Rheum*. **30**, 132–137.
357. Kaufman, K. M., Kelly, J., Gray-McGuire, C., Asundi, N., Yu, H., Reid, J., Baird, T., Hutchings, D., Bruner, G., Scofield, R. H., Moser, K., and Harley, J. B. (2001). Linkage analysis of angiotensin-converting enzyme (ACE) insertion/deletion polymorphism and systemic lupus erythematosus. *Mol. Cell. Endocrinol*. **177**, 81–85.
358. Kamboh, M. I., Manzi, S., Mehdi, H., Fitzgerald, S., Sanghera, D. K., Kuller, L. H., and Atson, C. E. (1999). Genetic variation in apolipoprotein H (beta2-glycoprotein I) affects the occurrence of antiphospholipid antibodies and apolipoprotein H concentrations in systemic lupus erythematosus. *Lupus* **8**, 742–750.
359. Gushiken, F. C., Arnett, F. C., Ahn, C., and Thiagarajan, P. (1999). Polymorphism of beta2-glycoprotein I at codons 306 and 316 in patients with systemic lupus erythematosus and antiphospholipid syndrome. *Arthritis Rheum*. **42**, 1189–1193.
360. Graninger, W. B. (1992). Transcriptional overexpression of the proto-oncogene bcl-2 in patients with systemic lupus erythematosus. *Wien. Klin. Wochenschr*. **104**, 205–207.
361. Miret, C., Font, J., Molina, R., Garcia-Carrasco, M., Filella, X., Ramos, M., Cervera, R., Ballesta, A., and Ingelmo, M. (1999). Bcl-2 oncogene (B cell lymphoma/leukemia-2) levels correlate with systemic lupus erythematosus disease activity. *Anticancer Res*. **19**, 3073–3076.
362. Johansson, C., Castillejo-Lopez, C., Johanneson, B., Svenungsson, E., Gunnarsson, I., Frostegard, J., Sturfelt, G., Truedsson, L., Lofstrom, B., Alcocer-Varela, J., Lundberg, I., Gyllensten, U. B., Alarcon-Segovia, D., and Alarcon-Riquelme, M. E. (2000). Association analysis with microsatellite and SNP markers does not support the involvement of BCL-2 in systemic lupus erythematosus in Mexican and Swedish patients and their families. *Genes Immun*. **1**, 380–385.

5

GENETIC SUSCEPTIBILITY AND CLASS III COMPLEMENT GENES

John P. Atkinson
Peter M. Schneider

INTRODUCTION

The great interest in this genetic region of chromosome 6 in humans relates to the now several decades old observation that individuals with complete deficiency of C4 or C2 come to medical attention with the clinical syndrome of systemic lupus erythematosus (SLE). Thus, these two proteins along with their friends (C1q, C1r, C1s) in the early part of the classical pathway are the only known single protein deficiency states that commonly (~90% in case of C1q and C4 deficiency) develop SLE. The problem that has been so elusive to solve is how these deficiency states in turn lead to SLE. One focus in this chapter will be on this question. After providing background data on the structure and function of the class III genes and their protein products, we will first review the older data relative to the long-appreciated role of the complement system in immune complex (IC) handling and then move into the more recent results on the role of the complement system in the immune response and in the processing of cellular debris. The new tools of this field, namely knockout mice, have been particularly helpful in more elegantly and firmly establishing an important role for the complement system in innate and adaptive immunity as well as in preventing autoimmunity.

The class III, or complement (C), region of the human major histocompatibility complex (MHC) is located between class I (B, C, A) and class II (DP, DQ, DR) regions on the short arm of chromosome 6 (Fig. 1). Complement genes in this 130-kb cluster include C4A, C4B, factor B, and C2. C4A and C4B are duplicated (>99% homologous) genes. The C2 and factor B genes encode serine proteases, which are approximately 30% homologous, indicating a more ancient duplication. C4 and C2 are required for the classical (CP) and lectin (LP) pathways, whereas factor B is necessary for alternative pathway (AP) activation (Fig. 2). Upon CP activation by IC, C4 is cleaved by the C1s subcomponent of the first component of complement (C1). For the LP, mannan (or mannose)-associated serine proteases (MASPs), which are homologous to C4r and C1s, cleave C4. The larger proteolytic fragment of C4, C4b, has the transient capacity to attach covalently to the target antigen (Ag) and to the activating antibody (Ab) (Fig. 3). Bound C4b has two roles: an opsonic one in which it is a ligand for complement receptors and an enzymatic one in which it serves as the noncatalytic domain of the CP C3 and C5 convertases. C2 and factor B are serine protease precursors and play identical roles in their respective activation pathways. After proteolytic cleavage by C1s or MASPs, C2a combines with C4b to form the classical pathway C3 convertase. Factor B birds to C3b and then it is cleaved by factor D, forming the alternative pathway C3 convertase. C2a and factor Bb are the catalytic (serine protease) domains of both C3 and C5 convertases.

As noted, attention was drawn to this gene cluster because of its location within the MHC and because inherited deficiencies of C2 (C2def) and C4 (C4def) were associated with SLE (see Chapter 6). A deficiency of any one of the early components of the classical

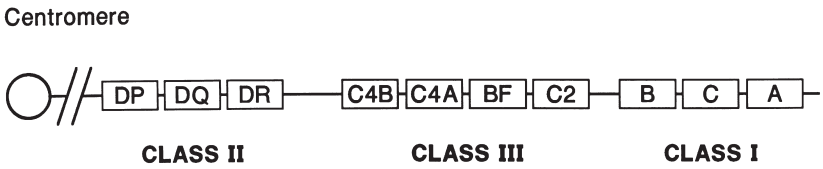


FIGURE 1 The human major histocompatibility complex on the short arm of chromosome 6. The class III or complement gene cluster, which covers a distance of 130 kb, includes complement activation components C4 (two highly homologous genes termed C4A and C4B), factor B (also noted as BF), and C2. From Liszewski *et al*. [152], with permission.

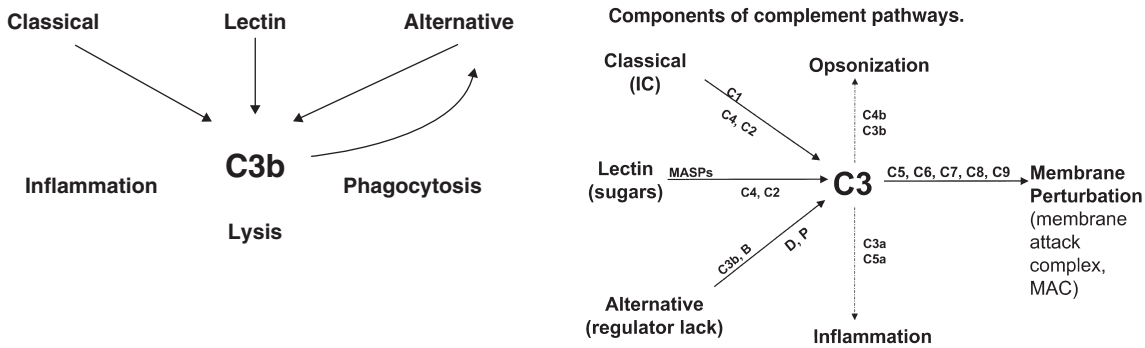


FIGURE 2 The three pathways of complement activation. Deposition of clusters of C3b on a target is the primary goal. The alternative pathway also serves as a feedback loop such that C3b deposition can be amplified.

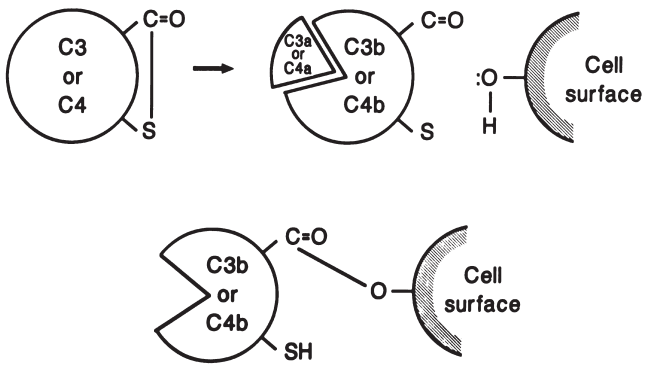


FIGURE 3 The thioester bond of C3 and C4. Activation of the thioester bond in C4 and C3 occurs in association with their proteolytic cleavage and liberation of the C4a and C3a fragments. Condensation with a hydroxyl group (formation of an ester linkage) is shown. Amino groups on the target surface can also be bound (formation of an amide linkage). From Hughes-Jones [153], with permission.

pathway is the only single protein deficiency state currently known that causes SLE in humans. Initially, this striking disease association was attributed by many observers to the class I or class II markers inherited with the C deficiency. While there may be an independent contribution to disease susceptibility from genetic markers that are in linkage disequilibrium, such as HLA class II genes, C deficiency itself is key, as summarized below.

1. C4def and C2def are inherited on distinct genetic (MHC) backgrounds. Moreover, C4def is inherited on different MHC backgrounds in, for example, Asians compared to Caucasians and yet the SLE association still holds. Therefore, it is not the company it keeps (i.e., other MHC-linked genes) that produce the disease association but the C deficiency itself.
2. C1qdef and C1r/C1sdef also predispose to SLE [1, 2]. The associations of SLE with C1q (>90% deficient individuals have SLE) and C1r/C1s deficiency are even a little stronger than that for C4def and SLE [3]. These subunits of C1 are, of course, immediately upstream of C4 and C2 in the classical pathway, strongly implying that it is activation of the cascade itself that is critical. Further and in support of the first point, the structural genes for these C1 subunits are at separate genetic loci, outside the MHC [1–7].
3. A partial deficiency of C4, especially of C4A, predisposes an individual to SLE in some populations [3, 5–7]. In the Caucasian SLE population, C4Adef is observed about two-thirds of the time on the DR3-B8 haplotype while the remaining one-third are associated

with a *diverse* array of haplotypes. Moreover, in Asian populations, C4Adef appears to be a risk factor for SLE as well, yet the DR3-B8 haplotype is unknown. Thus, a problem in interpretation related to linkage disequilibrium (in other words, which genes are the disease-promoting ones) has been further resolved by studies in ethnically diverse populations [5–7].

4. The major biologic function of these early components of the classical C pathway, C1, C4, and C2, and the pivotal C3 component *is to bind to antigens and thereby facilitate antigen processing (the immune response) and immune complex (IC) formation and handling* [8–14]. SLE is the prototypic spontaneously occurring human disease in which many of the clinical manifestations are mediated by the deposition of the IC in undesirable locations (e.g., kidney). Consequently, on the one hand, patients have an illness, SLE, in which the IC is mediating much of the tissue damage and, on the other hand, they possess an inherited deficiency of proteins, one of whose functions is to process IC.

5. Acquired C4 and/or C2def, particularly in the setting of hereditary angioedema, have been reported in association with SLE, again suggesting that it is the protein deficiency per se that is the critical factor (see Chapter 6).

Taken together, these data establish deficiency of an early component of the CP as a predisposition to the development of SLE. Next, we will provide some caveats to this statement and deal with several apparent discrepancies.

1. Since the major function of the early components of the CP is to activate C3 so its fragments can deposit on IC, the expectation is that C3 deficiency would be an even greater predisposing deficiency state for SLE. However, most patients (numbers are small, however) with C3 deficiency present with recurrent pyogenic infections and occasionally glomerulonephritis but certainly not with the SLE-like picture as one sees with C1q, C4, or C2 deficiency. Several explanations are possible. One is that C4b (a structural and functional homolog of C3b) is sufficient such that autoimmunity is largely avoided. Along this line of reasoning, Michael Carroll and colleagues have proposed a theory that C4b binding to immunocompetent autoreactive bone marrow B cells inhibit their activation (a negative feedback to prevent autoimmunity) [15]. While this is an attractive hypothesis [3], there is as yet no clear idea as to how this takes place and C1q-deficient mice did not demonstrate a defect in B-cell tolerance [16].

2. Another theory, championed by Mark Walport and colleagues, is the so-called "garbage hypothesis" [3, 17]. It centers on a role for the CP in the clearance of cell debris, including apoptotic cells. If intracellular debris is not cleaned up efficiently (analogous to IC containing foreign antigens), then modifications could occur to DNA/protein, RNA/protein, and other cellular constituents such that they now become immunogenic. With this argument, one, however, would also expect C3 deficiency to be a cause of SLE. Perhaps C1q and C4b binding to the "garbage" are sufficient to prevent autoimmunity.

3. Two other issues that remain problematic are the striking female predominance and the antibody specificity for nuclear material. The "garbage hypothesis" does provide a potential explanation for the antibody specificity question (see later) [3, 17] whereas the gender issue continues to defy a plausible explanation.

4. A point also emphasized by the Walport and Botto group is that C1qdef has a higher frequency of SLE (90%) than C4def (80 to 90%) or C2def (10 to 30%) [3, 18]. This hierarchical relationship suggests that the binding of C1q to apoptotic material is most critical in preventing SLE. These data also imply an interaction with C1q receptors, a field that is in flux as to the identification and function of such proteins. Also, C1r/C1s def (8/14 cases described to date have SLE) [3, 17] and SLE in C4def and C2def take place in the setting of "normal C1q."

5. Therefore, a more plausible hypothesis would seem to be that CP activation through at least C4 is the most critical issue in preventing SLE. The initial approach therefore that many of us took to unravel how the C deficiency led to SLE was to analyze the genetics, structure, and function of C4 and C2 and the receptors and regulators that interact with these two components. The more recent approach has been to utilize gene targeting (knockouts) of C1q, C4, or C2 in the mouse. Both types of studies have contributed much to our understanding of complement activation and its association with SLE. We will first summarize the earlier studies and then the mouse model systems.

FOURTH COMPONENT OF COMPLEMENT (C4)

Structure

C4 is an abundant (15 to 45 mg/dl) 200,000-Da, three-chain, disulfide-linked, plasma glycoprotein (Fig. 4) [19–21]. It is synthesized by the liver as a single chain precursor (pro-C4), which undergoes proteolytic cleavages at two sites to give rise to the three chain protein: α, 95,000; β, 70,000; and γ, 35,000. The secreted protein possesses three N-linked complex sugar residues on the α chain and one N-linked high mannose type on the β chain [22]. The COOH terminus of the α chain

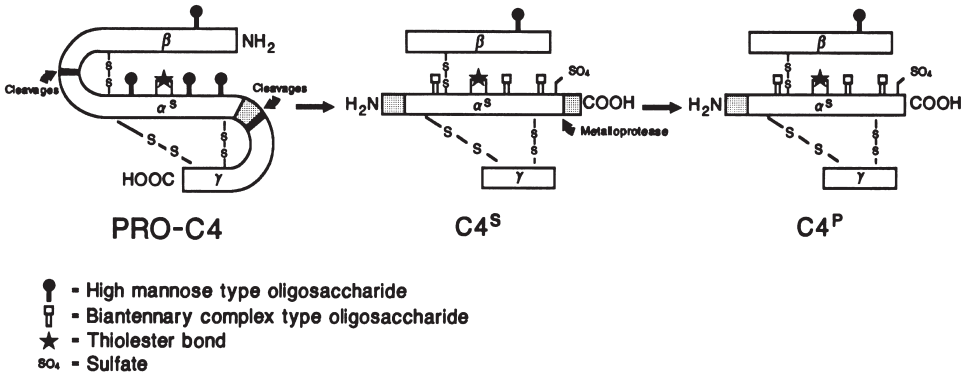


FIGURE 4 Schematic diagram of the structural features of human C4. Pro-C4 is the intracellular, single chain precursor of C4. Following a series of posttranslational modifications that include sulfation, glycosylation, and proteolytic cleavage, a three chain protein is secreted ($C4^s$). The major form of C4 in plasma, however, is $C4^p$. This form differs from $C4^s$ by removal of a 22 amino acid peptide from the carboxyl terminus of the α chain. The shaded area at the NH_2 terminus of the α chain represents the C4a fragment. Modified from Chan and Atkinson [22], with permission.

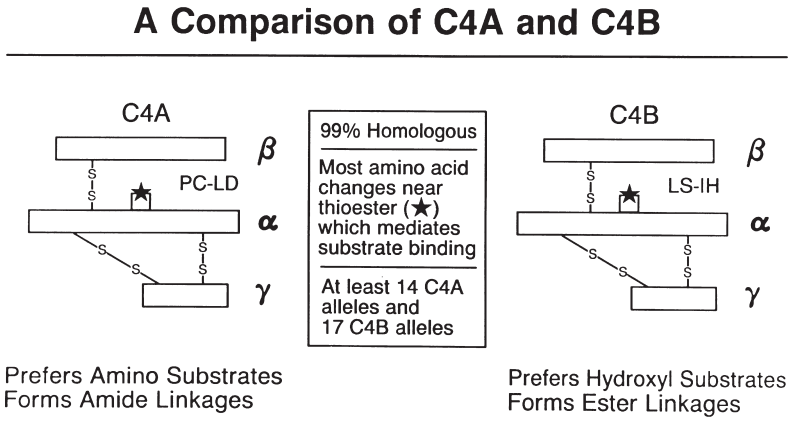


FIGURE 5 Although highly homologous, C4A and C4B differ in their binding specificities for substrates. From Liszewski and Atkinson [154], with permission.

undergoes two remarkable posttranslational modifications. The first is sulfation of two or three tyrosine residues [23], a modification that increases severalfold the efficiency of its cleavage by C1s [24]. The second modification, following secretion into plasma, involves removal of a 22 amino acid peptide from the carboxyl terminus [25, 26]. This cleavage exposes a highly charged, acidic stretch of amino acids, including the sulfation site, that likely is a recognition motif for C1s and MASPs. Thus, truncation and sulfation are designed to improve the efficiency of C4 cleavage by C1s and presumably by the MASPs as well.

Function

C4 is the first component of the classical C pathway to become directly bound to antigen in IC (C1 is bound through C1q to the Fc of Ab). Upon cleavage of the amino terminus of the α chain to liberate C4a by C1s or MASPs, C4b undergoes a molecular rearrangement. In so doing, a thioester bond in the central portion of the α chain is broken (Figs. 2 and 5). This thioester is formed over a four amino acid peptide between a γ-carboxyl group of glutamic acid and a sulfhydryl of cysteine [27, 28]. A reactive carbonyl species is formed that is capable of interacting with a hydroxyl group to form an ester linkage or with an amino group to form an amide bond. The result, in either case, is the covalent attachment of multiple, clustered C4bs to the target.

As outlined earlier, C4b serves two roles in the classical C pathway. First, C4b is a ligand (opsonic role) for complement receptor type one (CR1; CD35; C3b/C4b or immune adherence receptor) of peripheral blood cells (erythrocytes/B lymphocytes/monocytes/granulocytes) and B lymphocytes and follicular-dendritic cells in immune organs [29–31]. Second, C4b

is the noncatalytic domain of the classical pathway C3 and C5 convertases (enzymatic role) (Fig. 2). C4b, bound covalently to the target antigen, captures the C2a cleavage fragment of C2. This bimolecular enzyme complex, C4bC2a, is the classical pathway C3 convertase. C2a is a serine protease with very limited substrate specificity; i.e., it cleaves C3 but only if C2a is associated with C4b. If a C3b becomes bound covalently to an acceptor site on C4b, the enzyme complex changes its specificity from C3 to C5 (C4b2a3b is the classical pathway C5 convertase). Thus, the serine protease domain of C2 cleaves two proteins, C3 and C5.

C4A and C4B

Plasma C4 is composed of two distinct but highly homologous (>99%) isotypes, C4A and C4B (Fig. 5). C4A and C4B are each composed of 1725 amino acids [19, 32, 33]. Eight amino acid differences in positions near the thioester are responsible for the electrophoretic (fast vs slow), serologic (Chido and Rodgers blood group antigens), and hemolytic (C4B is several-fold more active than C4A in a whole complement assay) differences [34, 35]. In addition to their isotypic variations, C4A and C4B each demonstrate additional polymorphisms, defined initially by electrophoresis, giving rise to at least 14 C4A and 17 C4B alleles [36, 37]. Those alleles that have been sequenced result from single amino acid differences in the region containing the thioester (also known as the C4d fragment) [38], but additional amino acid exchanges may also exist outside this region, as demonstrated by the hemolytically inactive C4A6 allele [39].

The C4A gene is 22.3 kb long and lies about 10 kb from a similarly sized C4B gene (C4B long). A smaller 16-kb C4B gene (C4B short), lacking a 6.3-kb intron near the 5′ end of the gene, occurs in approximately one-third of Caucasians and in a higher frequency of African-Americans [5, 19]. This gene size variation is due to an ancient insertion of a complete endogenous retrovirus with strong homology to the HERV-K family [40, 41]. As this retroviral insertion is not present on all MHC haplotypes, it may have functional implications related to disease associations of certain MHC haplotypes. It has been shown that the insertion may regulate the expression of exogenous retroviral sequences, indicating the possibility of protecting the host by antisense inhibition [42].

Deletions and duplications of C4 and the nearby 21-hydroxylase genes are common [4, 5, 19, 33, 38, 43–50]. The most intensively analyzed deletion in this region occurs on the DR3-B8 haplotype (Fig. 6) and results in a loss of most of the C4A and all of its closely linked cytochrome P450 21-hydroxylase steroidogenesis pseudogene (CYP21A). Duplications and deletions in this area occur, probably as a result of unequal crossing over, which is promoted by the high homology of C4A and C4B and other members of the associated gene cluster (Tables 1 and 2). A *de novo* C4B-CYP21B gene deletion has been investigated, providing clear evidence for unequal crossing over as the basic mechanism for modular variation [49]. On a substantial number of haplotypes there are various combinations of one, two, or

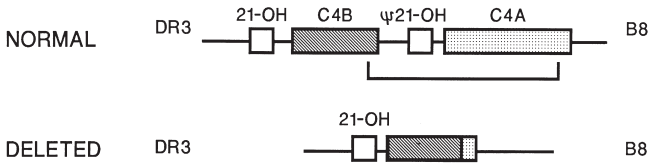


FIGURE 6 On the DR3-B8-extended haplotype, a deficiency of C4A is caused by a 30-kb deletion. (Top) What *should* be present; however, the DR3-B8 haplotype is always associated with this deletion. Most of the gene for C4A and a portion of C4B are deleted along with an intervening pseudogene, 21-hydroxylase A (ψ21-OH). Approximate limits of the deletion are indicated, although the precise end points remain to be determined. From Kemp *et al*. [77], with permission.

TABLE 1 Extended HLA Haplotypes with Null Alleles[a] of C4A and C4B

C4A-CYP21A gene deletion
HLA-B8, Cw7, DR3, C4A*Q0,[b] C4B1
HLA-B44, Cw5, DR3, C4A*Q0, C4B1
HLA-A2, B44, Cw5, DR4, C4A*Q0, C4B1
HLA-A2, B51, Cw2, DR1, C4A*Q0, C4B1

C4A null alleles (without gene deletion)
HLA-A2, B51, Cw2, DR1, C4A*Q0, C4B1
HLA-B60, Cw3, DR6, C4A*Q0,[c] C4B1(2)
HLA-A1, B35, Cw4, CA*Q0, C4B5

CYP21A-C4B gene deletion
HLA-A2, B7, DR2, C4A3, C4B*Q0
HLA-B18, DR3(4), C4A3, C4B*Q0
HLA-B35, Cw4, DR1, C4A3, C4B*Q0
HLA-A3, B60, Cw3, DR1, C4A3, C4B*Q0

C4B-CYP21B gene deletion
HLA-B47, Cw6, DR7, C4A91, C4B*Q0
HLA-A3, B40, Cw3, DR4, C4A3, C4B*Q0
HLA-A1, B8, DR1, C4A4, C4B*Q0
HLA-A24, B44, C4A4, C4B*Q0

C4B null alleles (without gene deletion)[d]
HLA-B44, DR4(5), C4A3, C4B*Q0
HLA-B35, DR1, C4A3, C4B*Q0
HLA-B35, DR1(4), C4A3,2, C4B*Q0

[a] No protein produced by the allele.
[b] Quantity zero or total lack of a protein product.
[c] C4A nonexpression is due to a 2-bp insertion in exon 29 [43].
[d] C4B*Q0 due to gene conversion from C4B to C4A isotype [45].

TABLE 2 Common Extended MHC Haplotypes in Caucasians[a]

Extended haplotype	Frequency in Caucasians (%)	Heterozygous persons in population (%)	Homozygous persons in population (%)
HLA-(A1), B8, SC01, DR3	9.2	16.7	0.85
HLA-(A3), B7, SC31, DR2	6.8	12.7	0.46
HLA-(A29), B44, FC31, DR7	3.0	5.8	0.09
HLA-(A2), B44, SC30, DR4	2.9	5.7	0.09
HLA-(A1), Bw57, SC61, DR7	2.0	3.9	0.04
HLA-(A33), B14, SC21, DR1	1.4	2.7	0.02
HLA-(A26), B38, SC21, DR4	0.9	1.8	0.01
HLA-(A2), B15, SC33, DR4	0.9	1.8	0.01
HLA-(A3), B35, FC3,20, DR1	0.9	1.8	0.01
Total	28.1	53.0	1.6

[a] In addition to showing HLA-B and -DR results, the haplotypes listed include the complement alleles of factor B, C2, C4A, and C4B in that arbitrary order. Thus, the abbreviation SC01 (= complotype) stands for the S allele of factor B, the C allele of C2, an absence of C4A (= Q0), and the C4B allele 1. The HLA-A allele associated most frequently with each haplotype is shown in parentheses. Modified from Kruskall *et al*. [155].

three C4A and C4B genes [46, 47, 50, 51]. C4A and C4B null alleles also result from intact but noncoding C4A or C4B, as has been shown for the HLA-B60-linked C4A null allele. This is caused by a 2-bp insertion in exon 29 leading to a frame shift mutation and a premature stop codon in exon 30 [52]. In addition to this more commonly observed deficient allele, a few cases with other mutations have been described as well [53–55]. Gene conversion, a change in a C4B allele to that of a C4A allele, probably has occurred in several noncoding C4B null alleles, providing another explanation for C4A null genes [45]. Of the extended HLA haplotypes bearing the C4 null gene (Table 2), the DR3-B8 haplotype, carrying the C4A deletion, is most common in Caucasians.

A molecular genetic and immunochemical analysis of C4A and C4B genes/proteins was carried out in 150 normal Caucasians [50]. Results indicate substantial variation in this locus with approximately two-thirds of the population carrying C4A and C4B genes on both chromosomes. This means that approximately one-third of the population have a partial deficiency of C4A or C4B (about 15% of each). A homozygous deficiency of C4A or C4B was found in 1/150 and 2/150 individuals, respectively. The interested reader should review the paper from the Yu laboratory, which contains a wealth of genetic information relative to the C4A/C4B locus and describes the methods required to rigorously assess C4def [50].

A completely C4def individual (C4def, double homozygote) possesses null alleles at all four loci (two C4A and two C4B) and therefore produces no C4 (Table 3). This is a rare situation with approximately 30 individuals reported in the literature (80 to 90%) with SLE [3, 18, 56, 57]. More commonly, individuals are homozygous for null alleles at *either* the C4A *or* the C4B locus, often with reduced serum C4 levels. Heterozygous C4A- or C4Bdef individuals have one null allele at either of the two loci, with serum C4 levels usually in the lower end of the normal range. Other combinations, such as homozygous C4Adef and heterozygous C4Bdef, have been described [18, 56].

C4A binds more avidly to amino groups than does C4B (Fig. 5) [58, 59]. As a result, C4A is more efficient at handling certain types of immune complexes [9, 10, 12–14, 60]. In contrast, C4B binds more efficiently to hydroxyl groups. Its greater hemolytic activity in the total complement (CH_{50} or THC) assay relates to the fact that sheep erythrocyte, the target antigen in this lysis assay, is a hydroxyl-rich substrate. Differences in affinities of C4A and C4B for substrates result from amino acid changes in the vicinity of the thioester bond [19, 33, 35, 61, 62]. This arrangement likely provides a survival advantage, allowing for a wider range of substrates to be bound efficiently by C4. There is also a variation in the ability of C4A and C4B to serve as a ligand for the C3b/C4b receptor [63], which provides a second explanation for differences in IC processing between C4A and C4B-coated antigens.

Serum C4 Concentration

The total C4 concentration results from expression of the protein product of two C4A genes and two C4B genes. Individuals deficient in one or more genes are common and although they do have reduced total C4

TABLE 3 Classification of C4 Deficiency

Genetic background	Phenotype		Serum C4[a]	THC[b]
Normal	C4A C4A	C4B C4B	Normal	Normal
Complete deficiency	C4A-Q0[c] C4A-Q0	C4B-Q0 C4B-Q0	None	Very low (0 to <10)
Partial deficiency				
C4A homozygous	C4A-Q0 C4A-Q0	C4B C4B	Variable, but usually low	Normal
C4B homozygous	C4A C4A	C4B-Q0 C4B-Q0	Variable, but usually low	Normal or slightly low
Heterozygous C4A or C4B deficiency				
C4A heterozygous	C4A-Q0 C4A	C4B C4B	Normal	Normal
C4B heterozygous	C4A C4A	C4B-Q0 C4B	Normal	Normal
Mixed C4A and C4B deficiency	C4AQ0 C4A	C4B-Q0 C4B	Variable, but usually low	Normal or slightly low

[a] Serum C4 levels, as determined by antigenic or functional assays.
[b] Total hemolytic complement refers to the standard whole complement assay that measures the ability of serum to lyse sensitized sheep erythrocytes. This assay requires the nine components of the classical pathway to be present to obtain a normal titer. Also termed CH_{50} assay.
[c] A null allele (i.e., quantity zero or no detectable protein).

levels, the concentration is usually still in the "normal" range. One group has provided data to suggest that the remaining genes are upregulated when one or more C4 null genes are present [64–66]. Also, C4, an acute-phase protein, may increase 1.5- to 3-fold during an inflammatory response [67]. In normal individuals and in SLE patients, there does not appear to be preferential activation (utilization) of one C4 type versus another [66]. This result is in keeping with the evidence that C1s cleaves C4A and C4B with equal efficiency [58, 59]. Thus, in an individual with two C4B genes and one C4A gene, two out of every three molecules of C4 activated would be derived from C4B. However, this does not necessarily mean that two out of every three C4 fragments bound to the IC will be derived from C4B in view of their different ability to react with substrates.

C2 AND FACTOR B

The C2 and factor B (BF) genes are only 421 base pairs apart and reside about 30 kb from the C4A locus (Fig. 1). The C2 and factor B genes encode for mature proteins of 734 and 939 amino acids, respectively [19, 68]. Factor B and C2 consist of three distinct domains: the serine protease, the von Willebrand type A and three of complement control protein repeating motifs (Fig. 7). In structure and function, C2 and factor B are homologous. Two common polymorphic protein variants of C2, as well as a null allele (C2Q0), have been described. Most individuals (>95%) are homozygous for the common (C2C) variant [69]. A restriction fragment length polymorphism (RFLP) subdivides this C2C allele [19]. There are two common factor B alleles, F and S, with gene frequencies of 0.27 and 0.71, respectively,

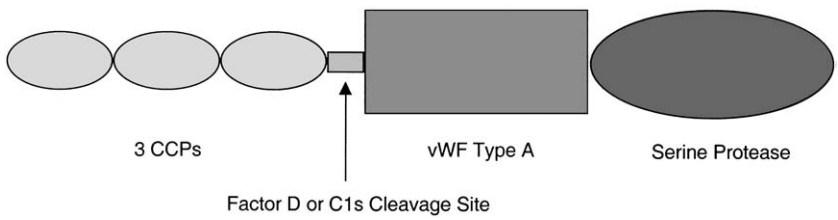


FIGURE 7 Domain structure of factor B and C2. CCP, complement control protein repeat (~60 amino acids each with two disulfide bonds); VWF type A; von Willibrand factor repeat domain.

two less common alleles, F1 and S07, and 14 rare variants [69]. However, there is no association between factor B deficiency and SLE. A patient with homozygous factor BF deficiency has not yet been observed, as this deficiency would most likely result in a severe impairment of the innate immune defense.

Clinical aspects of the association between C4 and C2def and SLE are reviewed in **Chapter 6**. The points to keep in mind for the discussion to follow are that C4def and C2def are inherited on distinct MHC haplotypes and they are part of an activation cascade. Animals and humans lacking an early component of the classical C pathway, up to and including C3, have a markedly abnormal (reduced) clearance of antibody-coated erythrocytes [70], which is representative of a general problem with the handling of IC or apoptotic cells or any other process requiring CP activation.

PARTIAL C4 DEFICIENCY AND SUSCEPTIBILITY TO SYSTEMIC LUPUS ERYTHEMATOSUS

In view of the association between complete complement deficiency and SLE, investigators naturally assessed this relationship in partial C deficiency. In the early 1980s, two groups, one in England [71] and one in Australia [72–74], first reported an association between partial C4 deficiency and susceptibility to SLE. This observation has been confirmed and extended (Table 4) [3, 18, 57].

Although total C4 deficiency is rare, one of every eight or nine Caucasian SLE patients has a partial deficiency in which C4A is lacking (Table 4). This produces a relative risk for SLE of more than eightfold for the C4A-deficient state [7, 71–90]. Fifty to 70% of these C4A-deficient individuals are on the DR3-B8 haplotype [46, 47, 77, 91]. This is the most common, extended haplotype in Caucasians (Table 2), possessing an approximately 30-kb deletion that encompasses both the C4A and the 3′ adjacent CYP21A genes [46, 47] (Fig. 6).

There is clear evidence for a significant increase of the C4A*Q0 allele in SLE patients. However, the association of homozygous C4Adef is not stronger than that for heterozygous deficiency among the patient population, indicating that a threshold might exist for the availability of functionally active C4A in serum to prevent autoimmunity. Also, a technical difficulty in some of the studies relates to evaluating the C4 phenotypes in SLE patients without studying their relatives. In cases where the C4 null allele cannot be confirmed by RFLP studies at the DNA level due to the absence of gene deletions (e.g., in Asian patients), heterozygous C4 deficiency is only reliably detected in family studies by following the segregation of null alleles [1, 3]. Table 4 presents data for several races on the gene frequency of C4A and C4B null alleles, as well as significant associations with HLA class II alleles of DR and DQ loci. There is an association of C4A deficiency with SLE in all Caucasoid populations studied, except for the French and Spanish populations, who exhibit an association with C4B deficiency. C4AQ0 allele frequencies are 0.25 to 0.41 in patients vs 0.10 to 0.23 in controls. As already mentioned, the C4AQ0 allele is linked to the common DR3-B8 haplotype. The DR3 allele is also significantly increased in patients with SLE, although in some studies, the C4A null allele was not increased when the DR3-positive individuals were subtracted from the patient group [82, 92]. In contrast, a survey among 188 American patients to detect nonexpressed C4A null alleles not linked to DR3 by screening for the 2-bp insertion in the C4A gene [52] found that this mutant C4AQ0 allele was significantly increased in particular among SLE patients of Caucasoid origin [93]. The C4B null association in the French and Spanish studies [94, 95] might reflect a different MHC association, as the haplotype HLA-A30, B18, DR3, DQ2, C2C, BF1, C4A3BQ0 is significantly increased only in SLE and insulin-dependent diabetes patients of southern European ancestry. Thus, the strong linkage disequilibrium of C4 null alleles with extended MHC haplotypes makes it difficult to ascertain the genetic contribution of the individual loci within these haplotypes to disease susceptibility in the Caucasoid population.

To help resolve this issue, association studies have also been carried out in other ethnic groups with a different MHC background, particularly in African and Asian populations. In African-Americans, the C4A null allele is also increased in patients with frequencies between 0.15 and 0.2 vs 0.04 and 0.08 in controls. As African-Americans have been influenced by genetic admixture from Caucasoids, data from native African populations would be highly desirable, but are not available yet except for HLA-DR. However, these admixture effects can be excluded for Asians. Except for one study, Chinese [81, 88, 96], Korean [97], and Japanese [79, 98] SLE patients also exhibit a significant increase of C4A null alleles. C4A alleles in Asians are not due to the common deletion of the C4A-CYP21A gene pair observed in Caucasoids [97, 98]. Also, the observed HLA-DR2 association in Chinese and Korean SLE patients appears to be independent from the presence of C4 null alleles. These data further imply that it is the C4A protein deficiency itself that predisposes an individual to SLE.

Although the association of complete and partial C deficiency and SLE is accepted by most investigators, there are dissenting opinions. For example, relative to

TABLE 4 C4Q0 and HLA Class II Associations with SLE in Several Races

Population	C4A*Q0 gene frequency		C4B*Q0 gene frequency		HLA class II			
						gene frequency		
	SLE	Control	SLE	Control	Allele	SLE	Controls	Ref.
American Caucasoid	0.25	0.10	0.09	0.11	DR 3	0.21	0.13	80
American Caucasoid	0.25	0.13	N/A[a]		N/A			77
Australian Caucasoid	0.32	0.20	0.15	0.10	N/A			74
Australian Caucasoid	0.32	0.17	0.23	0.20	DR 3	0.32	0.20	159
Australian Caucasoid	0.30	0.15	0.12	0.17	N/A			6
British Caucasoid	0.38	0.08	0.09	0.14	DR 3	0.41	0.09	71
British Caucasoid (DR3 negative)	0.08	0.05	0.25	0.13	N/A			82
British Caucasoid	0.31	0.20	N/A		DR 3 DQA1* 0501	0.38 0.40	0.18 0.14	160
English/Irish Caucasoid	0.41	0.22	N/A		DR 3	0.41	0.19	161
French Caucasoid	0.05	0.11	0.37	0.13	DR 3	0.27	0.13	84
German Caucasoid	0.29	0.12	0.10	0.14	DR 3 DR 2	0.27 0.26	0.13 0.16	92
Scandinavian Caucasoid	0.31	0.14	N/A		DR 3 DQA1* 0501	0.29 0.36	0.14 0.16	105
Spanish Caucasoid[b]	0.12	0.19	0.29	0.16	DR 3 DQA1* 0501	0.43 0.58	0.16 0.16	95
Mexican Mestizo	0.21	0.18	0.15	0.13	DR 3 DR 7	0.16 0.18	0.07 0.07	162
African-American	0.20	0.07	0.17	0.07	N/A			80
African-American	0.18	0.08	0.16	0.12	N/A			85
African-American	0.15[c]	0.04	N/A		n.s.[d]			89
South African Black	N/A		N/A		DR 2 DQB1*0602	0.19 0.30	0.07 0.15	103
Hong Kong Chinese	0.31	0.16	0.17	0.15	DR 2	0.38	0.21	81
Mainland Chinese	0.40	0.17	0.22	0.07	N/A			88
Southern Chinese	n.s.		n.s.		DR 2	0.27	0.09	96
Korean	0.21	0.13	0	0.02	DR 2 (15) DR 9	0.15 0.13	0.07 0.06	97
Japanese	0.34	0.21	0.32	0.30	N/A			87
Japanese	0.35	0.13	0.14	0.19	N/A			163

[a] Not available.
[b] Only patients with diffuse proliferative glomerulonephritis.
[c] C4/CYP21 gene deletions only (RFLP studies).
[d] No significant association detected.

complete Cdef, most C2def individuals do not have SLE or a related rheumatic syndrome (Table 5). Also, the frequency of SLE in C2def family members or probands with SLE is less than 30%, suggesting a possible ascertainment bias, as individuals with SLE have their C values determined more often than healthy persons or patients with other autoimmune diseases. While the frequency of C2def in SLE could be somewhat inflated [3], the association with C1q, C1r, C1s, or C4 deficiency cannot be accounted for by such an explanation. In a population survey, Japanese investigators examined complement activity in over 150,000 individuals [99, 100]. Total deficiency of early components was not observed, indicating that an ascertainment bias cannot explain the association [1, 3, 10]. Also, C2def may not be as severe a defect because there is a means to bypass this component in the cascade [43, 101]. Another explanation is that IC fixation by activated C4b alone leads to binding to CR1, which may be sufficient to solubilize and process some immune complexes.

TABLE 5 C2 Deficiency and SLE

C2 deficiency in SLE					C2 deficiency in Normals		
n	Homozygous	Heterozygous	Total	(%)	*n*	(%)	Ref.
137	1	7	9	(6.7)	509	(1.2)	156
106	2	7	9	(8.5)	N/A[a]	N/A	157
29	0	1	1	(3.4)	42	(0.9)	71
248	1	5	6	(2.4)	N/A	(1.4)	90
62	0	1	1	(1.6)	76	(1.3)	6
122	2	2	4	(3.3)	427	(1.4)	158

[a] Not available.

The C4A deficiency association with SLE has not held up in all populations. As pointed out previously, in French and Spanish Caucasians, in southern Chinese, and, most notably, in Mexican Mestizos (see Table 4), the frequency of C4A deficiency in SLE patients was not significantly greater than in the control population. Furthermore, it has also be taken into consideration that 40 to 50% of all SLE patients do not carry a C4 null gene. Therefore, a more complex situation with several independent risk alleles has to be considered (Table 4). In German Caucasoid patients, the MHC class II allele DR2 was increased significantly [90], and DR2 was also identified as a risk factor in another group of Caucasoid patients [102], in South Africa Blacks [103], in Chinese [81, 103], and in Koreans [97]. Finally, the closely linked HLA-DQA and -DQB loci appear to exhibit significant associations in Caucasoids (DQA1*0501, which is in linkage disequilibrium with the DR3-B8 haplotype [95, 104, 105] and in Africans DQB1*0602 [103]). Thus, it can be concluded that in addition to C4 deficiency, class II genes represent independent pathogenetic risk factors, most likely related to modulation of the immune response.

ACQUIRED C4 AND/OR C2 DEFICIENCY AND SUSCEPTIBILITY TO SYSTEMIC LUPUS ERYTHEMATOSUS

Inherited or acquired deficiency of the C1 inhibitor (angioedema syndrome associated with C1 inhibitor deficiency) is associated with an increased frequency of autoimmunity, especially SLE [106] (see Chapter 6). With a deficiency of the C1 inhibitor, C1 cleaves, in an unchecked fashion, its natural substrates, C4 and C2, causing a marked reduction in their serum levels. In some patients with C1 inhibitor deficiency, C4 and C2 concentrations are chronically so reduced that it simulates a C4/C2def state. A similar situation exists with autoantibodies that stabilize C3 convertases (nephritic factors). Such individuals may have chronically low levels and usually present with either lupus (if the classical pathway convertase is stabilized) or glomerulonephritis (if the alternative pathway convertase is stabilized) [10]. Evidence has also shown that the mechanism whereby hydralazine and isoniazid induce SLE may be mediated through inhibition of C4 function [107]. The concentrations of hydralazine and isoniazid (but not procainamide) that inhibited the activity of C4 by 50% were in the range of those obtained during therapy. Perhaps related to this observation was the finding of an increased frequency of C4A null alleles in hydralazine-induced SLE patients [107].

PARTIAL C2 DEFICIENCY AND SUSCEPTIBILITY TO SLE

Homozygous C2 deficiency is associated with SLE **(see Chapter 6)**. Moreover, the clinical picture may be suggestive of the deficient state. These patients tend to have an early childhood onset of a milder disease process with prominent photosensitive dermatologic manifestations, speckled anti nuclear antibodies [which on further testing demonstrate that the antibody specificity is for the Ro (SSA) antigen], and a family history of SLE. Although an informative subset, complete C2D accounts for less than 1% of all SLE patients. Heterozygous C2 deficiency may be slightly increased in lupus patients compared with controls (Table 5), although a recent comprehensive review concluded that there is probably no association [3]. Heterozygous C2D occurs in 1 to 2% of Caucasians, usually on part or all of the A10 (A25)-B18-DR2 haplotype [108].

IMMUNE COMPLEX PROCESSING AND THE COMPLEMENT SYSTEM

The primary goal of the humoral immune response is to bind (recognition function) and process (effector function) foreign antigens. Immune complex formation with C activation is a beneficial and essential part of this process [8–12, 14, 109–113]. A new concept in this arena is that this scheme may also be important for the handling of host cellular debris [3, 17, 18, 114–116].

C4b and C3b deposit on the IC and thereby maintain the solubility of the IC [11]. The interaction of Ag with Ab may produce aggregates of increasing size in a network of cross-linked complexes. As the size of the IC increases, so does the possibility of precipitation and cryoglobulin activity. C3 serves a critical role in IC formation by limiting their size thereby preventing precipitation. This "maintenance of IC solubility" requires the intercalation of C3b into the IC. A precipitated IC, especially in the vascular bed of organs such as the kidney, induces a local inflammatory reaction (glomerulonephritis, vasculitis). A deficiency of one or more CP components, up to and including C3, predisposes to inappropriate formation and deposition of the IC. Sera from patients deficient in C1q, C1r/C1s, C4, C2, or C3 do not efficiently solubilize preformed IC or inhibit the precipitation of the IC [3, 8–12, 14, 109, 111–113, 117]. Also, particularly with certain types of antigens, C4A is more efficient in preventing IC precipitation than C4B [118, 119]. A corollary here is that the processing of cellular debris, including apoptotic cells, may be a special case of this IC processing [3, 114]. Increasing evidence points to the CP playing a role in the safe disposal of damaged, degenerative, effete, and apoptotic cells.

C4b and C3b are ligands for C receptors [29–31]. This interaction provides a means to ingest the IC that form in tissue at sites of inflammation and to bind, transport, and transfer IC that enter or form in the circulation [110]. In tissue, such IC are ingested by dendritic and mononuclear cells and granulocytes, and the former, especially with migration to a regional lymph node, initiate the immune responses through their antigen-processing activities. In the vascular system, IC become bound to cells (Fig. 8), primarily erythrocytes (a phenomenon known as *immune adherence*), and are then transported to the liver and spleen. In the liver, the IC is transferred to Kupfer cells and the antigen is destroyed. In the spleen, the IC is transferred to follicular-dendritic cells and mononuclear phagocytes for processing. The erythrocyte returns to the circulation, deficient in some of its C3b receptors, but also ready to "shuttle" additional IC. This mechanism probably evolved to facilitate the clearance of IC that arise in the normal course of a humoral immune response to infectious organisms. Analysis of the steps in the pathway indicates that "pathologic" IC may arise from (1) excessive IC formation that overwhelms clearance pathways, (2) inefficient CP activation because of deficient antibody response, e.g., a weak IgG or an IgA response (rather than IgG), (3) C1, C4, C2, or C3 deficiency, or (4) C receptor anomalies. Again, this same set of problems would play out if the CP was involved in the clearance of cellular debris [3, 17, 115, 116, 120, 121].

TARGETED GENE KNOCKOUTS

Pertinent to the preceding discussion, C1q, C4, factor B, and C2/factor B-deficient mice have been produced in the mouse [3, 122, 123]. The major lessons learned from these knockouts have been as follows.

1. On most genetic backgrounds, C1q or C4def mice do not develop spontaneous autoimmunity (namely autoantibodies and a SLE phenotype). When SLE did develop spontaneously, it was on a background of low-level autoimmunity. The message is that the genetic background is extremely important and that C1q or C4d *alone* is unable to cause autoimmunity in most strains of mice (in contrast to the outbred human population).
2. In lupus mouse models, C4def or C1qdef substantially enhanced the phenotype. Disease onset was generally earlier and ANA titers were higher with a greater frequency of DNA antibodies. There was also more pronounced GN.
3. Factor B and C2/factor B knockouts did not develop spontaneous autoimmunity (on any genetic background tested). The effect of these combined deficiencies on lupus mouse models was mixed. In several cases, there was no disease amelioration [3, 123], whereas in another there was lessening in the glomerulonephritis and vasculitis (end organ damage) [124].
4. C3def mouse did not develop spontaneous lupus and it had little or no effect on the lupus mouse models. This is a surprising result, as the dogma would be that C3 activation would mediate some of the tissue damage.

In the following section, more details are given relative to these deficient mice.

C4

High titers of ANA developed spontaneously in C4 KO mice [15, 125] on an autoimmune permissive background. Further, in the Chen study [125], by 10 months of age all female and most male mice had a high-titer ANA and IC deposition in the glomerulus. In these two studies C4 KO mice were also crossed with complement

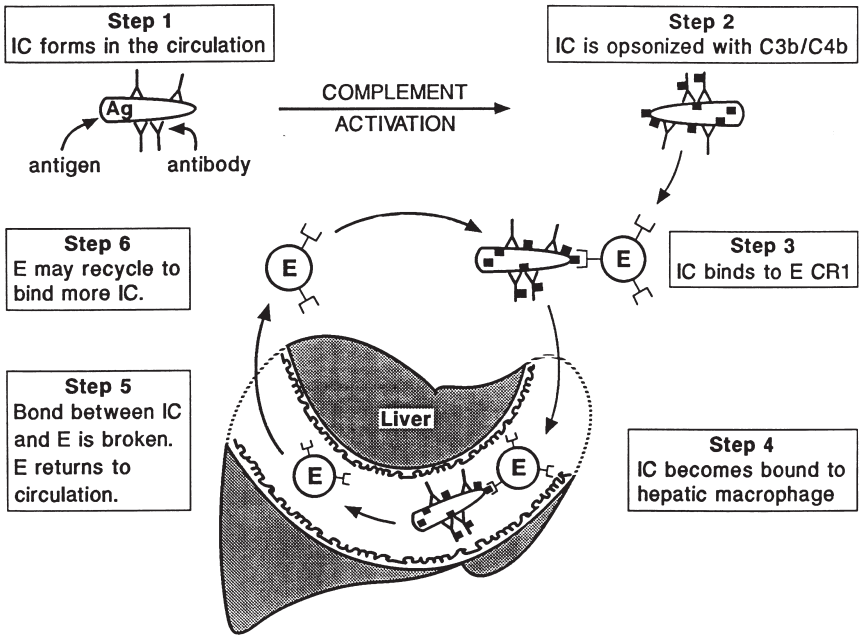


FIGURE 8 Erythrocyte processing of immune complexes (IC) or the "EPIC phenomenon." This diagram delineates a pathway for processing immune complexes (IC). Complement-coated IC adhere to the erythrocyte (E) C3b/C4b receptor, also termed *complement receptor type 1* (CR1). This binding of IC through C3b and its receptor to erythrocytes in most primates and to platelets in most nonprimates is known as the *immune adherence phenomenon*. The erythrocyte transports, like a shuttle or ferry, the IC to the liver and spleen. In the liver IC are transferred to macrophages and destroyed. In the spleen IC are transferred to B lymphocytes, macrophages, and follicular-dendritic cells for antigen processing, leading to a humoral immune response. The erythrocyte returns to circulation, possibly stripped of some of its receptors. This model was initially defined in baboons by Hebert and colleagues, but the essential elements of the scheme have now been described in humans, other primates, guinea pigs, and rabbits. Several different antigen–antibody systems, including tetanus–antitetanus, DNA–anti-DNA, and BSA–anti-BSA, have been analyzed. Adapted from Hebert and Cosio, [110], with permission.

receptor 1 and 2 KO mice, and these mice had a disease phenotype like C4 KO alone. The authors concluded that C4 deficiency causes lupus-like autoimmunity through a mechanism independent of CR1/CR2. In other words, C4b did not need to bind to these complement receptors to mediate its protective effect against the development of SLE. The investigators favor a direct regulatory effect on autoreactive B cells. Two other points were made in these studies that are worth noting. In Prodeus experiments, CR1/CR2 deficiency markedly worsened the disease phenotype (like C4def), whereas C3def had no effect (by itself or in conjunction with C4def).

On another genetic background, C4 KO mice did not spontaneously develop a SLE phenotype [15, 126]. However, if the C4def was bred into a strain that develops SLE, a more *severe* SLE like-illness developed [126]. The authors of this report also believe that this may be related to defects in the central tolerance of B cells. Of note, in all of these C4-deficient animals, C1q levels are normal and therefore its presence does not prevent the autoimmune phenotype from evolving.

In lupus-prone mice, animals null for both C3 and C4 were studied [126]. Interestingly, the disease process was similar in C4def and the combined C3/C4-deficient animals. Thus, severe SLE developed in the absence of C3. These data have two straightforward implications. One is that C3 activation is not required to cause SLE in the mouse, and the second is that C4 contributes much more to SLE susceptibility than C3. It should be remembered that the complement system is involved in susceptibility to as well as in mediating tissue damage

in SLE. Further, human and mouse SLE may have distinct causes and pathogenesis. Nevertheless, in both human and mouse, C4 (and C1q) provides an important protective role against the development of SLE.

Factor B/C2

A combined factor B and C2 [127] or a factor B alone [128] targeted gene deletion mouse did not develop spontaneous autoimmunity. Of interest, factor B/C2-deficient mice crossed into C1qdef did not prevent SLE from developing [129]. Because these animals cannot activate C3, these data again show that C3 is not required for the development of lupus phenotype. In a different model system, factor B deficiency was crossed into lupus-prone mice. The disease phenotype was milder [124]. Because autoantibody levels were similar, these results suggest that factor B plays a role primarily on the effector side of the equation, presumably via alternative pathway amplification of complement activation.

C1q

C1q, of course, is not part of complement gene cluster on chromosome 6 but it is also part of the pathway (as is C4 and C2) required for CP activation. About 50% of mice on a mixed genetic but autoimmune-prone background developed high titers of ANA [16, 130, 131]. By 8 months of age, 25% of the mice had glomerulonephritis with immune deposits. The authors also demonstrated larger numbers of apoptotic bodies in diseased glomeruli. Their hypothesis is that increased numbers of apoptotic cells are present because of a C-dependent clearance defect for processing autoantigens. It is difficult to accept, however, that C1qdef is the whole story in view of data with C4def mice (in the setting of normal C1q). Perhaps it is hierarchy, as pointed out in another publication [16] by these authors, in which C1q > C4 >>> C2 in leading to SLE. To buttress this argument, they point out that C1q could have two roles. One is to serve as a ligand for C1q receptors on phagocytes and the other being to activate the CP, thereby providing additional ligands for the disposal of apoptotic cells.

In a more recent study of C1qdef, the effect of the genetic background on disease expression was further employed [130]. In two strains, the autoimmune phenotype was not altered (worsened or improved), but in a third strain the disease process accelerated with the production of more autoantibodies and a more severe and earlier onset of glomerulonephritis.

FURTHER SPECULATION ON THE ASSOCIATION BETWEEN SYSTEMIC LUPUS ERYTHEMATOSUS AND COMPLEMENT DEFICIENCY

As discussed earlier, C is activated by IgG or IgM, and C activation through C3 is required to efficiently eliminate many infectious agents. This cannot be accomplished readily in a C-deficient host and therefore would potentially predispose such an individual to more prolonged exposure to infectious or damaged self-materials.

One problem thus faced by a C-deficient host is defective clearance of foreign antigens or damaged self constituents. As discussed in the preceding section, the lack of C3b deposition on an IC leads to impaired antigen localization such as to follicular areas of the spleen. This appears to be the critical factor in the poor antibody response of such individuals [132–134]. For example, if C4A, C2, or C3def guinea pigs or humans are immunized intravenously with ØXC174, a relatively low-titer IgM response is produced. Moreover, a secondary IgG response is not made, even with repetitive intravenous immunizations. A failure to localize the antigen to the follicular area of the spleen probably accounts for the defective immune response. The primary and secondary immune response can be rescued in C4def guinea pigs by adding purified human C4A but not C4B or an adjuvant with the primary immunization [62]. This presumed failure of antigen presentation is explained more simply by C4A being required for efficient opsonization of this antigen. A plausible scenario then for deficient antibody production in C deficiency is defective antigen localization. CR1 (the C3b/C4b receptor) and CR2 (C3d receptor) are expressed by B lymphocytes and follicular-dendritic cells. On both cell types they facilitate the localization of C-coated IC to B cells and enhance B-cell activation [30, 31, 135]. In these model systems, immunization with increased amounts of antigen lead to a normal immune response.

In a C-deficient host there are several avenues to a vasculitic state. Perhaps the simplest is the failure to efficiently eliminate an infectious material. Prolonged antigenemia could result (with or without significant tissue damage to the organ, which harbors the infection). Chronic antigenemia is the setting for impaired IC formation, such as at Ab-Ag equivalence or slight Ag excess. In experimental serum sickness models, such IC are prone to deposit in glomeruli and other tissues. Clinical examples of this type of problem are chronic serum hepatitis B and C infections [136]. Although we do not understand the reason for the poor antibody response

and failure to eliminate the virus, the consequences are clear: chronic antigenemia, IC formation, and IC deposition in undesirable sites, resulting in glomerulonephritis or a more generalized vasculitic syndrome.

Hepatitis B and C are surely not the only such infections of humans that produce this type of a syndrome. More likely, hepatitis is the one that we recognize because of its means of transmission and because of sensitive serologic and biochemical markers for these infections. Complement deficiency would contribute to and potentiate other defects in the immune response. In this context, it is interesting to note that C4A deficiency also appears to play a major role in nonresponders to HBsAg vaccination. It has been demonstrated that this nonresponse is significantly associated with C4A null alleles independent from the presence of the DRB1*0301 allele [137, 138]. This line of reasoning also suggests that some forms of SLE actually represent immune deficiency syndromes. Lupus associated with C deficiency or hypogammaglobulinemia are two examples.

In a C-deficient host, even normal amounts of IC (such as those that form because of passive absorption of bacterial antigens from epithelial surfaces) or in association with infections or associated with physiologic tissue turnover and cell death would have a greater likelihood of being processed inappropriately. The prevention of IC precipitation in vascular structures, their adherence to erythrocytes or leukocytes, and their transport to and deposition in the liver and spleen would all likely be impaired. Thus, if such individuals develop a condition leading to chronic antigenemia, they would be at greater risk for mishandling IC than a normal host. Lachmann and Walport [10] have noted that autoantibodies arise transiently in association with infections such as infectious mononucleosis and following tissue necrosis such as in myocardial or pulmonary infarctions. They suggest that a deficiency of effector mechanisms, especially immune complex processing, induces pathogenic inflammatory reactions at extrahepatic sites. A vicious cycle then ensues as intracellular antigens are exposed and become autoantigens, possibly modified by the inflammatory response with cell injury or death; i.e., the normal decay of an immune response does not occur.

DETECTION OF COMPLEMENT DEFICIENCY

A complete deficiency of a C-activating component is usually first suggested by the finding of a very low or zero whole complement (THC or CH_{50}) titer [139, 140]. This assay requires C1–C8 to be present to give a measurable titer and C9 to give a normal titer. Partial component deficiency is more difficult to ascertain. For example, a 50% reduction of C4 or C1 or most other components is not reflected by a 50% reduction in a THC. Usually, in this case the THC is within the normal range. The possibility of a partial C4 deficiency is often first suggested by a lower than expected antigenic level of C4 in a patient with SLE or a related vasculitic/autoimmune syndrome. A typical clinical example is a lupus patient with a persistently low C4 but who is in clinical remission and has a normal C3. The clinical question then becomes whether this represents an inherited partial C4A or C4B deficiency or "subclinical" complement activation by IC. Separation of these two possibilities can be difficult but a semiquantitative assessment by electrophoretic separation of C4A and C4B can be helpful [37, 141–145]. Several commercial laboratories now test for the presence of and quantitate the concentration of C4A and C4B. HLA typing may also be useful, as two-thirds of C4A-deficient alleles in the Caucasian population are on the DR3B8 haplotype.

Several experimental issues, however, may complicate these determinations of partial C4 deficiency, and improved means for detecting such deficiencies are needed. Monoclonal antibodies specific for C4A or C4B are available [46, 147], and several reports describe capture assays to measure the antigenic levels of C4A and C4B [64–66, 146]. Finally, molecular probes or polymerase chain reaction-based assays provide a means to assess RFLP variants and detect patterns specific for deletions such as the DR3-B8 haplotype (by standard Southern blotting or pulsed field gel electrophoresis) [19, 47, 69, 148–150]. If possible, genetic typing at the protein and/or DNA level of the patient and his relatives in family studies allows reliable assignment of C4 null alleles [69, 151]. To summarize, at present, the detection of complete complement deficiency states or homozygous C4A or C4B deficiency can be accomplished easily and such data are of utility to the clinician in caring for lupus patients. Partial C4A or C4B or C2 deficiency is more complicated to assess and is currently of no clinical utility.

References

1. Walport, M. J., Davies, K. A., Morley, B. J., and Botto, M. (1997). Complement deficiency and autoimmunity. *Ann. N. Y. Acad. Sci.* **815**, 267–281.
2. Sjoholm, A. G. (1991). Inherited complement deficiency states and disease. *Complement Inflamm.* **8**, 341–346.
3. Pickering, M. C., Botto, M., Taylor, P. R., Lachmann, P. J., and Walport, M. J. (2001). Systemic lupus erythematosus, complement deficiency, and apoptosis. *Adv. Immunol.* **76**, 227–322.

4. Tokunaga, K., Saueracker, G., and Kay, P. H. (1988). Extensive deletions and insertions in different MHC supratypes detected by pulsed field gel electrophoresis. *J. Exp. Med.* **168**, 933.
5. Arnett, F. C., and Moulds, J. M. (1991). HLA class III molecules and autoimmune rheumatic diseases. *Clin. Exp. Rheumatol.* **9**, 289.
6. Christiansen, F. T., Zhang, W. J., and Griffiths, M. (1991). MHC encoded complement deficiency, ancestral haplotypes and SLE: C4 deficiency explains some but not all of the influence of the MHC. *J. Rheumatol.* **18**, 1350.
7. Ng, Y. C., and Walport, J. M. (1988). Immunogenetics of SLE and primary Sjogren's syndrome. *Baillieres Clin. Rheumatol.* **2**, 623.
8. Schifferli, J. A., and Peters, D. K. (1983). Complement, the immune-complex lattice and the pathophysiology of complement-deficiency syndromes. *Lancet* **2**, 957–959.
9. Atkinson, J. P. (1986). Complement activation and complement receptors in systemic lupus erythematosus. *Springer Semin. Immunopathol.* **9**, 179–194.
10. Lachmann, P. J., and Walport, M. J. (1987). Deficiency of the effector mechanisms of the immune response and autoimmunity. *In* "Autoimmunity and Autoimmune Disease" (D. Evered and J. Whelan, eds.), p. 129. Wiley, New York.
11. Medof, M. E. (1988). Complement-dependent maintenance of immune complex solubility. *In* "The Complement System" (K. Rother and G. O. Till, eds.), pp. 418–443. Springer-Verlag, Berlin.
12. Atkinson, J. P. (1988). Complement deficiency: Predisposing factor to autoimmune syndromes. *Am. J. Med.* **85**, 45–47.
13. Atkinson, J. P. (1992). Immune complexes and the role of complement. *In* "Systemic Vasculitis" (E. C. LeRoy, ed.), pp. 525–546. Dekker, New York.
14. Schifferli, J. A., and Taylor, R. P. (1989). Physiological and pathological aspects of circulating immune complexes. *Kidney Int*. **35**, 993–1003.
15. Prodeus, A. P., Goerg, S., Shen, L. M., Pozdnyakova, O., Chu, L., Alicot, E. M., Goodnow, C. C., and Carroll, M. C. (1998). A critical role for complement in maintenance of self-tolerance. *Immunity* **9**, 721–731.
16. Cutler, A. J., Cornall, R. J., Ferry, H., Manderson, A. P., Botto, M., and Walport, M. J. (2001). Intact B cell tolerance in the absence of the first component of the classical complement pathway. *Eur. J. Immunol.* **31**, 2087–2093.
17. Navratil, J. S., and Ahearn, J. M. (2000). Apoptosis and autoimmunity: Complement deficiency and systemic lupus erythematosus revisited. *Curr. Rheumat. Rep.* **2**, 32–38.
18. Sullivan, K. E. (1998). Complement deficiency and autoimmunity. *Curr. Opin. Pediatr*. **10**, 600–606.
19. Campbell, R. D., Law, S. K. A., and Reid, K. B. M. (1988). Structure, organization and regulation of the complement genes. *Annu. Rev. Immunol.* **6**, 161.
20. Atkinson, J. P. (1989). The complement system. *In* "General Immunology" (H. Eisen, ed.), pp. 147–166. Lippincott, Philadelphia.
21. Chan, A. C., Karp, D. R., Shreffler, D. C., and Atkinson, J. P. (1984). The twenty faces of the fourth component of complement (C4). *Immunol. Today* **5**, 200–203.
22. Chan, A. C., and Atkinson, J. P. (1985). Oligosaccharide structure of human C4. *J. Immunol.* **134**, 1790–1798.
23. Hortin, G., Chan, A. C., Fok, K. F., Strauss, A. W., and Atkinson, J. P. (1986). Sequence analysis of the C-terminus of the α-chain of the fourth component of human complement: Identification of the site of its extracellular cleavage. *J. Biol. Chem.* **261**, 9065–9069.
24. Hortin, G. L., Farries, T. C., Graham, J. P., and Atkinson, J. P. (1989). Sulfation of tyrosine residues increases activity of the fourth component of complement. *Proc. Natl. Acad. Sci. USA* **86**, 1338–1342.
25. Chan, A. C., Mitchell, K. R., Munns, T. W., Karp, D. R., and Atkinson, J. P. (1983). Identification and partial characterization of a novel form of the fourth component of human complement: Evidence that the secreted form is different from the major plasma form. *Proc. Natl. Acad. Sci. USA* **80**, 268–272.
26. Lublin, D. M., Liszewski, M. K., Post, T. W., Arce, M. A., Le Beau, M. M., and Rebentisch, M. B. (1988). Molecular cloning and chromosomal localization of human membrane cofactor protein (MCP): Evidence for inclusion in the multi-gene family of complement-regulatory proteins. *J. Exp. Med.* **168**, 181–194.
27. Tack, B. F. (1983). The beta-cys-gamma-glu thioester bond in human C3, C4, and alpha$_2$-macroglobulin. *Springer Semin. Immunopathol.* **6**, 259.
28. Law, S. K. (1983). Non-enzymic activation of the covalent binding reaction of the complement protein. *Biochem. J.* **211**, 381.
29. Ross, G. D., Cain, J. A., and Lachmann, P. J. (1985). Membrane complement receptor type three (CR_3) has lectin-like properties analogous to bovine conglutinin and functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. *J. Immunol.* **134**, 3307–3315.
30. Ahearn, J. M., and Fearon, D. T. (1989). Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv. Immunol.* **46**, 183–219.
31. Hourcade, D., Holers, V. M., and Atkinson, J. P. (1989). The regulators of complement activation (RCA) gene cluster. *Adv. Immunol.* **45**, 381–416.
32. Belt, K. T., Carroll, M. C., and Porter, R. R. (1984). The structural basis of the multiple forms of human complement component C4. *Cell* **36**, 907.
33. Yu, C. Y., Belt, K. T., and Giles, C. M. (1986). Structural basis of the polymorphism of human complement component C4A and C4B: Gene size, reactivity and antigenicity. *EMBO J.* **5**, 2873.
34. Yu, C. Y., Campbell, R. D., and Porter, R. R. (1988). A structural model for the location of the Rodgers and the Chido antigenic determinants and their correlation with the human complement component C4A/C4B isotypes. *Immunogenetics* **27**, 399.
35. Carroll, M. C., Fathallah, D. M., Bergamaschini, L., Alicot, E., and Isenman, D. E. (1990). Substitution of a single amino acid (aspartic acid for histidine) converts

the functional activity of human complement C4B to C4A. *Proc. Natl. Acad. Sci. USA* **87**, 6868.
36. Mauff, G., Alper, C. A., Dawkins, R., Doxiadis, G., Giles, C. M., Hauptmann, G., Rittner, C., and Schneider, P. M. (1990). C4 nomenclature statement. *Complement Inflamm.* **7**, 261–268.
37. Mauff, G., Brenden, M., and Braun-Stilwell, M. (1990). Relative electrophoretic migration distances for the classification of C4 allotypes. *Complement Inflamm.* **7**, 277–281.
38. Belt, K. T., Yu, C. Y., Carroll, M. C., and Porter, R. R. (1985). Polymorphism of human complement component C4. *Immunogenetics* **21**, 173–180.
39. Anderson, M. J., Milner, C. M., Cotton, R. G., and Campbell, R. D. (1992). The coding sequence of the hemolytically inactive C4A6 allotype of human complement C4 reveals that a single arginine to tryptophane substitution at B-chain residue 458 is the likely cause of the defect. *J. Immunol.* **148**, 2795.
40. Dangel, A. W., Mendoza, A. R., Baker, B. J., Daniel, C. M., Carroll, M. C., Wu, L. C., and Yu, C. Y. (1994). The dichotomous size variation of human complement C4 genes is mediated by a novel family of endogenous retroviruses, which also establishes species-specific patterns among old world primates. *Immunogenetics* **40**, 425.
41. Chu, X., Rittner, C., and Schneider, P. M. (1995). Length polymorphism of the human complement component C4 gene is due to an ancient retroviral integration. *Exp. Clin. Immunogenet.* **12**, 74.
42. Schneider, P. M., Witzel-Schlomp, K., Rittner, C., and Zhang, L. (2001). The endogenous retroviral insertion in the human complement C4 gene modulates the expression of homologous genes by antisense inhibition. *Immunogenetics* **53**, 1–9.
43. Steuer, M., Mauff, G., and Adam, C. (1989). An estimate on the frequency of duplicated haplotypes and silent alleles of human C4 protein polymorphism. Investigations in healthy Caucasoid families. *Tissue Antigens* **33**, 501.
44. Partanen, J., and Campbell, R. D. (1989). Restriction fragment analysis of non-deleted complement C4 null genes suggests point mutations in C4A null alleles but gene conversions in C4B null alleles. *Immunogenetics* **30**, 520.
45. Braun, L., Schneider, P. M., and Giles, C. G. (1990). Null alleles of human complement. Evidence for pseudogenes at the C4A locus and gene conversion at the C4B locus. *J. Exp. Med.* **171**, 129–140.
46. Carroll, M. C., Palsdittir, A., and Belt, K. T. (1985). Deletion of complement C4 and steroid 21-hydroxylase genes in the HLA class III region. *EMBO J.* **4**, 2547.
47. Schneider, P. M., Carroll, M. C., and Alper, C. A. (1986). Polymorphism of the human complement C4 and steroid 21-hydroxylase genes: Restriction fragment length polymorphisms revealing structural deletions, homoduplications, and size variants. *J. Clin. Invest.* **78**, 650.
48. Yu, C. Y. (1998). Molecular genetics of the human MHC complement gene cluster. *Exp. Clin. Immunogenet.* **15**, 213–230.
49. Yang, Z., Mendoza, A. R., Welch, T. R., Zipf, W. B., and Yu, C. Y. (1999). Modular variations of the human major histocompatibility complex class III genes for serine/threonine kinase RP, complement component C4, steroid 21-hydroxylase CYP21, and tenascin TNX (the RCCX module): A mechanism for gene deletions and disease associations. *J. Biol. Chem.* **274**, 12147–12156.
50. Blanchong, C. A., Zhou, B., Rupert, K. L., Chung, E. K., Jones, K. N., Sotos, J. F., Zipf, W. B., Rennebohm, R. M., and Yu, C. Y. (2000). Deficiencies of human complement component C4A and C4B and heterozygosity in length variants of RP-C4-CYP21-TNX (RCCX) modules in Caucasians: The load of RCCX genetic diversity on major histocompatibility complex-associated disease. *J. Exp. Med.* **191**, 2183–2196.
51. Schneider, P. M. (1990). C4 DNA RFLP reference typing report. *Complement Inflamm.* **7**, 218.
52. Barba, G., Rittner, C., and Schneider, P. M. (1993). Genetic basis of human complement C4A deficiency: Detection of a point mutation leading to nonexpression. *J. Clin. Invest.* **91**, 1681.
53. Fredrikson, G. N., Gullstrand, B., Schneider, P. M., Witzel-Schlomp, K., Sjoholm, A. G., Alper, C. A., Awdeh, Z., and Truedsson, L. (1998). Characterization of non-expressed C4 genes in a case of complete C4 deficiency: Identification of a novel point mutation leading to a premature stop codon. *Hum. Immunol.* **59**, 713–719.
54. Lokki, M. L., Circolo, A., Ahokas, P., Rupert, K. L., Yu, C. Y., and Colten, H. R. (1999). Deficiency of human complement protein C4 due to identical frameshift mutations in the C4A and C4B genes. *J. Immunol.* **162**, 3687–3693.
55. Rupert, K. L., Moulds, J. M., Yang, Y., Arnett, F. C., Warren, R. W., Reveille, J. D., Myones, B. L., Blanchong, C. A., and Yu, C. Y. (2002). The molecular basis of complete complement C4A and C4B deficiencies in a systemic lupus erythematosus patient with homozygous C4A and C4B mutant genes. *J. Immunol.* **169**, 1570–1578.
56. O'Neil, K. M. (2000). Complement deficiency. *Clin. Rev. Allergy Immunol.* **19**, 83–108.
57. Welch, T. R., and Frenzke, M. (2001). Glomerulonephritis associated with deficiencies and polymorphisms of complement components encoded in the class III region of the MHC. *Front. Biosci.* **6**, D898–D903.
58. Law, S. K., Dodds, A. W., and Porter, R. R. (1984). A comparison of the properties of two classes, C4A and C4B, of the human complement component C4. *EMBO J.* **3**, 1819.
59. Isenman, D. E., and Young, J. R. (1984). The molecular basis for the difference in immune hemolysis activity of the Chido and Rodgers isotypes of human complement component C4. *J. Immunol.* **132**, 3019–3027.
60. Schifferli, J. A., Steiger, G., and Paccaud, J. (1986). Difference in the biological properties of the two forms of the fourth component of human complement. *Clin. Exp. Immunol.* **63**, 473.
61. Dodds, A. W., Ren, X. D., Willis, A. C., and Law, S. K. (1996). The reaction mechanism of the internal thioester in the human complement component C4. *Nature* **379**, 177.

62. Finco, O., Li, S., Cuccia, M., Rosen, F. S., and Carroll, M. C. (1992). Structural differences between the two human isotypes affect the humoral immune response. *J. Exp. Med.* **175**, 537.
63. Gatenby, P. A., Barbosa, J. E., and Lachman, P. J. (1990). Differences between C4A and C4B in the handling of immune complexes: The enhancement of CR1 binding is more important than the inhibition of immunoprecipitation. *Clin. Exp. Immunol.* **79**, 158.
64. Moulds, J. M., Arnett, F. C., and Giles, C. G. (1990). A novel immunoassay for the quantitation of human C4 gene products. *Complement Inflamm.* **7**, 95.
65. Moulds, J. M., Warner, N. B., and Arnett, F. C. (1991). Quantitative and antigenic differences in complement component C4 between American Blacks and Whites. *Complement Inflamm.* **8**, 281.
66. Moulds, J. M., Warner, N. B., and Arnett, F. C. (1994). Complement components C4A and C4B levels in systemic lupus erythematosus: Quantitation in relation to C4 null status and disease activity. *J. Rheumatol.* **21**, 577.
67. Kulics, J., Circolo, A., Strunk, R. C., and Colten, H. R. (1994). Regulation of synthesis of complement protein C4 in human fibroblasts: Cell- and gene-specific effects of cytokines and lipopolysaccharide. *Immunology* **82**, 509–515.
68. Campbell, R. D. (1987). Molecular genetics of C2 and Factor B. *Br. Med. Bull.* **43**, 37.
69. Schneider, P. M., and Rittner, C. (1997). Complement genetics. *In* "Complement: A Practical Approach" (A. W. Dodds and B. Sim, eds.), pp. 165–199. Oxford Univ. Press, Oxford.
70. Jaffe, C. J., Atkinson, J. P., and Frank, M. M. (1976). The role of complement in the clearance of cold agglutinin-sensitized erythrocytes in man. *J. Clin. Invest.* **58**, 942–949.
71. Fielder, A. H. L., Walport, M. J., and Batchelor, J. R. (1983). Family study of the major histocompatibility complex in patients with systemic lupus erythematosus: Importance of null alleles of C4A and 4B in determining disease susceptibility. *Br. Med. J.* **286**, 425.
72. Christiansen, F. T., Uko, G., and Dawkins, R. L. (1982). Complement allotyping in systemic lupus erythematosus: Association with C4A null. *In* "Immunogenetics in Rheumatology" (R. L. Dawkins, ed.), pp. 229–234. Excerpta Medica, Amsterdam.
73. Dawkins, R. L., Christiansen, F. T., and Kay, P. H. (1983). Disease associations with complotypes, supratypes and haplotypes. *Immunol. Rev.* **70**, 5.
74. Christiansen, F. T., Dawkins, R. L., and Uko, G. (1983). Complement allotyping in SLE: Association with C4A Null. *Aust. N. Z. J. Med.* **13**, 483.
75. Maddison, P. J., Provost, T. T., and Reichlin, M. (1981). Serologic findings in patients with ANA-negative SLE. *Medicine* (*Baltimore*) **60**, 87.
76. Provost, T. T., Arnett, F. C., and Reichlin, M. (1983). Homozygous C2 deficiency, lupus erythematosus, and anti-Ro (SSA) antibodies. *Arthritis Rheum.* **26**, 1279.
77. Kemp, M. E., Atkinson, J. P., Skanes, V. M., Levine, R. P., and Chaplin, D. D. (1987). Deletion of C4A genes in patients with systemic lupus erythematosus. *Arthritis Rheum.* **30**, 1015–1022.
78. Reveille, J. D., Arnett, F. C., and Wilson, R. W. (1985). Null alleles of the fourth component of complement and HLA haplotypes in familial systemic lupus erythematosus. *Immunogenetics* **21**, 299.
79. Tokunaga, K., Imoto, K., and Akaza, T. (1985). Haplotype study on C4 polymorphism in Japanese: Association with MHC alleles, complotypes, and HLA-complement haplotypes. *Immunogenetics* **22**, 359.
80. Howard, P. F., Hochberg, M. C., and Bias, W. B. (1986). Relationship between C4 null genes, HLA-D region antigens, and genetic susceptibility to systemic lupus erythematosus in Caucasian and Black Americans. *Am. J. Med.* **81**, 187.
81. Hawkins, B. R., Wong, K. L., and Wong, R. W. (1987). Strong association between the major histocompatibility complex and systemic lupus erythematosus in southern Chinese. *J. Rheumatol.* **14**, 1128.
82. Batchelor, J. R., Fielder, A. H. L., and Walport, M. J. (1987). Family study of the major histocompatibility complex in HLA DR3 negative patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **70**, 364.
83. Granados, J., Oliveras, I., and Melin, H. (1987). Further evidence of the role of complement genotypes in susceptibility to systemic lupus erythematosus (SLE) obtained from Mexican family studies. *Arthritis Rheum.* **30**(Suppl.), S21.
84. Gougerot, A., Stoppa-Lyonnet, D., and Poirier, J. C. (1987). HLA markers and complotypes: Risk factors in systemic lupus erythematosus. *Ann. Dermatol. Venereol.* **114**, 329.
85. Wilson, W. A., Perez, M. C., and Armatis, P. E. (1988). Partial C4A deficiency is associated with susceptibility to systemic lupus erythematosus in Black Americans. *Arthritis Rheum.* **31**, 1171.
86. Dahlquist, S. R., Beckman, G., and Beckman, L. (1988). Serum protein markers in systemic lupus erythematosus. *Hum. Hered.* **38**, 44.
87. Yukiyama, Y., Tokunaga, K., and Takeuchi, F. (1988). Genetic polymorphism of complement in patients with systemic lupus erythematosus II. The fourth (C4) and the seventh (7) components of complement. *Jpn. J. Rheumatol.* **1**, 271.
88. Zhao, X.-Z., Zhang, W. J., and Tian, Y. W. (1989). Allotypic differences and frequencies of C4 null alleles (C4Q0) detected in patients with systemic lupus erythematosus (SLE). *Chin. Sci. Bull.* **34**, 237–240.
89. Olsen, M. L., Goldstein, R., and Arnett, F. C. (1989). C4A gene deletion and HLA associations in Black Americans with systemic lupus erythematosus. *Immunogenetics* **30**, 27.
90. Hartung, K., Baur, M. P., Coldewey, R., Fricke, M., Kalden, J. R., Lakomek, H. J., Peter, H. H., Schendel, D., Schneider, P. M., Seuchter, S. A., *et al*. (1992). Major histocompatibility complex haplotypes and complement C4 alleles in systemic lupus erythematosus: Results of a multicenter study. *J. Clin. Invest.* **90**, 1346–1351.

91. Goldstein, R., Arnett, F. C., McLean, R. H. (1988). Molecular heterogeneity of complement component C4-null and 21-hydroxylase genes in systemic lupus erythematosus. *Arthritis Rheum.* **31**, 726.
92. Hartung, K., Albert, E., Baur, M., Coldewey, F., Kalden, J. R., Lakomek, H. J., Peter, H. H., Schendel, D., Schneider, P. M., Seuchter, S., Stangel, W., and Deicher, H. (1989). MHC haplotypes and complement C4 alleles in systemic lupus erythematosus: Results of a multicenter study. *J. Clin. Invest.* **90**, 1346.
93. Sullivan, K. E., Kim, N. A., Goldman, D., and Petri, M. A. (1999). C4A deficiency due to a 2 bp insertion is increased in patients with systemic lupus erythematosus. *J. Rheumatol.* **26**, 2144–2147.
94. Gougerot, A., Stoppa-Lyonnet, D., Poirier, J. C., Schmid, M., Busson, M., and Marcelli, A. (1987). HLA markers and complotypes: Risk factors in systemic lupus erythematosus?. *Ann. Dermatol. Venereol.* **114**, 329–334.
95. DeJuan, D., Martin-Villa, J. M., Gomez, R., Vicario, J. L., Corell, A., Martinez-Laso, J., Benmammar, D., and Arnaiz-Villena, A. (1993). Differential contribution of C4 and HLA-DQ genes to systemic lupus erythematosus susceptibility. *Hum. Genet.* **91**, 579.
96. Doherty, D. G., Ireland, R., Demaine, A. G., Wang, F., Veerapan, K., Welsh, K. I., and Vergani, D. (1992). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in southern Chinese. *Arthritis Rheum.* **35**, 641.
97. Hong, G. H., Kim, H. Y., and Takeuchi, F. (1994). Association of complement C4 and HLA-DR alleles with systemic lupus erythematosus in Koreans. *J. Rheumatol.* **21**, 442–447.
98. Yamada, M., Watanabe, A., and Mimori, A. (1990). Lack of gene deletion for complement C4: A deficiency in Japanese patients with systemic lupus erythematosus. *J. Rheumatol.* **17**, 1054.
99. Fukumori, Y., Yoshimura, K., Ohnoki, S., Yamaguchi, H., Akagaki, Y., and Inai, S. (1989). A high incidence of C9 deficiency among healthy blood donors in Osaka, Japan. *Int. Immunol.* **1**, 85–89.
100. Inai, S., Akagaki, Y., and Moriyama, T. (1989). Inherited deficiencies of the late-acting complement components other than C9 found among healthy blood donors. *Int. Arch. Allergy Appl. Immunol.* **90**, 274–279.
101. Farries, T. C., Steuer-Knutzen, K. L., and Atkinson, J. P. (1990). Evolutionary implications of a new bypass activation pathway of the complement system. *Immunol. Today* **11**, 78–80.
102. Fronek, Z., Timmerman, L. A., and Alper, C. A. (1990). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus. *Arthritis Rheum.* **33**, 1542.
103. Rudwaleit, M., Tikly, M., Gibson, K., Pile, K., and Wordsworth, P. (1995). HLA class II antigens associated with systemic lupus erythematosus in Black South Africans. *Ann. Rheum. Dis.* **54**, 678–680.
104. Davies, K. A., Erlendsson, K., Beynon, H. L. C., Peters, A. M., Steinsson, K., Valdimarsson, H., and Walport, M. J. (1993). Splenic uptake of immune complexes in man is complement-dependent. *J. Immunol.* **151**, 3866–3873.
105. Skarsvag, S. (1995). The importance of C4A null genes in Norwegian patients with systemic lupus erythematosus. *Scand. J. Immunol.* **42**, 572.
106. Agnello, V. (1986). Lupus diseases associated with hereditary and acquired deficiencies of complement. *Springer Semin. Immunopathol.* **9**, 161–178.
107. Sim, E., Gill, E. W., and Sim, R. B. (1984). Drugs that induce systemic lupus erythematosus inhibit complement component. *Lancet* **1**, 422.
108. Johnson, C. A., Densen, P., Wetsel, R. A., Cole, F. S., Goeken, N. E., and Colten, H. R. (1992). Molecular heterogeneity of C2 deficiency. *N. Engl. J. Med.* **326**, 871–874.
109. Atkinson, J. P. (1992). Genetic susceptibility and class III complement genes. "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), pp. 87–102. Churchill Livingstone, New York.
110. Hebert, L. A., and Cosio, F. G. (1987). The erythrocyte-immune complex-glomerulonephritis connection in man. *Kidney Int.* **31**, 877–885.
111. Schifferli, J. A., Ng, Y. C., Paccaud, J. P., and Walport, M. J. (1989). The role of hypocomplementaemia and low erythrocyte complement receptor type 1 numbers in determining abnormal immune complex clearance in humans. *Clin. Exp. Immunol.* **75**, 329.
112. Kahl, L. E., and Atkinson, J. P. (1988). Autoimmune aspects of complement deficiency. *Clin. Aspects Autoimmun.* **2**, 8–20.
113. Walport, M. J., and Lachmann, P. J. (1988). Erythrocyte complement receptor type 1, immune complexes, and the rheumatic diseases. *Arthritis Rheum.* **31**, 153–158.
114. Taylor, P. R., Carugati, A., Fadok, V. A., Cook, H. T., Andrews, M., Carroll, M. C., Savill, J. S., Henson, P. M., Botto, M., and Walport, M. J. (2000). A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells in vivo. *J. Exp. Med.* **7**, 359–366.
115. Navratil, J. S., Korb, L. C., and Ahearn, J. M. (1999). Systemic lupus erythematosus and complement deficiency: Clues to a novel role for the classical complement pathway in the maintenance of immune tolerance. *Immunopharmacology* **42**, 47–52.
116. Botto, M. (2001). Links between complement deficiency and apoptosis. *Arthritis Res.* **3**, 207–210.
117. Schifferli, J. A., Steiger, G., and Hauptmann, G. (1985). Formation of soluble immune complexes by complement in sera of patients with various hypocomplementemic states. *J. Clin. Invest.* **76**, 2127–2133.
118. Schifferli, J. A., Hauptmann, G., and Paccaud, J. P. (1987). Complement-mediated adherence of immune complexes to human erythrocytes: Differences in the requirements for C4A and C4B. *FEBS Lett.* **213**, 415.
119. Paul, I., Skanes, V. M., and Mayden, J. (1988). C4-mediated inhibition of immune precipitation and differences in inhibitory action of genetic variants, C4A3 and C4B1. *Complement* **5**, 110.
120. Sturfelt, G., Bengtsson, A., Klint, C., Nived, O., Sjoholm, A., and Truedsson, L. (2000). Novel roles of complement

in systemic lupus erythematosus: Hypothesis for a pathogenetic vicious circle. *J. Rheumatol.* **27**, 661–663.
121. Salmon, M., and Gordon, C. (1999). The role of apoptosis in systemic lupus erythematatosus. *Rheumatology* (*Oxford*) **38**, 1177–1183.
122. Reilly, C. M., and Gilkeson, G. S. (2002). Use of genetic knockouts to modulate disease expression in a murine model of lupus, MRL/lpr mice. *Immunol. Res.* **25**, 143–153.
123. Holers, V. M. (2000). Phenotypes of complement knockouts. *Immunopharmacology* **49**, 125–131.
124. Watanabe, H., Garnier, G., Circolo, A., Wetsel, R. A., Ruiz, P., Holers, V. M., Boackle, S. A., Colten, H. R., and Gilkeson, G. S. (2000). Modulation of renal disease in MRL/lpr mice genetically deficient in the alternative complement pathway factor B. *J. Immunol.* **164**, 786–794.
125. Chen, Z., Koralov, S. B., and Kelsoe, G. (2000). Complement C4 inhibits systemic autoimmunity through a mechanism independent of complement receptors CR1 and CR2. *J. Exp. Med.* **192**, 1339–1352.
126. Einav, S., Pozdnyakova, O. O., Ma, M., and Carroll, M. C. (2002). Complement C4 is protective for lupus disease independent of C3. *J. Immunol.* **168**, 1036–1041.
127. Taylor, P. R., Nash, J. T., Theodoridis, E., Bygrave, A. E., Walport, M. J., and Botto, M. (1998). A targeted disruption of the murine complement factor B gene resulting in loss of expression of three genes in close proximity, factor B, C2, and D17H6S45. *J. Biol. Chem.* **273**, 1699–1704.
128. Matsumoto, M., Fukuda, W., Circolo, A., Goellner, J., Strauss-Schoenberger, J., Wang, X., Fugita, S., Hidvegi, T., Chaplin, D. D., and Colten, H. R. (1997). Abrogation of the alternative complement pathway by targeted deletion of murine factor B. *Proc. Natl. Acad. Sci. USA* **94**, 8720–8725.
129. Mitchell, D. A., Taylor, P. R., Cook, H. T., Moss, J., Bygrave, A. E., Walport, M. J., and Botto, M. (1999). Cutting edge: C1q protects against the development of glomerulonephritis independently of C3 activation. *J. Immunol.* **162**, 5676–5679.
130. Mitchell, D. A., Pickering, M. C., Warren, J., Fossati-Jimack, L., Cortes-Hernandez, J., Cook, H. T., Botto, M., and Walport, M. J. (2002). C1q deficiency and autoimmunity: The effects of genetic background on disease expression. *J. Immunol.* **168**, 2538–2543.
131. Botto, M., Dell-Agnola, C., Bygrave, A. E., Thompson, E. M., Cook, H. T., Petry, F., Loos, M., Pandolfi, P. P., and Walport, M. J. (1998). Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature Genet.* **19**, 56–59.
132. Ellman, L., Green, I., and Judge, F. (1971). *In vivo* studies in C4-deficient guinea pigs. *J. Exp. Med.* **134**, 162.
133. Bottger, E. C., Hoffmann, T., and Hadding, U. (1985). Influence of genetically inherited complement deficiencies on humoral immune response in guinea pigs. *J. Immunol.* **135**, 4100.
134. Ochs, H. D., Wedgwood, J., and Frank, M. M. (1983). The role of complement in induction of antibody responses. *Clin. Exp. Immunol.* **53**, 208.
135. Ross, G. D., Yount, W. J., Walport, M. J., Winfield, J. B., Parker, C. J., Fuller, C. R., Taylor, R. P., Myones, B. L., and Lachmann, P. J. (1985). Disease-associated loss of erythrocyte complement receptors (CR1, C3b receptors) in patients with systemic lupus erythematosus and other diseases involving autoantibodies and/or complement activation. *J. Immunol.* **135**, 2005–2014.
136. Duffy, J., Lidsky, M. D., and Sharp, J. T. (1976). Polyarthritis, polyarteritis and hepatitis B. *Medicine* (*Baltimore*) **55**, 19.
137. Milich, D. (2002). Influence of C4A deficiency on nonresponse to HBsAg vaccination: A new immune response gene. *J. Hepatol.* **37**, 396.
138. Hohler, T., Stradmann-Bellinghausen, B., Starke, R., Sanger, R., Victor, A., Rittner, C., and Schneider, P. (2002). C4A deficiency and nonresponse to hepatitis B vaccination. *J. Hepatol.* **37**, 387.
139. Ross, S. C., and Densen, P. (1984). Complement deficiency states and infection: Epidemiology, pathogenesis and consequences of Neisserial and other infections in an immune deficiency. *Medicine* (*Baltimore*) **63**, 243–273.
140. Atkinson, J. P., Kaine, J. L., Holers, V. M., and Chan, A. C. (1986). Complement and the rheumatic diseases. *In* "Immunobiology of the Complement System: An Introduction for Research and Clinical Medicine" (G. D. Ross, ed.), pp. 197–211. Academic Press, Orlando.
141. Uko, G., Christiansen, F. T., and Dawkins, R. L. (1986). Serum C4 concentrations in the monitoring of systemic lupus erythematosus: Requirements for C4 allotyping. *Rheumatol. Int.* **6**, 111–114.
142. Zhang, W. J., Kay, P. H., and Cobain, T. J. (1988). C4 allotyping on plasma or serum: Application to routine laboratories. *Hum. Immunol.* **21**, 165.
143. Kramer, J., Gyodi, E., and Fust, G. (1989). Usefulness of densitometry in typing of human complement component C4. *Immunogenetics* **29**, 121.
144. Sim, E., and Cross, S. J. (1986). Phenotyping of human complement component C4, a class-III HLA antigen. *Biochem. J.* **239**, 763.
145. Hourcade, D., Miesner, D. R., Bee, C., Zeldes, W., and Atkinson, J. P. (1990). Duplication and divergence of the aminoterminal coding region of the complement receptor 1 (CR1) gene. *J. Biol. Chem.* **265**, 974–980.
146. Chrispeels, J., Bank, S., Rittner, C., and Bitter-Suermann, D. (1989). Sandwich enzyme-linked immunosorbent assays for the quantification of the C4 isotypes (C4A and C4B) in human plasma. *J. Immunol. Methods* **125**, 5.
147. Hsiung, L.-M., Mason, D. W., and Dodds, A. (1987). A monoclonal antibody which can distinguish between the two isotypes of human C4. *Mol. Immunol.* **24**, 91.
148. Dawkins, R. L., Tokunaga, K., and Saueracker, G. (1989). MHC gene arrangements on disease associated supratypes revealed by pulsed field gel electrophoresis. *In* "Immunobiology of HLA" (B. Dupont, ed.), Springer Verlag, New York.
149. Palsdottir, A., Fossdal, R., and Arnason, A. (1987). Heterogeneity of human C4 gene size: A large intron (6.5 kb) is present in all C4A genes and some C4B genes. *Immunogenetics* **25**, 299.

150. Yu, C. Y., and Campbell, R. D. (1987). Definitive RFLPs to distinguish between the human complement C4A/C4B isotypes and the major Rodgers/Chido determinants: Application to the study of C4 null alleles. *Immunogenetics* **25**, 383.
151. Mauff, G., Luther, B., Schneider, P. M., Rittner, C., Stradmann-Bellinghausen, B., Dawkins, R., and Moulds, J. M. (1998). Reference typing report for complement component C4. *Exp. Clin. Immunogenet.* **15**, 249–260.
152. Liszewski, M. K., Kahl, L. E., and Atkinson, J. P. (1989). The functional role of complement genes in SLE and Sjogren's syndrome. *Curr. Opin. Rheumatol.* **1**, 347–352.
153. Hughes-Jones, N. E. (1986). The classical pathway. *In* "Immunobiology of the Complement System" (G. D. Ross, ed.), p. 21. Academic Press, San Diego.
154. Liszewski, M. K., and Atkinson, J. P. (1991). The role of complement in autoimmunity. *In* "Systemic Autoimmunity" (M. R. Reichlin and P. Bigazzi, eds.), pp. 13–37. Dekker, New York.
155. Kruskall, M. S., Alper, C. A., and Yunis, E. J. (1990). HLA-homozygous donors and transfusion-associated graft-versus-host disease. *N. Engl. J. Med.* **322**, 1005.
156. Glass, D., Raum, D., Gibson, D., Stillman, J. S., and Schur, P. H. (1976). Inherited deficiency of the second component of complement: Rheumatic disease associations. *J. Clin. Invest.* **58**, 853–861.
157. Schur, P. H. (1982). Complement and lupus erythematosus. *Arthritis Rheum.* **25**, 793–798.
158. Sullivan, K. E., Petri, M. A., Schmeckpeper, B. J., McLean, R. H., and Winkelstein, J. A. (1994). Prevalence of a mutation causing C2 deficiency in systemic lupus erythematosus. *J. Rheumatol.* **21**, 1128–1133.
159. Dunkley, H., Gatenby, P. A., and Hawkins, B. R. (1987). Deficiency of C4A is a genetic determinant of systemic lupus erythematosus in three ethnic groups. *J. Immunogenet.* **14**, 209.
160. Davies, E. J., Hillarby, M. C., Cooper, R. G., *et al.* (1993). HLA-DQ, DR and complement C4 variance in systemic lupus erythematosus. *Br. J. Rheumatol.* **32**, 870.
161. Schur, P. H., Marcus-Bagley, D., Awdeh, Z., Yunic, E. J., and Alper, C. A. (1990). The effect of ethnicity on major histocompatibility complex complement allotypes and extended haplotypes in patients with systemic lupus erythematosus. *Arthritis Rheum.* **33**, 985.
162. Granados, J., Vargas-Alarcon, G., Andrade, F., *et al.* (1996). The role of HLA-DR alleles and complotypes through the ethnic barrier in systemic lupus erythematosus in Mexicans. *Lupus* **5**, 194.
163. Dunkley, H., Gatenby, P. A., and Hawkins, B. R. (1987). Deficiency of C4A is a genetic determinant of systemic lupus erythematosus in three ethnic groups. *J. Immunogenet.* **14**, 209.

6

COMPLEMENT DEFICIENCY AND SYSTEMIC LUPUS ERYTHEMATOSUS

György Ábel
Vincent Agnello

The exact etiology of systemic lupus erythematosus (SLE) is not known. Evidence from human and animal studies suggests that multiple factors, including genetic, endocrine, and environmental components, are involved. Hereditary complement deficiency, especially deficiency of an early complement component of the classical pathway, is a genetic factor associated with SLE and SLE-like disease in humans and most recently in mouse models with "knockout" of these components. In addition to hereditary deficiencies of complement, acquired deficiencies of the early components that persist over long periods of time may be similarly associated with SLE and SLE-like disease. The clinical observation that both genetic and acquired deficiencies of the early components are associated with SLE first suggested that deficiencies of the early complement components lead to SLE and SLE-like disease in some patients and that these components had distinct biologic activities that were part of the host's defense to this disease [1, 2]. Since those initial observations, clinical data accumulated on patients with early complement component deficiencies (reviewed in this chapter), the delineation of the genetics of the early complement components, and complement component "knockout" mice models (reviewed in Chapter 5) have proved evidence supporting this hypothesis.

This chapter reviews the association of hereditary and acquired complement deficiency states with SLE and SLE-like disease. The emphasis is on clinical manifestations in patients with these deficiencies, the differences in many patients from classic SLE, and the implication of these differences for current hypotheses on the specific role of the early complement components in SLE. The chapter begins with a description of the complement system. Next, the biological activities and the associations of deficiencies of the complement system with disease are described to provide the context for this review on the early components. The genetics of complement components and the clinical manifestations of genetic and acquired complement deficiencies are then reviewed with the exception of the genetics of the C4 component of complement are reviewed in the previous chapter (Chapter 5). The chapter concludes with a speculative discussion of the role of complement deficiency in SLE, including recent studies and hypotheses on the role of early complement components in apoptosis.

THE COMPLEMENT SYSTEM

The complement system consists of numerous proteins: plasma proteins that are inactive enzyme precursors, plasma or cell-bound regulators proteins, and cell membrane glycoproteins, which serve as specific receptors for complement protein cleavage fragments (Tables 1–3). Twelve proteins are present in plasma as inactive precursors, which can be activated by a complex series

of highly specific biochemical reactions. Seven of the components (C1r, C1s, C2, C3, C5, B, and D) have proteolytic activity when activated. Activation for C2, C3, C4, and C5 involves limited proteolytic cleavage and results in the generation of biologically active peptide fragments. Eight additional plasma and cell-bound proteins have been identified that regulate the activation and degradation of all complement proteins and cleavage fragments.

TABLE 1 Proteins of the Complement System

	Molecular weight	Serum concentration (μg/ml)
Classical pathway		
C1q	460,000	65
C1r	83,000	50
C1s	88,000	40
C4	206,000	640
C2	117,000	25
C3	180,000	1,600
Alternate pathway		
C3	180,000	1,600
Factor B	93,000	200
Factor D	24,000	1
Lectin-binding pathway		
MBL	32,000 (subunit)	1
MASP-1	100,000	7
MASP-2	75,000	??
Membrane-attack pathway		
C5	180,000	80
C6	128,000	75
C7	121,000	55
C8	154,000	55
C9	79,000	60

Classical Pathway Activation

Activation of the complement system is initiated in three different ways (Fig. 1). There is a marked difference in the mode of activation for the three different pathways. Antibody–antigen complexes initiate activation of the classical pathway, whereas alternative pathway activation occurs in the absence of antibody. The classical activation pathway involves proteins C1q, C1r, C1s, C2, C4, and C3. The C1 component consists of three subunits: C1q, C1r, and C1s. The C1q molecule consists of six units of polypeptide chains, A, B, and C in triple helices that form a bouquet-like structure with six globular "flowers" at the carboxyl-terminus end, and a stalk that has a collagen-like composition at the amino-terminal end. The six globular structures each contain a combining site that are receptors for a specific sequence found on the Fc portion of IgG1, IgG2, IgG3, and IgM. The collagen portion can bind a variety of polyanionic substances, including DNA [3, 4] that provides a potential nonimmune activation pathway. Immunoglobulins, when complexed to an antigen, will bind C1q and cause activation of the molecule. Activated C1q generates enzymatic activity in C1r, which cleaves C1s and generates the first major enzyme in the complement system, C1 esterase (C1). C1 esterase then cleaves C4 and C2, whose major cleavage products form a bimolecular complex, C4b2a, which has C3 convertase enzymatic activity. The C3 convertase cleaves C3 and generates a small fragment, C3a, and a larger fragment, C3b. Attachment of C3b to the C4b2a complex forms the C4b2a3b complex, which exhibits C5 convertase activity. The generation C5 convertase leads to activation of the membrane attack complex.

TABLE 2 Complement Regulatory Proteins in Serum

Protein	Abbreviation	Molecular weight	Serum concentration (μg/ml)	Regulatory action
C1 esterase inhibitor	C1-INH	90,000	180	Binds to activated C1r, C1s, inactivates C1qrs esterase
C4 binding protein	C4bp	560,000	250	Enhances decay of C4b2a and cleavage of C4 by I
Factor I	I	88,000	34	Inactivates C4b and C3b
Factor H	H	150,000	500	Enhances decay of C3bBb and cleavage of C3b by I
Anaphylatoxin inactivator		310,000	35	Inactivates C3a, C4a, and C5a
S protein (vitronectin)	S	83,000	300	Binds to and inhibits the C5b-7 complex
SP40, 40 (clusterin) (apolipoprotein J)	Apo J	80,000	75	Binds to C5b-6 and prevents conversion to C5b-7
Properdin	P	220,000	20	Enhances stability of C3 and C5 convertases

TABLE 3 Cell Membrane Recognition and Regulatory Proteins

Protein	Molecular mass (kDa)	Cell type[a]	Complement protein bound	Protein function
CR1 (CD35)	160, 190, 220, or 250	PMNs, RBCs, T and B lymphocytes, eosinophils, monocytes, macrophages, dendritic cells, glomerular podocytes	C3b, C4b, iC3b, C1q	Control of C3 breakdown, immune complex binding
CR2 (CD21)	145	B lymphocytes, NK cells, follicular dendritic cells	C3d, C3dg, iC3b, C3b, C3c	Regulation of B cells, EBV receptor
CR3 (CD11b/CD18)	165, 95 dimer	PMNs, monocytes, macrophages,	iC3b	Mediates phagocytosis, NK cells, cytotoxic T cells enhances antibody synthesis
CR4 (CD11c/CD18)	150, 95	PMNs, monocytes, macrophages, NK cells, cytotoxic T cells	C3d, g	Unknown
MCP (CD46)	45в70	Lymphocytes, PMNs, monocytes, platelets, endothelial cells, epithelial cells, fibroblasts	C3b, C4b	Control of C3 cleavage
DAF (CD55)	70	Lymphocytes, PMNs, monocytes, platelets, endothelial cells, epithelial cells, RBCs, fibroblasts	C4b2a, C3bBb	Decay of C3 and C5 convertases
MIRL (CD59)	19	RBCs, renal cells, all WBCs, endothelial cells, fibroblasts	C5b-8	Prevents formation of MAC complex
HRF	65	RBCs	C8, C9	Prevents insertion of MAC into cell membranes
C3a/4a receptor	?	PMNs, mast cells, macrophages	C3a, C4a	Binding anaphlatoxins
C5aR (CD88)	50	PMNs, eosinophils, monocytes, macrophages, mast cells, platelets, vascular endothelial cells	C5a	Binding anaphylatoxin
gC1q (globular)	33	PMNs, B cells, monocytes, macrophages	C1q heads	Mediates phagocytosis
C1qRp (collagen) of adhesion molecules	126	Myeloid cells, monocytes, macrophages, vascular endothelium	C1q tails	Mediates phagocytosis, upregulation
cC1qR complex	60	Wide cell distribution, WBCs, platelets, fibroblasts, smooth muscle, vascular endothelium	C1q tails, MBL, SP-A, SP-B (collectins)	Mediates phagocytosis, immune binding
C3e R	?	PMNs	C3e	Release of PMNs from bone marrow
Factor H receptor	?	PMNs, B cells, macrophages	H	?
Factor Ba receptor	?	PMNs, B cells, macrophages	Ba	?

[a] NK, natural killer; WBC, white blood cells.

Alternate Pathway Activation

Activation via the alternate pathway involves factors B, D, and C3, but the specific molecular interactions are not as clearly understood as for the classical pathway. No specific recognition sites analogous to the antibody–C1q interaction have been identified for the alternate pathway. It is known that activation occurs on the surface of certain fungi, bacteria, lymphoblastoid cells, and virus-infected mammalian cells, which have repetitive polysaccharide or lipopolysaccharide structures and lack sialic acid. Alternate pathway activation is mediated by C3, which contains a thioester bond that may hydrolyze spontaneously and form covalent bonds with certain types of chemical compounds. This may lead to the conversion of C3 to a C3b-like molecule, which interacts with B and magnesium. This C3B complex is then cleaved by B and is then assembled

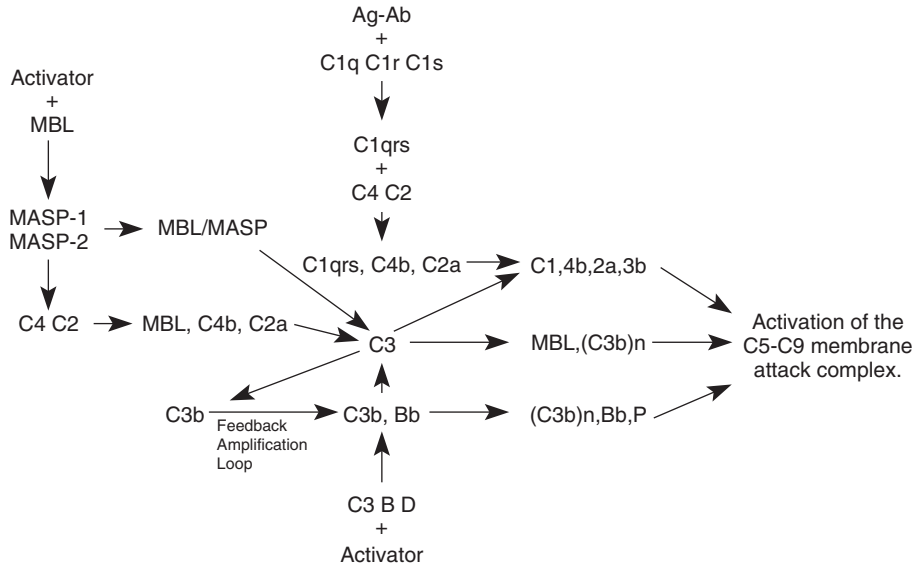


FIGURE 1 Simplified scheme of the pathways for complement activation. Table 1 lists the symbols used for each complement component. Activation fragments are designated by lowercase letters.

into larger complexes containing multiple molecules (C3bBb)n, and the C3 convertase molecule is generated. The C3 convertase molecule then cleaves additional C3 molecules, which also bind B and ultimately generates the C5 convertase that leads to the activation of the membrane attack complex.

The activation pathways utilize different complement components but are similar in that both pathways generate C3 convertase and C5 convertase enzymes. The C3 convertases generated are C4b2a and C3bBb. These are structurally similar complexes in that the C2 and factor B proteins have very similar amino acid sequence and are encoded by two genes closely linked on chromosome 6 thought to have arisen by gene duplication. The C5 convertases C4b2a3b and (C3bBb) are also very similar in that both contain activated C3b molecules. The cleavage of C5 by esterases generated by either pathway initiates the activation of the remaining complement proteins C5–9 and the assembly of the membrane attack complex.

Mannan-Binding Lectin Activation Pathway

A third pathway of complement activation was first reported by Ikeda *et al.* [5] where mannan-binding lectin (MBL) was demonstrated to activate the classical pathway. MBL is a protein with a complex structure composed of both a collagenous region and a lectin-binding domain. MBL has a "bouquet-like" structure with some structural similarity and multiple binding domains similar to C1q and IgM. MBL binds to a wide variety of sugars, including *N*-acetylglucosamine, mannose, *N*-acetylmannosamine, fucose, maltose, glucose, galactose, and *N*-acetylgalactosamine. This lectin-binding activity is weak with single sugars but the functional avidity becomes very strong with repeating sugar structures such as those found on a variety of bacterial cell surfaces, yeasts, and viruses, including human immunodeficiency virus (HIV)-1, HIV-2, and influenza A. In plasma, MBL is associated with a second protein, mannose-associated serine protease (MASP), which has a similar structure and function to C1r and C1s. A second protease, MASP2, which has structural homology to MASP, C1r, and C1s, may also be associated with the MBL/MASP molecule [6]. When the MBL/MASP is activated by binding to multiple sugar molecules, the serine protease is capable of cleaving both C4 and C2, thereby activating the complement pathway in a manner analogous to that of the C1qrs complex. In addition, the MBL/MASP complex can also cleave C3 directly and activate the complement pathway in this method [7]. This MBL/MASP activation is downregulated by α_2-macroglobulin in a manner analogous to the C1 esterase inhibitor (C1-INH) control mechanism [8, 9]. *In vitro* MBL can also associate with the activated C1r–C1s complex and cause C4 and C2 activation.

Membrane Attack Complex Activation

The membrane attack complex utilizes complement components C5, 6, 7, 8, and 9. Initially, cleavage of C5 by C5 convertase produces a small fragment C5a and a larger fragment C5b. The latter forms a stable trimolecular complex with C6 and C7. The complex has the

ability to bind to biological membranes. C7, C8, and C9 are then adsorbed to the complex. After initiation by the enzymatic cleavage of C5, the self-assembly of the membrane attack complex occurs without further enzymatic activity. The complex, when fully assembled, forms a cylindrical structure that extends through the entire lipid bilayer. Electron microscopy has been used to demonstrate that the structure has a circular shape with a central hole. Assembly of the attack complex disrupts the cell membrane and results in cell lysis. Leakage of the cell membrane occurs with C8 binding and is accelerated with the addition of C9.

Regulation and Control of Complement Activation and Cell Lysis

A large number of proteins have been identified that influence the initial activation, amplification, cell membrane deposition, and biological effects of the complement components (Table 2). Nine plasma proteins influence the activation, assembly, and stability of complement esterase enzymes in the fluid phase, whereas four transmembrane proteins influence the activity and stability of C3b and the deposition and stability of the membrane attack complex on cell membranes. The plasma proteins that influence the classical and alternate pathway activation steps are (1) C1-INH, which binds to both activated C1r and C1s and causes disassembly of the activated C1qrs complex; (2) C4BP, which causes decay of the C4b2a complex and increases the C4b cleavage by factor I; (3) H, which causes decay of the C3bBb complex and increases the C3b cleavage by factor I; (4) I, which degrades C4b and C3b; (5) S protein, which binds to C5b–7 and prevents binding of the complex to cell membranes; (6) decay accelerating factor (DAF), which causes decay of the C3 and C5 convertases; (7) serum apolipoprotein SP40, 40 (clusterin) binds to C5b–6 and prevents binding of C7, and also binds to the soluble SC5b–9 complex in solution; and (8) properdin, which binds to C3 and C5 convertases of the alternate pathway and stabilizes them. The first eight proteins all exert negative effects on the activation and amplification of the complement system, whereas properdin significantly, positively amplifies alternative pathway activation. Two other proteins that have factor H-like activity have been identified in plasma: factor H-like protein 1 (FHL-1) and factor H-related 1 β-protein [10]. Both of these proteins have cofactor activity to enhance the factor I-mediated cleavage of C3b.

Six transmembrane glycoproteins, H, CR1, MCP (membrane cofactor protein), DAF, MIRL (membrane inhibitor of reactive lysis), and HRF (homologous restriction factor) have been identified and characterized that influence complement protein functions (Table 3). CR1, MCP, decay accelerating factor (DAF), and H all affect the binding and stability of C3b complexes to the cell membrane. H, DAF, and CR1 cause disruption of the C3bBb complex, the activity described as DAF. Factor H, MCP, and CR1 all cause degradation of C3b into fragments iC3b and C3f. CR1 and I then cause further degradation of iC3b into C3c and C3dg. These activities take place at the cell membrane, but secreted and transmembrane forms of factor H and DAF have been identified that may exert inhibitory effects in the plasma phase as well as at the cell membrane. Because DAF and MCP are found on many cell types, they are thought to be involved in protection of a variety of cells from cell lysis. HRF and MIRL inhibit the binding of the membrane attack complex (MAC) into the cell membrane, HRF binds to C9 and C9, and MIRL binds to C5b–9 to prevent polymerization of C9.

Seven additional transmembrane glycoproteins have been identified that bind complement components and mediate a variety of biological functions: CR2, CR3, CR4, C3aR, C5aR, cC1qR, gC1qR, and mannose-binding lectin receptor (MBLR) (Table 3). CR2 (CD21) found on B cells binds iC3b, C3d, g, and C3d-containing immune complexes. Immune complex binding to B cells has been shown to augment B-cell responses and increase the efficiency of antigen processing by B cells [11]. Coligation of the antigen receptor and CR2 on the B-cell membrane is also important in the immune response to T-dependent antigens, which is mediated by the CD21 ligand CD23 [12]. CR2 is also the B-cell membrane receptor for the Epstein–Barr virus (EBV). CR3 (CD11b/CD18) and CR4 (CD11c/CD18) both bind iC3b and immune complexes containing iC3b. Binding of the complexes causes cell activation and a variety of biological responses. Glycoprotein inositol (GPI)-linked proteins including CD14, CD16b, and CD87 are associated both physically and functionally with CR3. In a number of studies reviewed [13], studies have shown that glycoprotein inositol (GPI)-linked proteins utilize CR3 to signal across membranes. Studies have indicated that the CR3 surface protein may be a major binding site for *Borrelia burgdorferi* (Lyme spirochete) and *Mycobacterium tuberculosis* and thus play a major role in the binding of the organisms to phagocytes in the absence of immunoglobulin or complement components [14, 15]. C3aR and C5aR bind the anaphylatoxin complement fragments and mediate the biological activities of blood vessel dilation, smooth muscle contraction, and chemotaxis. There are at least three receptors that bind C1q: cC1qR, which binds to the collagen portion of the C1q molecule; gC1qR, which binds to the globular heads portion of the C1q molecule; and CR1, which binds C1q in addition to C3b. cC1qR has also been reported to bind mannan-binding lectin and

conglutinin. A second MBL-binding receptor (MBLR) has also been described that has sequence homology with calreticulin.

BIOLOGICAL ACTIVITIES OF THE COMPLEMENT SYSTEM

Cytolysis appears to be a main function of the complement system that requires a complete sequence of activation of most of the soluble complement components. In addition to cytolysis, a variety of other biological activities are mediated by proteins of the complement system. The major activities associated with various complement components are shown in Table 4. For activation of the membrane attack system, all components of the classical or alternative pathway are required. The cytolytic process is more efficient in the classical pathway. The actual lysis of the membrane is a function of C8 and C9 molecules binding to the cell membrane and C9 molecules polymerizing into a circular complex.

Opsonization of particles is a second major biological function of the complement system. Opsonization is mediated by the C1q, MBL, and the C3b split products of C3 generated during complement activation. Opsonic activity can be generated either by antibody–antigen complexes or in the absence of specific antibody by alternative pathway activation or MBL binding and complement activation [16]. Alternate pathway-activated opsonization and MBL-mediated opsonization are critical host defense systems for unimmunized individuals.

TABLE 4 Major Biological Activities of Complement Components

Activity	Component
Enhancement of phagocytosis	C3b, MBL
Viral neutralization	C1C4b
Viral lysis initiation	C1q
Clearance of apoptotic debris	C1q, C4b
Enhancement of yeast phagocytosis	C5b, MBL
Solubilization of immune complexes	C3, B, D, P, H, I
Clearance of immune complexes	C3b, CR1
Mediators of inflammation	
Chemotactic factor	C3a, C5a, C5b67
Anaphylatoxin	C3a, C5a
Enhanced vascular permeability	C2 fragment (C2 kinin)
Marrow leukocyte release	C3e
Cell lysis	C8, C9
Modulation of immune response	C4b, C3b, C3d, CR2

A second major function of complement is the clearance of soluble immune complexes [17]. This activity is mediated by receptors for the activated complement components that are found in a variety of cell types. The majority of immune complex clearance is usually done via binding to the CR1 receptor present on red blood cells (RBCs). RBCs transport the complexes to the spleen where they are removed by macrophages and other cells of the reticuloendothelial system.

A major biological activity of the alternative pathway, which has been demonstrated *in vitro*, is that six components of the alternative pathway alone are capable of solubilizing insoluble immune complexes. This may represent a major physiologic mechanism for processing immune complexes and hence a host defense system to immune complex disease.

Other defense mechanisms may be mediated by the early components of the complement system. *In vitro* studies demonstrated that C1 and C4b are sufficient for the neutralization of certain viruses coated with nonneutralizing antibodies. Other studies have demonstrated a direct role for complement in the lysin of virally infected cells. In several studies, C1q bound directly to oncornaviruses in the absence of antibody. Although antigen–antibody complexes are the major mode of activating the classical pathway, evidence shows that C1 can be activated by diverse other substances in the complete absence of antibody [18, 19]. These substances include a wide variety of proteins, lipids, carbohydrates, and polyionic molecules.

Additional biological activities are mediated by other cleavage products of complement components. Patients with hereditary angioneurotic edema produce a peptide, C2 kinin, which enhances vascular permeability. The C2 peptide is generated by the cleavage of C2. C3a has both chemotaxic activity and anaphylatoxic activity. A breakdown product of C3b, C3e, appears to induce leukocyte release from the marrow. C5a has a relatively weak anaphylatoxic activity but a potent chemotaxic activity. C5b has been shown to enhance the phagocytosis of yeast. The complex C5b, 6, 7 has also been shown to have chemotaxic activity. Some complement fragments are also involved in modulation of the immune response by their specific receptors on lymphocytes.

The biological activities of the complement components are regulated and mediated by plasma and membrane-bound complement receptors. The anaphylatoxin inactivator (carboxypeptidase B) exerts a negative effect on C3a, C4a, and C5a, enzymatically cleaving a terminal arginine residue and inactivating the anaphylatoxin activity. At least 16 different transmembrane proteins have been identified that have specificity for activated complement fragments (Table 3). These recep-

tors bind complement components and then play a role in activation of the cells and mediating the biological roles of those cells in phagocytosis (CR1, CR3, CR4), B-cell function (CR2), anaphylatoxic responses (C3aR and C5aR), and lymphocyte migration (C3e). The biological functions mediated by CR4 and the receptors for C1q, C3aR, and C5aR have not yet been well characterized.

GENETICS OF COMPLEMENT COMPONENTS

Genes encoding proteins of the complement system are clustered on at least three different chromosomes (see also Chapters 4 and 5). The genes for C2, factor B, and two genes for C4, C4A and C4B, are located on the short arm of chromosome 6 in the major histocompatibility (HLA) complex [20–22] (see Fig. 2). In this chromosomal area are genes that control three different host defense systems: self-recognition (HLA-A, B, C), immune response (HLA-Dr, Dp, Dq), and complement (C2, C4A, C4B, B). Factor B and C2 genes are closely linked on the chromosome and are separated from each other by only 421 bases. They are about 30 kb from the C4A locus, which is separated from the C4B locus by 10 kb. As seen with the HLA class I and class II genes, the complement genes in the HLA complex show a high degree of polymorphism. This polymorphism is present at both protein and DNA levels. Because these four genes are closely linked, they are usually inherited as a single group and the polymorphisms defined by complotypes.

For patients with hereditary C2 deficiency, a single 28-bp mutation that results in a pseudogene incapable of producing C2 protein is present in essentially all Caucasian cases. The majority of patients have the complotype S042 and the HLA B18, DR2 phenotype [23]. In studies in Sweden [24], the prevalence of the 28-bp deletion was found to be 5.8% of SLE patients; 5 of 86 patients were heterozygous. In Brazilian studies [25], the prevalance was 6.6% in SLE patients and 2.2% for blood donors. In this study, patients with heterozygous C2 had an earlier disease onset, a higher prevalance of oral ulcerations, and a lower prevalance of anti-dsDNA antibodies compared to patients with normal C2 levels. In North American studies [26], the 28-bp deletion gene frequency was 0.0246 for SLE patients and 0.0070 for normal controls. The 28-bp deletion was not seen in 127 Black SLE patients or 194 Black controls.

For factor B, four allelic forms have been identified (S, F, F1, and S1) with gene frequencies of 0.71, 0.27, 0.01, and 0.01, respectively. Additional polymorphisms can also be demonstrated for factor B by restriction fragment length polymorphism (RFLP) [27]. Complotypes missing the factor B gene have not yet been described [28].

Polymorphism of C4 is very complex. There are two genes, C4A and C4B, that encode C4 proteins and are highly homologous at both protein and DNA sequence levels. For a review of the genetics of C4, see Chapter 5.

Polymorphism has also been demonstrated for the other complement proteins—C3, C6, C7, and C8 [28]—but is much more limited than that which exists for genes within the HLA complex. Polymorphism has not been identified at the protein level for C1q, C1r, C1s, C5, or C9. The C6, C7, C8, and C9 proteins are all structurally similar to each other; C6 and C7 are single polypeptides, and C8 and C9 are dimers containing α and β chains. The genes for C6, C7, and the α and β chain genes of C8 are located on chromosome 1; C6 and C7 genes are very closely linked. An increased incidence of deficiencies of C6 and C8 has been identified in Blacks and deficiencies of C7 and C9 in Japanese. Two types of C8 deficiencies have been identified: type I, in which there are low levels of the β chain but normal levels of the α chain (which is functionally normal), and type II, in which there are dysfunctional α subunits and low levels of β chains in the plasma [29]. The majority of C8 deficiencies are type I.

There are two polymorphic forms of C3: S and F. The C3F form has weakly been associated with glomerulonephritis. Studies comparing the S and F forms have demonstrated that the S form was slightly more efficient in hemolytic assays than the F form [30]. No differences were detected in the ability of the S and F forms to interact with factors H, 1, and CR1. The C3 protein has significant homology to both C4 and C5 proteins. The C3 gene is located on chromosome 19. C3 deficiencies

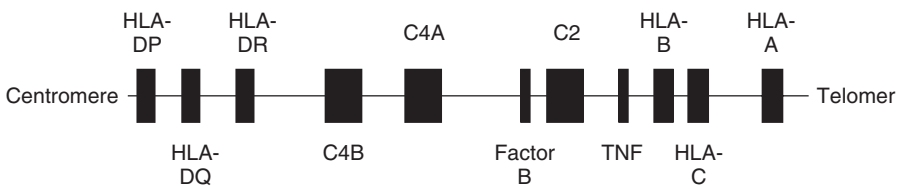


FIGURE 2 Simplified representation of the HLA gene complex of chromosome 6.

are very rare; in the single case analyzed at the DNA level, a stop codon was present within exon 18, leading to premature termination of the MRNA, and no C3 protein product was produced.

The C1 molecule is composed of one C1q, two C1r, and two C1s molecules. C1r and C1s are highly homologous proteins, each containing two short consensus repeats (SCRs; see later). The molecules contain low-density lipoprotein (LDL) receptor type B sequences and serine protease portions. The genes for C1r and C1s are closely linked within 50 kb and and are found on chromosome 12. The genetics of C1q have been reviewed previously [31]. Three different genes within 24 kb on chromosome 1 encode the A, B, and C, and chains of C1q. In contrast to other complement component genes, there is no polymorphism of the C1q genes. Single base mutations in one of the three genes are thought to be responsible for the C1q deficiency phenotype. Two types of C1q deficiencies have been identified. In type I, no C1q protein is assembled because premature chain termination takes place. In type II C1q deficiency, the quantity of C1 is normal but the molecule is nonfunctional. C1q molecules that cannot polymerize into the high molecular weight complex are formed and result in excess low molecular weight C1q in the plasma. Only individuals homozygous for the defective C1q genes have the C1q deficiency phenotype; heterozygous individuals have normal C1q and CH_{50} levels.

The MBL gene has four exons and encodes a peptide chain of 32 kDa. Three of these peptide chains associated together to form a basic subunit that has an N-terminal "neck" region, a collagenous triple helix "arm," and a C-terminal "flower" that is the lectin-binding site. MBL can exist as dimers, trimers, tetramers, pentamers, and hexamers of this basic subunit bound together by disulfide bonds in the "neck" region. The three-dimensional structure of the MBL molecule is very analogous to the C1q structure. In the human plasma, the majority of MBL is dimer, trimer, or tetramer in structure. The MBL gene contains four exons and is located on the long arm of chromosome 10 in a gene cluster that also contains the genes for SP-A and SP-D and a pseudogene SP-A. The DNA sequence has features typical for other acute-phase proteins in the 5N region, including a heat shock consensus element, three glucocorticoid responsive elements, and a sequence with similarity to that of serum amyloid A protein. The gene encodes protein with homology to a family of other proteins of the collectin family that includes pulmonary surfactant proteins SP-A and SP-D and conglutinin. Three different point mutations that cause immunodeficiency and significantly decreased MBL plasma levels have been identified [32, 33]. The mutations are in exon 1 at codons 52, 54, or 57 and cause amino acid substitutions that influence the protein function. The changes at 54 and 57 disrupt the structure of the collagen-like region and result in aberrant subunit structures that cannot form multimers. In addition, the codon 54 change results in the inability of the MASP protein to bind to the MBL protein and results in a lack of function of the complex [34]. The codon 54 and 57 mutations show dominant inheritance, and heterozygous individuals have MBL less than 10% of normal plasma levels. The change at position 52 introduces an additional cysteine residue and may result in extra disulfide bond formation. Patients heterozygous for the codon 52 mutation have MBL levels at 50% of normal plasma levels. Patients homozygous for any of the three mutations have undetectable levels of serum MBL. MBL deficiency is common with the actual frequency of the mutation dependent on the racial makeup of the population [35]. About 20% of the Caucasian population have at least one mutant allele, either codon 52 or 54 alleles. Studies on British and Danish Caucasian populations have shown gene frequencies of about 0.16 for the codon 54 allele and 0.06 for the codon 52 allele. The frequency in Caucasian populations of individuals with homozygous codon 52 is about 0.2%, homozygous codon 54 individuals about 2.0%, and heterozygous codon 52/54 alleles about 0.6%. The codon 57 allele is found in high frequency in African populations. In a study done in the West Africa country of Gambia, the gene frequency in native Africans for codon 57 mutations was 0.29, for codon 52 was 0.02, and for codon 54 was 0.003. Gambians who were racially Caucasians (British) and Hong Kong Chinese had codon 54 gene frequencies of 0.17 and 0.11 but did not have any 57 mutations present. Levels of MBL in the serum are also influenced by genetic elements in the promoter region of the MBL gene. Three haplotypes of protein expression, HY (high), LY (intermediate), and LX (low) phenotypes corresponding to the serum MBL levels, have also been identified [36]. These phenotypes are a result of two variants in the promoter region of the molecule that are in linkage disequilibrium located at codons –221 (X or Y) and –550 (H or L). The HY haplotype has a gene frequency of 0.38 in Caucasians and 0.11 in Africans, the the LY haplotype is found at 0.38 in Caucasians and 0.65 in Africans, and the LX haplotype is found at 0.24 in Caucasians and 0.24 in Africans.

The MASP gene has been mapped to the long arm of chromosome 3 and contains six exons. The encoded protein has a molecular mass of 100 kDa and contains an epidermal growth factor-like domain, two SCR repeats, and a serine protease domain similar in structure to that of C1r and C1s. In addition, the MASP molecule has a histidine loop structure present in many

serine proteases such as trypsin and chymotrypsin [37]. It is this histidine structure that enables the molecule to have specificity for C3 cleavage.

GENETICS OF COMPLEMENT REGULATORY MOLECULES

C1-INH is a protein of the serpin family of protease inhibitors. There are two known allelic variants of C1-INH. Two types of deficiencies have been characterized: type I, in which normal molecules are produced but in levels only 5–30% of normal, and type II, in which the protein is present in normal levels but is nonfunctional. RFLP analysis of DNA from deficient patients has shown wide genetic variability at the DNA level.

Factor I is a 88-kDa plasma glycoprotein composed of two chains, a light chain (38 kDa) and a heavy chain (50 kDa) containing LDL receptor domains, a CD5 domain, and sequence unique to factor I, and a segment with sequence homology with C6 and C7 proteins. The gene for factor I is located on chromosome 4 and spans 63 kb with 13 exons. Polymorphism of factor I has been identified in Japanese individuals [38] and hereditary deficiency of factor I has been reported for 23 individuals from 19 families [39]. Factor I DNA mutations have been studied for two families [40]. In the first family, a missense mutation led to the substitution of histidine by leucine in a semiconserved area near the enzyme active site. In the second, one allele had the same mutation as the patient in the first family (although there was no evidence that they were related) and the other allele had a splice site point mutation that led to deletion of the fifth exon from the mRNA transcript. Both affected individuals had no detectable circulating factor I protein.

The four transmembrane glycoproteins, factor H, CR1, MCP, and DAF, are highly homologous molecules with similar structures. Genes encoding these molecules are closely linked on chromosome 4 in the regulators of complement activation (RCA) gene cluster [41]. Analysis of genes and amino acid sequences have identified common domain-like structures with SCRs of about 60–70 amino acids; factor H contains 20 SCRs; CR1, 33 SCRs; and DAF, 4 SCRs [42]. Similar SCRs are also found in C4BP (56 SCRs); CR2, C2, and factor B (3 SCRs); and factor I (1 SCR). These highly homologous repeated sequences may be involved in the binding of C3. Because the transmembrane proteins have many SCRs, it is possible that a large number of C3 molecules interact with a single membrane protein. Factor H is transcribed into two messages, which are 4.3 and 1.8 kb. These are transcribed into a 155-kDa transmembrane protein with 20 SCR domains and a 45-kDa protein with 7 SCRs. All identified patients with factor H deficiency have been shown to have point mutations in either SCR 9 or SCR 16 domains, which modify cysteine residues and are predicted to profoundly influence the three-dimensional structure of the 155-kDa protein [43]. Two additional factor H-like proteins, FHL-1 and FHR-1-β, have similar structures to the smaller factor H protein and each has seven repetitive SCR segments. Structures of the four NH-terminal SCRs are critical for the function of the FHL-1 and factor H molecules [10].

Four allelic forms of CR1 (CD35) have been identified: A, B, C, and D [44]. The A (or F) allotype is found with a gene frequency of 0.87 in Caucasians, 0.74 in Blacks, and 0.98 in Asians. The B (or S) allotype is found with a gene frequency of 0.12 in Caucasians, 0.22 in Blacks, and 0.02 in Asians. The C allele is only found in Blacks at a frequency of 0.04 and the D allele in Caucasians at a frequency of 0.01. CR1 molecules produced by the four different alleles are different sizes: the A protein is 190 kDa, the B protein is 220 kDa, the C protein is 160 kDa, and the D protein is 250 kDa. When the gene structure for the A, B, and C alleles was determined, significant differences in the genes for the alleles were found [45, 46]. The A gene contained 39 exons that encoded four long homologous repeats (LHRs) each of which encodes seven SCR repeated sequences. The B gene contained an additional eight exons and a fifth LHR. The C gene was truncated and only contained three LHR segments. Because each of the SCR repeated sequences binds to C3 and C4 molecules, the allelic forms could bind different quantities of complement fragments. The two most common alleles can be easily distinguished from each other at a DNA level by a *Hin*dIII restriction polymorphism detected by RFLP analysis with a CR1.1 probe [47]. The A gene produces a 7.4-kb fragment, whereas the B gene produces a 6.9-kb fragment.

The binding site for the CR1 molecule is located in residues 727–768 on the C3 molecule. This area is also involved in the binding of C3 to factor H and factor B and involves at least two areas on the C3 molecule [48]. The allelic forms of C3, C3S and C3F, bind with equal affinity to the CR1 receptor [49]. The CR1 receptor also binds the C4 molecule. Studies with C4A and C4B show that erythrocyte-bound CR1 as well as recombinant soluble CR1 (sCR1) bind with a significantly higher affinity to C4A than to C4B [50, 51]. It was also demonstrated that CRP bound to soluble ligands can also bind to the RBC CR1 [52]. This suggests that CR1 may mediate a biological function of C-reactive protein (CRP).

The C4-binding protein (C4BP) is composed of multiple α chains (70 kDa) associated with a single β chain (45 kDa). C4BP is present in a variety of isoforms in

plasma, each of which has a different number of α chains present in the molecule. The relative level of each of the isoforms is determined by genetic factors that localize in the RCA gene cluster [53]. Two genes, C4BPA and C4BPB, have been identified. The β chain contains three SCRs and contains a binding site for the anticoagulant protein S in the SCR-1 region [54]. Enzyme assays have been developed to measure the serum levels of C4BP and the protein S–C4BP complex. Levels of both of these proteins have been shown to be increased in patients with membranous nephropathy, decreased in liver cirrhosis patients, and unchanged in patients with IgA nephropathy [55]. SLE patients have not yet been studied.

Receptors for the C3a and C5a molecules have been identified and cloned [56–58]. The C5a (CD88) and C3a receptors have similar structures, both are within the G-protein superfamily of similar receptors that include receptors for IL-8, Mip-1/Rantes, thrombin, formyl peptide, and platelet-activating factor. Each of these receptors has seven hydrophobic domains that span the cell membrane and have a serpentine topology. The C5a receptor structure is complex and contains at least two separate C5a-binding sites. Only one site needs to be activated to cause intracellular signaling [59]. The C3a receptor has 37% homology to the C5a receptor but differs from it by the presence of a large extracellular loop with greater than 160 amino acids that is between transmembrane domains 4 and 5. Expression of mRNA for C3a and C5a has been found in tissues throughout the body and in the central nervous system (CNS). At least two monoclonal antibodies have been studied that react with the C5a receptor and inhibit the biological effects of C5a, which may in the future be useful to control anaphylactic responses *in vivo* [60, 61].

The C1q receptor (collectin) has been identified as a single protein chain of 60 kDa. At an amino acid level it has homology to calreticulin, a component of the SS-A antigen, an *Onchocerca* antigen, and a murine melanoma antigen. The receptor is found on leukocytes, platelets, and endothelium where it binds to C1q, mannan-binding protein (MBP), lung surfactant protein A (SP-A), and conglutinin [62]. The protein has three domains, N, C, and central P, and recombinant studies have demonstrated that the C1q-binding site spans the N and P domains [63] and binds to the collagen stalk portion of the C1q molecule. The consequence of C1q binding to its receptor varies with the cell location, binding to monocytes enhances phagocytosis, whereas binding to granulocytes, eosinophils, endothelial cells, or smooth muscle cells generates toxic oxygen molecules. These consequences may aid in the destruction of foreign pathogens but may also damage host tissues [64]. C1qR has also been shown to prevent the association of C1q with C1r and C1s and functions to control complement activity [65]. A second C1q-binding protein receptor, gC1qR, has been identified that binds to the globular heads portion of C1q. The second protein receptor is present on vascular endothelium, platelets, neutrophils, and eosinophils and binds vitronectin in addition to C1q [66]. C1q may also bind to CR1 on leukocytes and RBCs [67].

DEFICIENCIES OF THE COMPLEMENT SYSTEM AND DISEASE

Complete hereditary deficiencies have been described for many of the complement system proteins and regulatory molecules, and a variety of disease manifestations have been associated with these deficiencies (Table 5). The diseases can be grouped into two major types: rheumatologic and infectious. The rheumatologic diseases associated most frequently with complement deficiencies are SLE and SLE-like disease, and the infectious diseases are bacterial diseases, especially life-threatening infections caused by neisserial organisms. However, individuals who are completely deficient for a complement component can be free of disease so the development of disease is undoubtedly multifactorial.

Susceptibility to Infection

The important role the complement components play in hostdefenses is demonstrated by the striking association between complement deficiencies and susceptibility to infection. Deficiencies that result in significant decreases in C3 (deficiencies in C3 or factor I), the alternate pathway proteins (factor D or properdin), or in the membrane attack complex (deficiencies in C5, C6, C7, C8, or C9) are strongly associated with infections with *Neisseria* or other pyogenic bacteria. In one study of patients with *Neisseric* meningitis, 11 of the 104 patients studied were found to be completely deficient for at least one complement component [68]. In a second study of 30 patients with meningococcemia or meningitis seen in one hospital over 20 years, 3 patients (10%) had complement deficiencies. One of the 3 patients had congenital C7 deficiency and the other 2 patients had SLE. The prevalence of complement deficiency in the meningococcal disease was 100 times greater than the prevalence in the normal population [69].

The role that complement proteins and receptors play in HIV infection has been studied extensively over the last few years. The complement system functions to limit viral spreading through the infected host by activating immune cells and mediating the lysis of viral

TABLE 5 Clinical Findings in Complete[a] Hereditary Deficiency of Complement Components[b]

Deficient component	Number of reported cases	Percent with infections	Rheumatologic clinical findings
C1q	42	24	MPGN, SLE, DLE, skin rash, SLE-like disease
C1r-C1s	14	22	SLE, SLE-like, glomerulonephritis
C4	25	16	SLE, SLE-like, DLE, Henoch–Schönlein and anaphylactoid purpura, cryoglobulinemia
C2	109	10	SLE, subacute cutaneous LE, glomerulonephritis, anaphylactoid purpura, vasculitis, dermatomyositis arthritis, arthralgias, rheumatic fever, cold urticar, inflammatory bowel disease, Hodgkin's disease, idiopathic atrophodermia, common variable immunodeficiency
C3	24	60	glomerulonephritis, SLE-like
C5	27	70	SLE
C6	77	84	DLE, SLE-like, Sjögren's syndrome, arthritis
C7	73	65	SLE, CREST syndrome, pyelonephritis, hematuria
C8	73	65	SLE, hepatosplenomegaly, sickle cell and thalassemia hemoglobinopathies
C9	18	8	SLE
C1 INH[a]	Hundreds, 25	Rare	Angioedema (hundreds); SLE, DLE, SLE-like (25 patients)
H	13	80	SLE, vasculitis
D	3	74	None
P	70	74	SLE, vasculitis
C4bp	3	None	Angioedema
I	23	100	SLE, vasculitis
MBL	Many	60	SLE?
CR3	<20	100	None
DAF	Many	None	Paroxysmal nocturnal hemoglobinuria

[a] Homozygous deficiencies, except for heteroxygous C1 INH.
[b] Data from Vyse *et al.* [39], Pickering *et al.* [91], and Whaley and Schweble [113].

particles and infected host cells. However, the HIV virus utilizes complement receptors to invade various cell types more efficiently and to activate viral replication in latently infected cells. These studies with HIV, which have been reviewed [70], demonstrate that complement proteins and regulatory proteins can play either a beneficial or a detrimental function in host defense against viruses.

Heterozygous and homozygous deficiency of MBL has been shown to be associated with several types of immunodeficiencies. A primary immunodeficiency characterized by defective yeast opsonization was described 20 years before the molecular defect that was identified as the codon 54 mutation in the MBL [71, 72]. Many studies have now been published, including two large studies of 229 and 345 children with unknown primary immunodeficiencies [73, 74], which have demonstrated significant associations for both homozygous and heterozygous MBL mutations with an increased risk of infection. In the largest study, of the 17 homozygous MBL-deficient patients identified, 13 presented with severe infections, including septicemia, cellulitis and boils, severe tonsillitis, and otitis media. Homozygous MBL mutations have also been reported to be a factor in susceptibility to *Mycobacterium tuberculosis* and *avium*, *Trypanosoma cruzi*, *Klebsiella*, *Cryptococcus neoformans*, other fungal infections, hepatitis B virus, and influenza A.

In several published studies, MBL deficiency has been seen in increased frequency in HIV-infected men. In one study from Copenhagen [75], 96 HIV-infected men were compared to 123 healthy Danish adults and 36 Danish homosexual HIV-uninfected men. The incidence of homozygous MBL mutations was 8% in the HIV-infected men and 0% in the control groups. Infected HIV men with the most rapid progression of their disease had the lowest levels of MBL in the serum. Interpretation of these studies is controversial and work is needed to further characterize the possible role of MBL deficiency in HIV disease.

Patients deficient in CR3, the cell membrane receptor for C3, have increased susceptibility to infectious

agents. In initial reported studies, about 20 patients suffering from severe recurrent infections caused by phagocyte dysfunction were identified that lacked the β chain of the CR3 molecule [76, 77]. These patients are missing CR3 molecules as well as the LFA-1 and CR4 membrane molecules, which share the same types of β chains. Granulocytes from these patients are defective in the ability to opsonize and adhere to foreign organisms and cell membrane. Lymphocyte function is also impaired in these individuals.

Complement receptors may also play a significant regulatory role in the activation or suppression of PMN responses to a variety of infectious agents. It has been found [77, 78] that iC3b-containing immune complexes that bind to CR3 caused PMN activation and a respiratory burst in peripheral blood PMNs. This demonstrated a possible significant role for complement fragments in PMN activation during an infection. In addition, a suppressive role for CR1 was also demonstrated because the PMN respiratory burst was enhanced significantly by the monoclonal antibody blockade of CR1 sites specific for C3b. The authors hypothesized that CR1 present on PMNs and RBCs in whole blood promote the degradation of immune complex-bound iC3b to C3dg. This biological function would prevent binding of the complement-containing complexes to the CR3 and downregulate the PMN activation by immune complexes.

Autoimmune Manifestations of Complement Deficiencies

SLE and other rheumatic diseases have been associated for the most part with deficiencies of the early components, but there are documented rheumatic disease cases associated with virtually all complement deficiencies described. A summary of identified deficiencies and associated rheumatologic diseases found in published case reports is given in Table 5. Hereditary complement deficiencies with a complete lack of a complement protein are seen in the population at low frequencies with the exception of C1-INH, C4A, or C4B deficiencies, which are seen more frequently. Screening studies done on serum from blood donors in Osaka, Japan, found an approximate frequency of 0.003% for C5, C6, C7, and C8 deficiencies [79]. In contrast, C4A and C4B deficiencies are seen much more frequently (see Chapter 5). Complement deficiencies have also been reported that are not hereditary deficiencies but rather are acquired conditions associated with the presence of anticomplement protein autoantibodies. The most frequently occurring form of acquired complement deficiency is a result of C1-INH deficiency.

TABLE 6 Homozygous C2 Deficiency: 52 Kindred Studies

Clinical findings	All cases	Nonindex cases
Normal	23	20
SLE	18	0
Atypical cutaneous LE	10	1
Infections	8	3
Glomerulonephritis	9	2
Anaphylactoid purpura	3	0
Vasculitis	2	0
Rheumatic fever	1	1
Polyarthritis	1	1
Idiopathic atrophoderma	1	1
Arthralgia	1	0
Dematomyositis	1	0
Inflammatory bowel disease	1	0
Cold urticaria	1	0
Hodgkin's disease	1	0
Variable immunodeficiency	1	0
Total	82	29

Atypical Cutaneous Lupus Erythematosus: A Hallmark of Deficiencies of Early Complement Components

SLE associated with homozygous deficiency of the early components of complement was the first of the associations of early complement deficiency with lupus disease to be reported [80]. A striking finding present in the first index case and subsequently confirmed was that some of the lupus disease in these patients, called *atypical cutaneous lupus erythematosus* for lack of a better term, differs from classic lupus in the following four ways: (1) increased incidence of discoid skin lesions, (2) an absence of renal disease, (3) low or absent titers of ANA and antibodies to native DNA, and (4) infrequent findings of immunoglobulin and complement in the skin lesions (see section on C2 deficiency and Table 6). These features were also found in some patients with complete deficiencies of C1q, C1r–C1s, and C4 that were reported later.

Hereditary C1q Deficiency

The last early complement component homozygous deficiency associated with SLE-like disease to be reported was C1q and in effect provided evidence for the hypothesis that all the early components, C1 through C4, were involved in host defenses to the development of SLE. Of the 42 patients reported, 39 (93%)

had a clinical syndrome similar to SLE [81–91]. Skin rash, predominantly with photosensitivity, occurred in 37 patients with negative lupus band tests in 3 of 9 cases reported. Glomerulonephritis was present in 16 patients with the membranoproliferative type present in 5 of the 8 cases characterized. Antinuclear antibodies were present in 24 of 35 patients tested but only 5 of 25 patients had antibodies to double-stranded DNA. Although patients with complete C1q deficiency had some features of lupus disease, the diagnostic criteria for SLE were usually not met in most cases. The female to male ratio was 1.3:1 in the C1q-deficient group compared to a ratio of 15:1 in classic SLE (see Chapter 7). Hereditary deficiencies of C1q can be subdivided into two major groups: those with normal levels of C1q and those with depressed levels of C1q protein in the blood. Only individuals with homozygous deficiency are affected (as described earlier in the genetics section).

Hereditary C1r–C1s Deficiency

There have been 14 patients with C1r–C1s patients reported in the literature [91–95]. C1r and C1s deficiencies usually occur together in affected individuals, perhaps because the C1r and C1s genes are located very close to each other on chromosome 12 where simultaneous deletions of both closely linked genes may occur. Some patients have been reported to have a complete absence of both C1r and C1s, whereas others have complete absence of one of the molecules and significantly decreased levels of the other. Eight of the 14 (57%) had lupus disease; all had skin rash, DLE was diagnosed in 2 and SLE in 2. Five of 8 had glomerulonephritis; only 1 was characterized and had mesangioproliferative glomerulonephritis. The female to male ratio was 1.7:1. ANA was negative or weakly positive in 3 of 8, and DNA antibodies were negative in 3 of 5 patients studied. LE band tests were not reported.

Hereditary C4 Deficiency

C4-deficient patients of two types have been characterized: complete C4 deficiency, in which both the C4A and C4B protein products are absent, and partial C4 deficiencies, in which C4 levels are decreased and one or more of the genes for either C4A or C4B genes are missing. Complete C4 deficiency is rare; among 25 reported patients, 21 (84%) had SLE or SLE-like disease [22, 96–103]. Seventeen patients had skin rash, predominately with photosensitivity; lupus band tests were negative in four of the five cases studied. Nine patients had mainly mild glomerulonephritis; the mesangial type was present in all seven cases characterized. ANA was negative or weakly positive in 10 of 20 patients tested. DNA antibody tests were negative in 9 of 11 patients tested. Anti-SS-A(RO) was positive in 6 of 8 patients tested. The female to male ratio of affected patients was 2:1. There were 2 asymptomatic patients and single case reports of Henoch–Schönlein purpura [104] and cryoglobulinemia [105] associated with complete C4 deficiency.

Partial deficiencies may exist in individuals who are missing one, two, or three of the C4 genes (see Chapter 5 for gene frequencies and risk factors for SLE-like disease for the C4 isotypes). Absence of both C4A genes is associated with an increased susceptibility to SLE. The clinical manifestation associated with homozygous C4A deficiency has been reported in several studies. In a study from Sweden [106], homozygous C4A deficiency was found in 13 of 80 SLE patients (16%). C4A-deficient patients had an increased incidence of photosensitivity but other clinical features were similar to the non-C4A-deficient patients. No differences were seen in the percentage of anti-dsDNA, Sm, RNP, SS-A, SS-B, rheumatoid factors, or cardiolipin antibodies for these patients. In a North American study [107] that included Caucasians and Blacks, patients with homozygous C4A deficiency had less proteinuria, lower levels of anti-dsDNA, Sm, SS-A, and cardiolipin antibodies and higher C3 levels. The C4A gene deletion was found in 23.4% of 121 patients with SLE and was associated with subacute cutaneous lupus erythematosus and Sjögren's syndrome.

One large family of 35 individuals from three generations in Iceland with C4A null alleles has been reported [108]. HLA typing and C4 allele typing were done by agarose–immunofixation techniques. Five of the family members had four or more criteria for SLE and an additional five members had clinical or laboratory evidence of SLE but did not fulfill four American College of Rheumatology (ACR) criteria. No other autoimmune diseases were seen in the family. The C4A null allele was highly associated with SLE in this family in that 9 of the 10 members with symptoms were C4A deficient. Interestingly, five different C4A null haplotypes were involved, including three that originated from nonconsanguineous spouses.

Absence of both C4B genes is associated with an increased risk of IgA nephropathy and glomerulonephritis [109]. Absence of at least one C4B gene, which leads to low levels of plasma C4, has been associated with dermatological diseases, specifically discoid lupus erythematosus, angioedema, and urticaria [110]. C4 deficiencies have also been shown to be associated with anti-SS-A antibodies and with anticardiolipin antibodies in Blacks [111, 112].

Hereditary C2 Deficiency

C2 deficiency is the most common homozygous complement component deficiency. The prevalence of either of C4 null genes is higher but the prevalence of complete C4 deficiency, inheritance of both null genes, is not (see Chapter 5). Larger numbers of patients with C1 INH deficiency than homozygous C2 patients have been reported because the inheritance of CI INH deficiency is autosomal dominant (Table 5). Homozygous C2 deficiency has an approximate prevalence of 1:20,000 in the western European Caucasian population. Over 100 patients with homozygous C2 deficiency have been reported [113]. C2 deficiency was the first complement deficiency reported to be associated with SLE. This disease association has been confirmed as shown in a study of the first 52 kindred reported with homozygous C2 deficiency (Table 6) [114]. Twenty-eight patients (34%) had lupus disease with a female to male ratio of 8:1. Eighteen patients (22%) were diagnosed as SLE but only 14 fulfilled ARA criteria for the diagnosis of SLE. The atypical aspects of the lupus disease present in the original index case [80] were also confirmed; 10 patients, a third of the patients, had atypical cutaneous LE. Idiopathic glomerulonephritis was present in 9 patients and recurrent infection in 8 patients; 7 of the 9 patients with glomerulonephritis were males. The 8 patients with recurrent infections were all young children. Frequent infections have also been noted as secondary diagnosis in several of the other childhood cases of homozygous C2 deficiency. The prevalence of lupus disease in this study most likely is inflated due to a patient selection bias, as the studies were mainly performed in SLE clinics. Among the nonindex cases (Table 6), a less biased population, only 1 of 29 (3.4%) C2 homozygotes had lupus disease; interestingly, the patient had atypical cutaneous LE. Hence, while the true prevalence of lupus disease among homozygous C2-deficient patients is not known, it is most likely considerably less than 34% and much lower than the very high prevalence of lupus disease associated with C1q, C1r–C1s, and C4 complete deficiencies.

Comparison of homozygous C2-deficient patients with SLE and atypical cutaneous LE (Table 7) illustrates the four differences noted earlier: an increased incidence of discoid skin lesions, absence of renal disease, low or absent titers of ANA and antibodies to native DNA, and infrequent findings of immunoglobulin and complement in the skin lesions. Glomerular lesions in patients with SLE varied in histological type and included membranoproliferative, mesangial, membranous, and focal sclerosis. Clinically and serologically, these cases resemble two subgroups of classic SLE: the ANA-negative SLE with photosensitivity group and the cutaneous lesions and subcutaneous lupus erythematosus (SCLE) group [115, 116]. The rash in atypical cutaneous lupus erythematosus patients (Fig. 3) bears striking resemblance to the rash in the antinuclear antibody (ANA)-negative group and to that in SCLE. The skin lesions have been described as nonscarring, papillosquamous, or annular polycyclic, with a characteristic distribution (i.e., the lesions spare the knuckles, inner aspects of the arm, axilla, lateral part of the trunk, and are rarely seen below the waist). Because the skin lesions differ from those in discoid lupus erythematosus in their clinical appearance and immunohistology, the diagnosis of the discoid type in some of these complement-deficient cases was inaccurate. The two subgroups of SLE and the complement-deficient patients with atypical cutaneous LE all share a high incidence of positivity for the anti-Ro (SSA) antibody [112, 115, 117].

TABLE 7 Homozygous C2-Deficient Patients: Comparison of SLE with Atypical Cutaneous Lupus Erythematosus

	SLE		Atypical cutaneous LE	
	Number studied	Number positive	Number studied	Number positive
Discoid LE lesion	17	4	10	10
Renal disease	17	5	10	0
ANA	17	17	10	2
dsDNA antibody	12	6	8	0
Ig, C′ in skin lesion	4	3	8	0

Whether there is an association of lupus disease with heterozygous C2 deficiency similar to that seen with heterozygous C4 deficiency (Chapter 5) is controversial [91, 118, 119]. There appears to be a slight increase in the prevalence of heterozygous C2 deficiency among SLE patients compared to controls (see Chapter 5). Moreover, in the study on 52 kindred with C2 deficiency cited earlier, the prevalence of lupus disease among nonindex heterozygous C2 deficiency cases was 0.6% compared to a prevalence of 3.4% among nonindex homozygous cases, suggesting that there may be an association of lupus disease with heterozygous deficiency that is six times less than that with homozygous C2 deficiency.

The effect of homozygous C2 deficiency on the clearance of immune complexes has been studied [120]. A patient with C2 deficiency was injected with hepatitis B surface antigen–anti-HbsAg immune complexes labeled with ^{123}I. The patient's uptake of complexes in the liver, spleen, and clearance of the complexes from

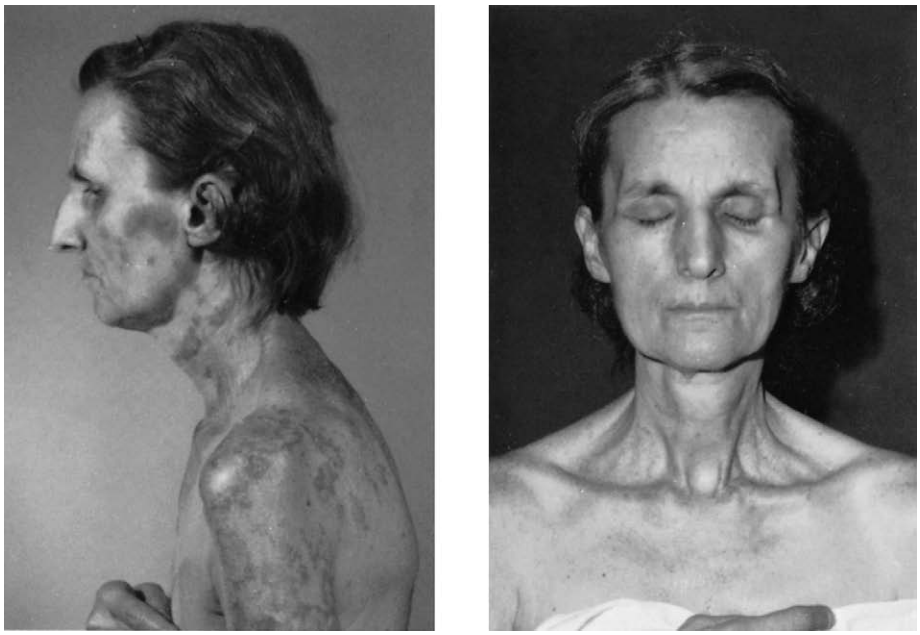


FIGURE 3 Homozygous C2-deficient patient. (*Left*) Characteristic rash. Note the annular polycyclic pattern. (*Right*) Patient after therapy. Rash cleared slowly but completely with topical steroids. There was no residual scarring.

the circulation was studied before and after fresh-frozen plasma treatment. When her C2 and CH50 levels were zero, the complexes were taken up rapidly by the liver and cleared from the circulation. No binding of the complexes was seen in the spleen or on RBCs via CR1. After treatment with FFP, which normalized her complement levels, the complexes cleared from the circulation more slowly, and 20% of the complexes were found in the spleen. These studies suggest that uptake of immune complexes by the spleen is complement dependent and that abnormal processing of immune complexes by complement-deficient patients may take place.

Hereditary C3, C5, C6, C7, and C8 Deficiencies

The major disease association with homozygous deficiencies of components C3 through C8 is infection (Table 5); few cases of SLE and SLE-like disease are associated with deficiencies of these components. The relative infrequency of these associations may indicate that no causal relationship exists, although such a relationship cannot yet be excluded because of the small numbers of cases of each deficiency studied.

Of 24 reported cases of homozygous C3 deficiency, three patients had SLE-like disease and seven had glomerulonephritis, proteinuria, or IgA nephropathy [39]. The SLE-like disease patients had rash and arthralgias or arthralgias and photosensitivity [121, 122]. All three had negative serologic assays for ANA, LE cells, and rheumatoid factor assays.

More typical SLE occurs among individuals with C5–C8 deficiencies. One case of homozygous C5 deficiency with SLE has been reported [123]. There were no unusual features of SLE in this case. The lupus erythematosus, ANA, and dsDNA antibody tests were positive. The prominent skin lesions were typical of lupus. There were deposits of immunoglobulin and complement along the dermal–epidermal junction. There are three cases of homozygous C6 deficiency with SLE or SLE-like disease [124–126]. One case had typical SLE, and one case had SLE-like disease with polyarthritis, pleurisy, and Raynaud's phenomenon. The lupus erythematosus and dsDNA tests were negative, the ANA was weakly positive, and an ssDNA test was positive. The third case had discoid lupus and Sjögren's syndrome with negative serologic test studies. Two cases of C7 deficiency have been reported with classical seropositive SLE [127]. Two cases of C8 deficiencies have been reported: one had classic seropositive SLE and the other had clinical criteria for SLE with negative serologic tests for ANA and lupus erythematosus cells and a positive rheumatoid factor [128, 129].

Hereditary C9 Deficiency

Initially, C9 deficiency was thought to be rare and not associated with disease. A further study of C9

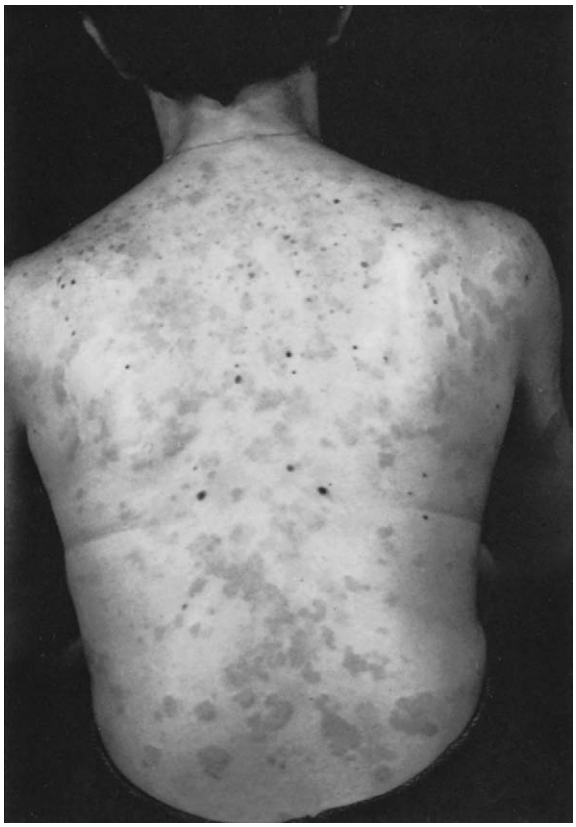


FIGURE 4 Patient with typical rash of hypocomplementemic urticarial vasculitis syndrome.

deficiencies, especially in ethnic Japanese, has demonstrated that the incidence of C9 deficiency in Japan is about 0.086%, much higher than in initial studies of other populations. Three cases of SLE have been reported in association with C9 deficiencies in Japan. The first reported patient had sicca syndrome accompanied by severe Raynaud's phenomenon [130]. Serologic tests for ANA and dsDNA were positive, as were assays for RNP, Sm, and SS-A (Ro) antibodies and immune complexes. The other two patients [131, 132] had low CH50 and absent C9 protein and function. One of the patients had recurrent urinary tract infections, pleuritis, pericarditis, proteinuria, and facial edema with a positive ANA of 1:640 and a dsDNA antibody of 28 U/ml. This patient had two other sisters with homozygous C9 deficiency with hypergammaglobulinemia and low titered ANAs (1:80). Their father was heterozygous for C9 deficiency with a positive ANA of 1:640. Four other C9-deficient patients with disease have been reported: one had sicca syndrome and the remaining three had rheumatoid arthritis. Cases of C9 deficiency associated with neisserial meningitis have also been identified.

Hereditary MBL Deficiencies

MBL protein deficiencies, which are a result of the gene mutations of the MBL gene, have been studied in SLE. Serum levels of MBL were measured in a study with 58 SLE, 92 HIV, 30 chronic liver disease, 20 rheumatoid arthritis, and 161 healthy controls [133]. Severely decreased levels of serum MBL protein were found in 12% of SLE patients, 4% of HIV patients, 3% of liver disease patients, no RA patients, and 4% of healthy controls. In one study [134], 102 Caucasian SLE patients were compared to 136 healthy controls to determine their MBL genotypes. The mutant adenine at nucleotide 230 (the codon 54 allele) was found in 41% of patients and 30% of controls, with the frequency of homozygosity for the SLE group of 10% compared to 7% for the control group. This study showed an increase but not a statistically significant frequency of MBL mutation in the SLE patient group. In addition, when DQA*0501/Dr3/C4A*Q0 gene studies were also done, the association with SLE for the presence of the C4 null allele and MBP genes was stronger than association of the C4 null allele alone. Three additional studies have been done with other racial populations. In a study of Chinese individuals in Hong Kong [135], 111 SLE patients and 123 healthy controls, 33% of patients (37) and 23% of controls (28) were positive for the codon 54 mutation. Two of the SLE patients were homozygous for the codon 54 mutation. Mean levels of MBL in the serum were about 10% of normal levels for the codon 54+ patients and controls, whereas MBL levels for SLE patients without the mutation were about 50% of the normal levels. A second study of a Black cohort with SLE and matched normal controls was done to determine the gene frequency and relative risk of the codon 54, codon 57, –550, –221, HY, LY, and HX gene frequencies [136].

Codon 54 mutations were found with a frequency of 0.163 in the SLE group and 0.087 in the control group; codon 57 mutations had a frequency of 0.125 in the SLE group and 0.047 in the control group. For the promoter mutations, mutations in the –550, –221, and the LX haplotype were found in increased frequency in the SLE group, whereas the HY haplotype was found in increased frequency in the controls. The LY haplotype was found in equal frequency in both groups. Homozygous LX/LX haplotypes were found in 11% of the SLE group but only in 2.6% of the controls. Overall, the prevalence of any of the MBL genetic deficiencies in the SLE population was 46% compared to 23% of the control group. This study also identified an additional promoter haplotype, HX, present in 3 of their SLE patients. In a third study of 50 SLE patients and 49

controls in Spain [137], codon 54 mutations were found in 52% of SLE patients and 31% of controls; codon 57 mutations were found in 6% of patients and 4% of controls. Homozygosy for codon 54 was found for two SLE patients and one control. C4A and B genes were also determined for this group of patients. The C4B allele was at increased frequency in the SLE group (37% versus 12%), and there was a stronger association for SLE with the C4A null allele and the MBL mutant alleles than for either mutation alone. In summary, several studies have now documented an increased frequency of MBL mutations that result in nonfunctional MBL proteins in SLE patients in a variety of racial groups. This association of MBL gene mutation with an increased risk for disease appears to demonstrate a gene dosage effect associated with C4A null alleles. Additional studies are necessary to further elucidate the mechanism of this increased risk for the development of SLE.

Combined Hereditary Complement Deficiencies

A combined heterozygous C4 and C2 deficiency has been reported for 15 individuals from six families [138]. About 30% of the people had SLE or another autoimmune disease. The C2 deficiencies were all due to the 28-bp deletion in the C2 gene, whereas eight of the C4 deficiencies were all due to heterozygous C4A null alleles and five were due to C4B null alleles (the other two C4 deficiencies could not be identified as null alleles). From the frequencies of C2, C4A, and C4B deletions, the expected combined C2 and C4 deletion frequency of the population is 0.001.

A family with four children has been identified in which three of the children are homozygous deficient for factor H and two of the three children are heterozygous deficient for C2. One of the two children with both C2 and factor H deficiency had classic SLE with nephritis. Two patients have been identified with combined homozygous C7 and C4B deficiency; one of the patients was normal and one had SLE [139]. The patient with SLE had a sister who was asymptomatic but was also homozygous C7 deficient. Additional patients have been identified with combined properdin and C2 deficiency, and DAF and C9 deficiency, but rheumatic disease was not reported for any of them.

Acquired Deficiencies of Complement

Two forms of acquired complement deficiency states with prolonged persistent complement depression have been associated with SLE-like disease similar to that seen in hereditary complement diseases. These syndromes are early complement component depression associated with chronic hypocomplementemic cutaneous vasculitis and C3 deficiency with mesangiocapillary glomerulonephritis or SLE.

TABLE 8 Forty-Seven Patients with Hypocomplementemic Urticarial Vasculitis Syndrome

Clinical and laboratory findings	Number studied	Number positive	Percent positive
Rash	46	46	100
Depression of C1–C3	47	46	98
Anti-C1q antibodies (C1q precipitins)	20	27	74
Arthralgias or nondeforming arthritis	34	23	68
Angioedema	46	26	56
Renal disease	42	12	29
Obstructive lung disease	42	10	24
Antinuclear antibodies (>1:20)	46	9	20

Hypocomplement Urticarial Vasculitis Syndrome

Hypocomplementemic cutaneous vasculitis is an acquired form of deficiency that leads to chronic decreases in complement components C1, C2, C4, and C3. The syndrome is now called hypocomplementemic urticarial vasculitis syndrome (HUVS) but in the past was identified as SLE-related syndrome, hypocomplementemic cutaneous vasculitis, chronic hypocomplementemic cutaneous vasculitis, or hypocomplementemic vasculitis urticaria syndrome. Patients with this syndrome have several distinct clinical features similar to those of SLE but do not fulfill SLE diagnostic criteria [140–159]. The typical patient is a young female with chronic rash, angioedema, and arthralgias. Table 8 contains a summary of the clinical and serologic studies of 47 patients (37 females, 6 males, 4 sex not reported) with SLE-related syndrome. Two patients developed pseudotumor cerebri [146, 155]. The rash, which is the most characteristic and prominent feature of the syndrome, was initially called erythema multiforme like because of the presence of classic target lesions [140]. More recently it has been called urticaria like but is very atypical urticaria because of the absence of itching and the persistent nature of the rash. Histologically, the skin lesions form a perivasculitis to a true leukocytoclastic vasculitis. The presence of

immunoglobulins and complement in the lesions also varies, but in most cases they are absent. Renal disease, when present, is usually mild to moderate, and obstructive lung disease, which may be severe, occurs in some patients. Studies done on isolated glomeruli basement membranes demonstrated a high level of antibody to C1q in the glomerular tissues [160]. When pulmonary function was measured in 17 patients, 11 of them had dyspnea and severe airflow obstruction [161]. Six of these patients died of respiratory failure.

The major serologic finding in the majority of patients is the presence of low molecular weight C1q precipitins, which have been demonstrated in most cases to be antibody to the C1q molecule [162]. These antibodies can be demonstrated in this syndrome as well as in SLE, findings that are consistent with the original description of the low molecular weight C1q precipitin that was reported to be present not only in the first case of HUVS, but in SLE patients as well [114]. The majority of patients can be distinguished from those with SLE serologically because the majority of patients with the syndrome have negative ANA tests, negative assays for lupus erythematosus cells, and negative assays for dsDNA. A small number of patients identified were found to have low titered antibodies to the Sm antigen. When patients with SLE were tested for anti-C1q antibodies by enzyme-linked immunosorbent assay (ELISA), 33.6% of 113 patients were positive [163]. Plasma titers of anti-C1q antibodies were inversely correlated with CH50 activity. Eighty-five percent of patients with severe complement consumption had positive results for anti-C1q antibodies. Only 14% of patients with no evidence of complement consumption had positive results for anti-C1q antibodies.

The most striking serologic feature of patients with the syndrome is the marked depression of serum complement. The pattern of complement depression resembles that in SLE except that there is severe depression of C1q and associated marked depression of C2 and C4 levels. Depression of C3 tends to be moderate, and all terminal components are present at normal levels. Normal C1 esterase inhibitor levels differentiate the syndrome from the hereditary and acquired forms of angioedema.

The antibodies present in patient serums have been shown to react with a variety of epitopes on the C1q molecule. By Western blot, antibodies can be detected that react with the B and C chains in patients with HUVS. Serum from HUVS also react with the CN-CN and AN-BN dimers of the collagenous C1q fragments. Patient serums from SLE patients were negative by Western blot and thus the antibodies must be directed toward conformational epitopes of bound C1q [164].

Patients with HUVS are similar to patients with hereditary complement deficiency and SLE in that the skin rash is prominent and the ANA test is negative. However, the rash in patients with HUVS does not resemble discoid or atypical cutaneous lesions. None of the inherited forms of deficiency of C1q have similar rashes and all are negative for antibodies to C1q. Hence, the relationship of this acquired complement deficiency to SLE appears to differ in major ways from the association of hereditary complement deficiencies and SLE.

Acquired C3 Deficiency and Systemic Lupus Erythematosus

A portion of patients with chronic mesangiocapillary glomerulonephritis will have associated depressed serum levels of C3. In a study of 21 patients with partial lipodystrophy, 17 were found to have low C3 with normal C4 and C2 concentrations [165]. Seven had chronic mesangiocapillary glomerulonephritis. Patients with depressed C3 levels have been shown to have a protein in their sera capable of cleaving C3 in normal serum. This protein has been termed *C3 nephritic factor* (C3Nef) and has been shown to be an autoantibody that stabilizes the C3 convertase of the alternative pathway.

A 34-year-old Caucasian woman with partial lipodystrophy since age 5 years has been reported with classic SLE [166]. The patient had pleuritis, polyarthritis, and a photosensitive rash on her arms. Lupus erythematosus cells, ANA, native DNA antibody, and rheumatoid factor tests were positive. C3 was markedly depressed, with normal levels of C1q, C2, and C4. C3Nef was demonstrated in the serum. A renal biopsy showed focal mesangial sclerosis without any electron-dense deposits on electron microscopy. This patient's lupus is classic SLE and bears little resemblance to the lupus-like syndrome seen in three of the homozygous C3-deficient cases except that all had skin rashes and three had photosensitivity. The significance of any of these observations remains to be determined.

Deficiencies of Regulatory Molecules

C1 Inhibitor Deficiency and Systemic Lupus Erythematosus

Genetic and acquired forms of C1-INH are associated with the syndrome of hereditary angioneurotic edema, which consists of recurrent bouts of noninflammatory swelling involving the subcutaneous tissues, intestinal walls, airways, and lungs. Twice as many males as females are affected. Two major types of the genetic

deficiency exist. In approximately 85% of patients, functional and antigenic assays show a low serum concentration of the inhibitor. In the variant form, which comprises approximately 15% of the cases, there is a normal or elevated concentration of a functionally inactive inhibitor. Two types of acquired deficiency have been identified: type I, which is associated with B-cell lymphoproliferative disease, and type II, which is characterized by the production of autoanti-C1-INH antibodies. A summary of the information from 22 patients has been published [167]. The autoantibodies are directed at epitopes at or near the reactive center of the molecule and prevent the inactivation of target proteases by the C1-INH. The levels of free antibody in the serum do not correlate with C4 levels, C1-INH functional assays, or clinical symptoms.

The clinical syndrome is very similar for the genetic and acquired forms of the disease. The severity of the clinical syndrome does not correlate directly with C1 inhibitor quantitative serum concentrations. Some affected individuals with a clear depression of C1 esterase inhibitor do not have clinical manifestations of disease. During attacks, patients usually develop detectable levels of free C1 esterase, which cannot be found in the circulation of normal individuals. C4 and C2, the substrates of C1 esterase, are chronically depressed in most patients. The chronic depression of C4 and C2 in these patients may be similar to levels seen in hereditary deficiency disorders.

A number of patients with hereditary deficiencies of C1 esterase and lupus disease have been reported [110, 168–186] (Table 9). Of 25 affected patients, 6 had SLE, 9 had discoid lupus erythematosus, and 10 had SLE-like disease. Three of the latter patients appeared to have atypical cutaneous LE; there were insufficient data on the remainder for assessment. Three of the 5 lupus bands tested were negative as found in the atypical cutaneous LE lesions. The female to male ratio was 7:1. As with the hereditary C2 deficiency and the C4 deficiency, the lupus disease is characterized by a high incidence of skin rash, which often has been diagnosed as discoid lupus erythematosus; some of these patients may have atypical cutaneous lupus erythematosus. Most skin biopsies of the lesions showed no immunoglobulin or complement deposits. In 7 of 19 and 5 of 11 patients the ANA and 5 DNA antibody tests were negative,

TABLE 9 Twenty-Five Patients with Hereditary Angioedema and Lupus

Number of cases	Sex	Clinical findings	ANA	Skin biopsy, immunofluorescent studies	Ref.
1	M	SLE	Negative	Not done	168
1	F	SLE-like	Positive	Not done	169
1	F	SLE-like	Negative	Not done	170
1	F	SLE-like	Negative	Not done	171
1	F	SLE-like	Positive	Not done	172
1	F	DLE	Negative	Not done	173
1	F	DLE	Positive	Not done	174
1	F	SLE-like	Weak positive	Not done	175
1	F	SLE-like	Positive	Not done	176
1	F	SLE-like	Negative	Not done	177
1	F	SLE	Not done	Not done	178
2	M	DLE	Weak positive	IgG, IgM, C3	
1	F	SLE	Positive	Not done	179
1	F	SLE	Positive	IgM in normal skin	184
3	F	DLE	Weak positive	Not done	180
1	F	DLE	Positive	IgG	181
1	F	SLE	Positive	Not done	182
1	F	SLE-like with SCLE[a]	Negative	Negative	186
1	F	SLE with DLE[a]	Positive	Negative	183
1	M	SLE-like with DLE[a]	Positive	IgM	
1	F	SLE-like with DLE[a]	Negative	Negative	185
1	F	DLE	Positive	Not done	206

[a] Possible atypical cutaneous LE.

respectively. Membranoproliferative glomerulonephritis was present in 4 of the 6 patients with SLE.

There are two published investigations of twins with hereditary C1 esterase deficiency. Identical male twins who both exhibited classic symptoms of hereditary angioedema and marked depression of C4 had discoid lupus that appeared through both clinical and immunologic studies to be typical discoid lupus erythematosus [178]. In the second set of twins, identical girls with a complete absence of C1 esterase inhibitor, one remains normal while the other, since age 6 years, has manifested classic symptoms of hereditary angioedema [179]. At age 14 years she developed classic SLE with a positive anti-dsDNA antibody and profuse proliferative glomerulonephritis. C4 levels in the affected twin were 4% of normal, whereas the twin without clinical hereditary angioedema or SLE had C4 levels that were chronically in the range of 10–15% of normal. The findings suggest that chronic low C4 is not sufficient to predispose to lupus disease in patients with C1 esterase deficiency disease; very low levels may be required. This may be analogous to the greater association of lupus disease with homozygous C4 deficiency than with the heterozygous states. Indirect evidence for a relationship between C4 levels and lupus skin lesions has come from treatment of two patients with danazol [186, 187]. Danazol, an impeded androgen, has been shown to increase C1 inhibitor levels and to control clinical symptoms in hereditary angioedema. In both patients, danazol therapy resulted in complete remission of the skin rash and elimination of photosensitivity accompanied by increases in the C1 esterase inhibitor and C4 levels. Discontinuation of danazol in both cases resulted in recurrence of the skin rash.

TABLE 10 Clinical Findings among Nonindex Cases from Kindred Studies of Homozygous and Heterozygous C2-Deficient Patients

	Number of individuals
Homozygous C2 deficient	
Normal	20
Infection	3
Discoid lupus erythematosus	1
Glomerulonephritis	2
Rheumatic fever	1
Polyarthritis	1
Idiopathic atrophoderma	1
Total	29
Heterozygous C2 deficient	
Normal	310
Vasculitis	2
SLE	1
Discoid lupus erythematosus	1
Infection	1
Glomerulonephritis	1
Juvenile rheumatoid arthritis	1
Asthma and eczema	1
Total	318

Factor I Deficiency

Twenty-three patients have been identified with homozygous factor I deficiency. Recurrent infections and meningitis infections with *S. pneumoniae*, *N. meningitidis*, *H. influenzae,* and otitis media were reported in 21 of the patients [188, 189]. One patient was asymptomatic and one patient had a fatal systemic vasculitis following penicillin therapy. Levels of plasma and RBC associated factor I were both decreased significantly in these patients. The absence of factor I was also shown to lead to significant increases in RBC-bound C3c and C3d antigens on patient red cells. In addition, there was continual formation of the alternate pathway convertase (C3bBb), which led to consumption of factor B and C3 in the serum and to the increased formation of C3b fragments. All leukocytes from these patients are also covered with C3b fragments. When peripheral blood cells from two patients with factor I deficiency were studied, CR1 levels were significantly lower and CR2 levels were somewhat reduced on B cells; CR1 levels on monocytes and granulocytes were normal or slightly elevated.

Factor H Deficiency

Fifteen individuals from seven families have been identified with homozygous factor H deficiency [190]. The disease is inherited as an autosomal-recessive trait and results in the uncontrolled cleavage of C3. This C3 cleavage depletes the factor B and properdin components of the alternative pathway and depletes C5. Patients with factor H deficiency have been associated with recurrent bacterial infections, including meningococcal meningitis, glomerulonephritis, SLE, and subacute cutaneous lupus erythematosus. In 21 relatives encompassing three generations for one reported patient, 10 had low factor H levels, which indicated probable heterozygous deficiencies in these relatives. Decreased factor H serum levels, C3 serum levels, C3 functional activity, factor B levels, and properdin levels were all strongly associated for these heterozygous relatives.

CR1 Deficiency

A quantitative deficiency of the number of CR1 molecules on white cells and red cells from SLE patients

has been reported in many studies. Because of the possibility that the level of expression on cell membranes is related to the CR1 genotype, studies have been done to determine the frequency of CR1 alleles in SLE patients. In a group of 63 French SLE patients and 158 normal controls, a significantly higher frequency of the S (B) allele was found in patients (51%) than for controls (26%) [191]. However, many other studies that have utilized the *Hin*dIII polymorphism detection method have not shown any association between SLE and the B allelle [44, 45, 192–194]. Studies have also shown that the CR1-C allele, smallest of the CR1 alleles, was not shown to be at increased frequency in SLE patients in either Black or Caucasian populations [195]. The quantitative number of CR1 receptors on lymphocytes, granulocytes, macrophages, and RBCs has been found to be decreased in patients with a wide variety of autoimmune diseases, including SLE, rheumatoid arthritis, hydralazine-induced lupus, discoid lupus erythematosus, primary phospholipid syndrome, essential mixed cryoglobulinemia, primary biliary cirrhosis, ulcerative colitis, and on cells in the MRL/lpr mouse SLE model. The decrease in receptor numbers has been correlated with disease activity and can be reversed on red cells by the production of new red cells stimulated by erythropoietin in some patients.

CR3 Deficiency

A single reported case of the association between SLE and CR3 deficiency has been reported [196]. The patient had classic SLE and an immune vasculitis. The patient had normal levels of CR3 on cell membranes but the molecule was nonfunctional. The CR3 was unable to interact with the C3bi ligand.

DAF Deficiency and Clinical Disease

Two types of deficiencies in the DAF (CD55) regulatory membrane molecule have been characterized. The acquired anemia condition of paroxysmal nocturnal hemoglobinuria (PNH) has been shown to be due to a lack of DAF and HRF20 (CD59) on red cell membranes of affected patients. A number of studies have demonstrated that the molecular defect in PHN is a clonal mutation that results in a defective PIG-A gene located on the X chromosome that encodes a glycosyltransferase. The deficiency of the enzyme leads to the inability of the cells to link a number of glycoproteins to the membrane via their usual glycosylphosphatidylinositol (GPI)-linked anchors. Expression on the red cell and white cells of over 20 glycoproteins is deficient. The lack of CD55 and CD59 on the red cell membrane results in an increased sensitivity to complement-mediated red cell lysis because of the increased uptake of C3b and increased levels of C5–9 complexes on the cell. This is due to a lack of the regulatory functions of CD55, which disassembles the C3 convertase, and the CD59, which restricts the binding of C8 and C9 and prevents assembly of the membrane attack complex. Inherited forms of CD55 and CD59 deficiency have also been identified. Three individuals have been identified who are deficient in the Cromer red cell blood group antigens (Inab phenotype) found on DAF molecules [197]. The red cells were lacking DAF molecules. No episodes of *in vivo* hemolysis were reported for the patients, but the red cells were extremely sensitive to *in vitro* lysis. In contrast, the few patients with inherited CD59 deficiency have all been associated with hemolytic episodes. PNH associated with rheumatic disease has not been reported, but PNH- and GPI-linked deficiencies have been demonstrated in aplastic anemia, hypercoagulable states, and increased susceptibility to bacterial infections.

SPECULATION ON THE ROLE OF EARLY COMPLEMENT COMPONENT DEFICIENCY IN SYSTEMIC LUPUS ERYTHEMATOSUS

There is now considerable evidence from studies on patients with hereditary deficiencies of individual complement components that complement has specific roles in host defense, independent of other systems. Specific roles have most clearly been defined for deficiencies of C3 and C5–8 that are associated with diseases caused by bacterial agents. In these instances the component is directly involved in a physiologic mechanism that eliminates the disease agent (i.e., opsonization and bacterial lysis). A hypothesis, proposed in the 1970s, on specific roles for early complement components in host defense was that these components protect against lupus diseases. Evidence reviewed in Chapter 5 and in this one has confirmed that the early complement proteins, C1 through C2, are directly involved in the host defense against lupus disease. It appears from the review of the prevalence of lupus disease with other complement components that these host defenses are related to biological activities of C1, C4, and C2 and are not dependent on activation of C3 or the rest of the complement cascade. Early reservation that the association of early complement component deficiency genes with lupus disease may not be a casual relationship, but rather a result of linkage to some other genes in the HLA complex involved in the pathogenesis has been dispelled as reviewed in Chapter 5. Other HLA genes, in particular the D/DR genes, may be involved in lupus

disease as described in Chapter 4 but independently of the complement genes.

Complete hereditary deficiency of a complement component, nature's "knockout" experiments, can provide insights to the biological function of individual components that cannot be gleaned from patients with complement activation where multiple components may be decreased or from *in vitro* studies. Much of the lupus disease associated with early complement component deficiencies lack hallmarks of classic SLE: female predominance, antibodies to dsDNA, or glomerulonephritis. The atypical cutaneous LE that lacks these features of classic SLE suggests that host defense mechanisms mediated by these components occur in the skin. For C1 and C4, these mechanisms appear to be independent of female hormones. Hence, the notion that early complement component deficiencies cause SLE is most likely incorrect. It appears more likely that these deficiencies cause specific aspects of lupus disease. Consistent with this assessment is the observation that atypical cutaneous LE constitutes 36% of the lupus disease in homozygous C2-deficient individuals but that the apparent equivalent form of disease in the general populations of patients with lupus disease, subacute lupus erythematosus, affects only 9% of lupus patients. This hypothesis is similar to that proposed for murine SLE [198], i.e., multiple genetic factors predispose to lupus disease, whereas some genes or absence or certain genes, as well as certain infectious agents, accelerate or enhance disease. The early complement component deficiency genes may be one of the accelerator or disease-enhancing groups of genes. In some deficient individuals, especially in females bearing other lupogenic genes, the result is classic SLE.

Several other clinical observations are relevant in considering hypotheses on the role of early complement component in the etiology of lupus disease. There is a greater prevalence of the disease in complete genetic deficiencies compared with partial ones. Similar disease associations occur in acquired deficiencies of early components as in the genetic deficiencies of these components, suggesting that the greater the deficiency of the complement protein the greater the susceptibility to disease. Initial observations that the prevalence of lupus disease with complete deficiency of C2 was lower than those of complete deficiency of C1 or C4 and a marked female predominance among affected patients with C2 but not C4 of C1 [114] have been confirmed.

At the time when the original hypothesis was formulated, the leading hypothesis on the etiology of SLE was that the disease was initiated by some yet unidentified viral agent. Hence, it was postulated that the early complement components were involved in specific host defenses to the putative viral agent. This hypothesis has not been totally excluded, but no viral etiologic agent has been identified in human SLE after 25 years of searching. A new major hypothesis on the etiology of SLE is that aberrant apoptosis leads to a breach of self-tolerance and autoimmunity. New evidence on the role of early complement components in the clearance of apoptotic cells has led to a new interesting hypothesis on how complement is involved in host defenses to lupus disease. The most widely accepted hypothesis (discussed in Chapter 5) is that these components are essential for the clearance of immune complexes and because SLE disease is mediated by immune complexes, deficiencies of these components lead to disease. This hypothesis appears untenable for two reasons: (1) the existence of other complement pathways for clearing immune complexes that bypass the early components and (2) the absence of evidence for increased immune complex disease in early component complement-deficient patients.

Complement has been implicated in the apoptosis processes involved in tolerance [199] except a preliminary report that C4 participates in negative selection of autoreactive cells in mouse model studies [200]. However, complement is involved in the clearance of apoptotic cell, and another mechanism for breaking tolerance may be the generation of autoantigen resulting from a defective clearance of apoptotic cells.

The aforementioned findings have led to the hypothesis by the Walport and Ahearn groups that C1q deficiency may predispose to autoimmune disease, that abrogation of tolerance leading to autoimmunity in SLE may be initiated in the skin lesions, and that the hierarchy of early complement component efficacy in the clearance of apoptotic cells mimics the hierarchy of prevalences of lupus disease among humans with deficiencies of these components. In the terminal phases of apoptosis the plasma membrane undergoes blebbing that results in cell surface expression of a variety of self-antigens involved in autoantibody formation in lupus disease, Ro (SS-A), La (SS-B), nucleosomes, and ribosomes [201]. It has been demonstrated *in vitro* that C1q binds independently of immunoglobulin directly to cell surface blebs on keratinocytes undergoing apoptosis following UV irradiation or viral infection [202]. In addition, a C1q knockout mouse model was shown to develop a lupus-like disease characterized by antinuclear antibodies and glomerulonephritis with increased numbers of glomerular apoptotic cells [203]. Further studies comparing the clearance of apoptotic cell in C1q and C4 knockout mice and glomerularnephritis in C1q and C2 knockout mice demonstrated a hierarchy in clearance with a greater deficit in C1q- than C4-deficient mice [204] and glomerulonephritis with an

excess of glomerular apoptotic bodies in C1q-deficient, but not C2-deficient mice [205], respectively.

The hypotheses are appealing because they are consistent with some of the clinical findings in the hereditary C1q deficiency, i.e., UV induced lesion lacking immunoglobulins and the most severe glomerulonephritis among patients with early complement component deficiencies. The lowest prevalence and least severe glomerulonephritis occur in C2-deficient patient; the prevalence in C4-deficient patients was similar to that among C1q-deficient patients but all were the milder mesangial lesions. Hence, the mouse C1q knockout mouse mimics the nature's C1q knockout experiment. However, there are several contrary observations. The characteristic skin lesion occurs in C4- and C2-deficient patients where C1q levels are normal. C1q alone is insufficient to cause lupus disease, as illustrated by normal individuals with C1q deficiency and the requirement for an autoimmune background in mice that develop lupus disease with deletion of the C1q gene. The development of lupus disease predominantly in females, in patients with prolonged depression of C4 and C2, and with normal levels of C1q in patients with C1INH deficiency also indicates that deficiency of C4 and C2 without a deficiency of C1q, along with female hormones, can produce susceptibility to lupus disease.

Thus far, studies have not been performed in human lupus to confirm the observations made on the role of apoptosis and C1q deficiency in mouse lupus models. It should also be empasized that the etiology of SLE is unknown so that any hypothesis on the role of early complement components is purely speculative. However, studies of patients with lupus disease and complement deficiencies have led to clues to possible etiologies and have provided a new understanding of the specific roles that complement components can play in host defense systems, as illustrated by studies on the role for C1q in the clearance of apoptotic cells.

References

1. Agnello, V., Ruddy, S., Winchester, R., Christian, C. L., and Kunkel, H. G. (1975). Hereditary C2 deficiency in systemic lupus erythematosus and acquired complement abnormalities in an unusual SLE-related syndrome. *In* "Proceedings of the 2nd International Workshop in Primary Immunodeficiency Disease in Man," Vol. XI, pp. 312–317. National Foundation March of Dimes.
2. Agnello, V. (1978). Complement deficiency states. *Medicine* **57**, 1.
3. Agnello, V., Carr, R. I., Koffler, D., and Kunkel, H. G. (1969). Gel diffusion reactions of C1q with aggregated γ globulin, DNA and various anionic substances. *Fed. Proc.* **28**, 696.
4. Uwatoko, S., and Mannik, M. (1990). The location of binding sites on C1q for DNA. *J. Immunol.* **144**, 3484–3488.
5. Ikeda, K., Sappoh, T., Kawasaki, N., Kawasaki, T., and Yamashina, I. (1987). Serum lectin with known structure activates complement through the classical pathway. *J. Biol. Chem.* **262**, 7451.
6. Thiel, S., Vorup-Jensen, T., Stover, C. M., *et al.* (1997). A second serine protease associated with mannan-binding lectin that activates complement. *Nature* **386**, 506.
7. Matsushita, M., and Fujita, T. (1995). Cleavage of the third component of complement (C3) by mannose-binding protein-associated serine protease (MASP) with subsequent complement activation. *Immunobiology* **194**, 443.
8. Storgaard, P., Nielson, E. H., Skriver, E., Anderson, O., and Svehag, S. E. (1995). Mannan-binding protein forms complexes with alpha-2-macroglobulin: A protein model for the interaction. *Scand. J. Immunol.* **42**, 373.
9. Terai, I., Kobayashi, K., Matsushita, M., Fujata, T., and Matsuno, K. (1995). Alpha-2 macroglobulin binds to and inhibits mannose-binding protein-associated serine protease. *Int. Immunol.* **7**, 1579.
10. Kuhn, S., Skerka, C., and Zipfel, P. F. (1995). Mapping of the complement regulatory domains in the human factor H-like protein 1 and in factor H1. *J. Immunol.* **155**, 5663.
11. Boackle, S. A., Holers, V. M., and Karp, D. R. (1997). CD21 augments antigen presentation in immune individuals. *Eur. J. Immunol.* **27**, 122.
12. Reljic, R., Cosentino, G., and Gould, H. J. (1997). Function of CD23 in the response of human B cells to antigen. *Eur. J. Immunol.* **27**, 572.
13. Stockinger, H. (1997). Interaction of GPI-anchored cell surface proteins and complement receptor type 3. *Exp. Clin. Immunogenet.* **14**, 5.
14. Cinco, M., Murgia, R., Presani, G., and Perticarari, S. (1997). Integrin CR3 mediates the binding of nonspecifically opsonized *Borrelia burgdorferi* to human phagocytes and mammalian cells. *Infect. Immun.* **65**, 4784.
15. Cywes, C., Hoppe, H. C., Daffe, M., and Ehlers, M. R. (1997). Nonopsonic binding of *Mycobacterium tuberculosis* to complement receptor type 3 is mediated by capsular polysaccharides and is strain dependent. *Infect. Immun.* **65**, 4258.
16. Fearon, D. T., and Austen, K. F. (1980). The alternative pathway of complementCa system for host resistance to microbial infection. *N. Engl. J. Med.* **303**, 259.
17. Schifferli, J. A., Ng, Y. C., and Peters, D. K. (1986). The role of complement and its receptor in the examination of immune complexes. *N. Engl. J. Med.* **315**, 488.
18. Agnello, V., Carr, R. I., Koffler, D., *et al.* (1969). Gel diffusion reactions of Clq with aggregated globulin DNA and various anionic substances. *Fed. Proc.* **28**, 696(A).
19. Cooper, N. R. (1983). Activation and regulation of the first complement component. *Fed. Proc.* **42**, 134.
20. Fu, S. N., Kunkel, H. G., Brusman, H. P., *et al.* (1974). Evidence for linkage between HL-A histocompatibility genes and those involved in the synthesis of the second component of complement. *J. Exp. Med.* **140**, 1108.

21. Allen, F. H., Jr. (1974). Linkage of HL-A and GBG. *Vox Sang.* **27**, 382.
22. Hauptman, G., Grosshans, E., and Heid, E. (1974). Lupus erythematosus syndrome and complete deficiency of the fourth component of complement. *Ann. Dermatol. Syph.* **101**, 479.
23. Truedsson, L., Alper, C. A., Awdeh, Z. L., *et al.* (1993). Characterization of type I complement C2 deficiency MHC haplotypes. Strong conservation of the complotype/HLA-B-region and absence of disease association due to linked class II genes. *J. Immunol.* **151**, 5856.
24. Truedsson, L., Sturfelt, G., and Nived, O. (1993). Prevalence of the type I complement C2 deficiency gene in Swedish systemic lupus erythematosus patients. *Lupus* **2**, 325.
25. Araujo, M. N., Silva, N. P., Andrade, L. E., *et al.* (1997). C2 deficiency in blood donors and lupus patients: Prevalence, clinical characteristics and HLA-associations in the Brazilian population. *Lupus* **6**, 462.
26. Sullivan, K. E., Petri, M. A., Schmeckpeper, B. J., McLean, R. H., and Winkelstein, J. A. (1994). Prevalence of a mutation causing C2 deficiency in systemic lupus erythematosus. *J. Rheumatol.* **21**, 1128.
27. Campbell, R. D. (1987). Molecular genetics of C2 and factor B. *Br. Med. Bull.* **43**, 37.
28. Raum, D., Donaldson, V. H., Rosen, F. S., *et al.* (1980). The complement system. *Curr. Top. Hematol.* **3**, 112.
29. Tedesco, F., Roncelli, L., Peterson, H., *et al.* (1990). Two distinct abnormalities in patients with C8 deficiency. *J. Clin. Invest.* **86**, 884.
30. Welch, T. R., Beischel, L., and Kleesattel, A. (1990). Functional consequences of the genetic polymorphism of the third component of complement. *J. Pediatr.* **116**, S92.
31. Petry, F. (1998) Molecular basis of hereditary Ciq deficiency. *Immunobiology* **199**, 286.
32. Kurata, H., Cheng, H. M., Kozutsumi, Y., Yokata, Y., and Kawasaki, T. (1993). Role of the collagen like domain of the human serum mannan-binding protein in the activation of complement and the secretion of this lectin. *Biochem. Biophys. Res. Commun.* **191**, 1204.
33. Lipscombe, R. H. J., Sumiya, M., Summerfield, J. A., and Turner, M. W. (1995). Distinct physiochemical characteristics of human mannose binding protein expressed by individuals of differing genotype. *Immunology* **85**, 660.
34. Matsushita, M., Ezekowitz, R. A., and Fujita, T. (1995). The Gly-54-Asp allelic form of human mannose-binding protein (MBP) fails to bind MBP-associated serine protease. *Biochem. J.* **311**, 1021.
35. Turner, M. W. (1995). Mannose-binding lectin: The pluripotent molecule of the innate immune system. *Immunol. Today* **17**, 532.
36. Madsen, H. O., Garred, P., Thiel, S., *et al.* (1995). Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. *J. Immunol.* **155**, 3013.
37. Sato, T., Endo, Y., Matsushita, M., and Fujita, T. (1994). Molecular characterization of a novel serine protease involved in activation of the complement system by mannose-binding protein. *Int. Immunol.* **6**, 665.
38. Rasmussen, J. M., Teisner, B., Brandslund, J., *et al.* (1986). A family with complement factor I deficiency. *Scand. J. Immunol.* **23**, 711.
39. Vyse, T. J., Spath, P. J., Davies, K. A., *et al.* (1994). Hereditary complement factor I deficiency. *Q. J. Med.* **87**, 385.
40. Vyse, T. J., Morley, B. J., Bartok, I., *et al.* (1996). The molecular basis of hereditary complement factor I deficiency. *J. Clin. Invest.* **97**, 925.
41. Hourcade, D., Holers, V. M., and Atkinson, J. P. (1989). The regulators of complement activation (RCA) gene cluster. *Adv. Immunol.* **45**, 381.
42. Ahern, J. M., and Fearon, D. T. (1989). Structure and function of the complement receptors CRI (CD35) and CR2 (CD21). *Adv. Immunol.* **46**, 183.
43. Ault, B. H., Schmidt, B. Z., Fowler, N. L., *et al.* (1989). Human factor H deficiency: Mutations in framework cysteine residues and block in H protein secretion and intracellular catabolism. *J. Biol. Chem.* **272**, 25168.
44. Van Dyne, S., Holers, V. M., Lubin, D. M., and Atkinson, J. P. (1987). The polymorphism of the C3b/C4b receptor in the normal population and in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **68**, 570.
45. Vik, D. P., and Wong, W. W. (1993). Structure of the gene for the F allele of complement receptor type 1 and sequence of the coding region unique to the S allele. *J. Immunol.* **151**, 6214.
46. Wong, W. W., Cahill, J. M., Rosen, M. D., *et al.* (1989). Structure of the human CR1 gene: Molecular basis of the structural and quantitative polymorphisms and identification of a new CR1-like allele. *J. Exp. Med.* **169**, 847.
47. Wilson, J. G., Wond, W. W. J., Murphy, E. E., Schur, P. H., and Fearon, D. T. (1987). Deficiency of the C3b/C4b receptor (CR1) of erythrocytes in systemic lupus erythematosus: Analysis of the stability of the defect and of a restriction fragment length polymorphism of the CR1 gene. *J. Immunol.* **138**, 2708.
48. Lambris, J. D., Lao, Z., Oglesby, T. J., *et al.* (1996). Dissection of CR1, factor H, membrane cofactor protein, and factor B binding and functional sites in the third complement component. *J. Immunol.* **156**, 4821.
49. Bartok, I., and Walport, M. J. (1995). Comparison of the binding of C3S and C3F to complement receptors type 1, 2, and 3. *J. Immunol.* **154**, 5367.
50. Gibb, A. L., Freeman, A. M., Smith, R. A., Edmonds, S., and Sim, E. (1993). The interaction of soluble human complement receptor type 1 (sCR1, BRL55730) with human complement component C4. *Biochim. Biophys. Acta* **1180**, 313.
51. Reilly, B. D., and Mold, C. (1997). Quantitative analysis of C4Ab and C4Bb binding to the C3b/C4b receptor (CR1, CD35). *Clin. Exp. Immunol.* **110**, 310.
52. Mold, C., Gurule, C., Otero, D., and Du Clos, T. W. (1996). Complement-dependent binding of C-reactive protein complexes to human erythrocyte CR1. *Clin. Immunol. Immunopathol.* **81**, 153.
53. Sanchez-Corral, P., Criado Garcia, O., and Rodriguez de Cordoba, S. (1995). Isoforms of human C4b-binding protein. I. Molecular basis for the C4BP isoform pattern

and its variations in human plasma. *J. Immunol.* **155**, 4030.
54. Harding, Y., and Dahlback, B. (1996). The amino-terminal module of the C4b-binding protein beta-chain contains the protein S-binding site. *J. Biol. Chem.* **271**, 20861.
55. Tamei, H., Hoshino, T., Yoshida, S., *et al.* (1995). One-step sandwich enzyme immunoassays for human C4b-binding protein (C4BP) and protein S-C4BP complex using monoclonal antibodies. *Clin. Chim. Acta* **234**, 115.
56. Ames, R. S., Li, Y., Sarau, H. M., *et al.* (1996). Molecular cloning and characterization of the human anaphylatoxin C3a receptor. *J. Biol. Chem.* **271**, 20231.
57. Gerard, C., and Gerard, N. P. (1994). C5A anaphylatoxin and its seven transmembrane-segment receptor. *Annu. Rev. Immunol.* **12**, 775.
58. Wetsel, R. A. (1995). Structure, function and cellular expression of complement anaphylatoxin receptors. *Curr. Opin. Immunol.* **7**, 48.
59. Siciliano, S. J., Rollins, T. E., DeMartino, J., *et al.* (1994). Two-site binding of C5a by its receptor: An alternative binding paradigm for G protein-coupled receptors. *Proc. Natl. Acad. Sci. USA* **91**, 1214.
60. Elsner, J., Oppermann, M., and Kapp, A. (1996). Detection of C5a receptors on human eosinophils and inhibition of eosinophil effector functions by anti-C5a receptor (CD88) antibodies. *Eur. J. Immunol.* **26**, 1560.
61. Morgan, E. L., Ember, J. A., Sanderson, S. D., *et al.* (1993). Anti-C5a receptor antibodies: Characterization of neutralizing antibodies specific for a peptide, C5aR-(9-29), derived from the predicted amino-terminal sequence of the human C5a receptor. *J. Immunol.* **151**, 377.
62. Malhotra, R. (1993). Collectin receptor (C1q receptor): Structure and function. *Behring Inst. Mitt.* **93**, 254.
63. Stuart, G. R., Lynch, N. J., Lu, J., *et al.* (1996). Localisation of the C1q binding site within C1q receptor/calreticulin. *FEBS Lett.* **397**, 245.
64. Tenner, A. J. (1993). Functional aspects of the C1q rptors. *Behring Inst. Mitt.* **93**, 241.
65. van den Berg, R. H., Faber-Krol, M., van Es, L. A., and Daha, M. R. (1995). Regulation of the function of the first component of complement by human C1q receptor. *Eur. J. Immunol.* **25**, 2206.
66. Lim, B. L., Reid, K. B., Ghebrehiwet, B., *et al.* (1996). The binding protein for globular heads of complement C1q, gC1qR: Functional expression and characterization as a novel vitronectin binding factor. *J. Biol. Chem.* **271**, 26739.
67. Klickstein, L. B., Barbashov, S. F., Liu, T., Jack, R. M., and Nicholson-Weller, A. (1997). Complement receptor type 1 (CR1, CD35) is a receptor for C1q. *Immunity* **7**, 345.
68. Schlessinger, M., Nave, Z., Levy, Y., *et al.* (1990). Prevalence of hereditary properdin. C7 and C8 deficiencies in patients with meningococcal infections. *Clin. Exp. Immunol.* **81**, 423.
69. Garty, B. Z., Nitzan, M., and Danon, Y. L. (1993). Systemic meningococcal infections in patients with acquired complement deficiency. *Pediatr. Allergy Immunol.* **4**, 6.
70. Madi, N., Steiger, G., Estreicher, J., and Schifferli, J. A. (1991). Immune adherence and clearance of hepatitis B surface Ag/Ab complexes is abnormal in patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **85**, 373.
71. Super, M., Thiel, S., Lu, J., Levinsky, R. J., and Turner, M. W. (1989). Association of low levels of mannan-binding protein with a common defect of opsonization. *Lancet* **II**, 1236.
72. Sumiya, M., Super, M., Tabona, P., *et al.* (1991). Molecular basis of opsonic defect in immunodeficient children. *Lancet* **337**, 1569.
73. Garred, P., Madsen, H. O., Hofmann, B., and Svejaard, A. (1995). Increased frequency of homozygosity of abnormal mannan-binding protein alleles in patients with suspected immunodeficiency. *Lancet* **346**, 941.
74. Summerfield, J. A., Sumiya, M., Levin, M., and Turner, M. W. (1996). Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. *Br. Med. J.* **314**, 1229.
75. Garred, P., Madsen, H. O., Balslev, U., *et al.* (1997). Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. *Lancet* **349**, 236.
76. Anderson, D. C., Schmalstein, F. C., Finegold, M. J., *et al.* (1985). The severe and moderate phenotypes of heritable Mac-1 LFA-1 deficiency: Their quantitative definition and relationship to leukocyte dysfunction and clinical features. *J. Infect. Dis.* **152**, 668.
77. Gallin, J. I. (1985). Leukocyte adherence-related glycoproteins LFA-1, Mo-1, and pl5O,95: A new group of monoclonal antibodies, a new disease, and a possible opportunity to understand the molecular basis of leukocyte adherence. *J. Infect. Dis.* **152**, 661.
78. Nielsen, C. H., Antonsen, S., Matthiesen, S. H., and Leslie, R. G. (1997). The roles of complement receptors type 1 (CR1, CD35) and type 3 (CR3, CD11b/CD18) in the regulation of the immune complex-elicited respiratory burst of polymorphonuclear leukocytes in whole blood. *Eur. J. Immunol.* **27**, 2914.
79. Inai, S., Akagaki, Y., Moriyama, T., *et al.* (1989). Inherited deficiencies of the late-acting complement components other than C9 found among healthy blood donors. *Int. Arch. Allergy Appl. Immunol.* **90**, 274.
80. Agnello, V., deBracco, M. M. E., and Kunkel, H. G. (1972). Hereditary C2 deficiency with some manifestations of systemic lupus erythematosus. *J. Immunol.* **108**, 837.
81. Berkel, A. L., Loos, M., Sanal, O., *et al.* (1979). Clinical and immunological studies in a case of selective complete C1q deficiency. *Clin. Exp. Immunol.* **38**, 52.
82. Leyva-Cobian, F., Mampaso, I. M. F., Sanchez-Bayle, M., *et al.* (1980). Familial C1q deficiency associated with renal and cutaneous disease. *Clin. Exp. Immunol.* **44**, 173.
83. Uenaka, A., Akimoto, T., Aoki, T., *et al.* (1982). A complete selective C1q deficiency in a patient with discoid lupus erythematosus (DLE). *Clin. Exp. Immunol.* **48**, 353.
84. Minta, J. O., Winkler, C. J., Biggar, W. D., *et al.* (1982). A selective and complete absence of C1q in a patient with vasculitis and nephritis. *Clin. Immunol. Immunopathol.* **22**, 225.

85. Ziccardi, R. J., and Cooper, N. R. (1977). The subunit composition and sedimentation properties of human C1. *J. Immunol.* **118**, 2047.
86. Müller-Eberhard, H. J. (1979). The molecular dynamics and biochemistry of complement. *In* "Modulation of Protein Function" D. E. Atkinson and C. F. Fox (eds.), p. 219. Academic Press, San Diego.
87. Muller-Eberhard, H. J. (1975). Complement. *Annu. Rev. Biochem.* **44**, 697.
88. Topaloglu, R., Bakkaloglu, A., Slingsby, J. H., *et al.* (1996). Molecular basis of hereditary C1q deficiency associated with SLE and IgA nephropathy in a Turkish family. *Kidney Int.* **50**, 635.
89. Kirschfink, M., Petry, F., Khirwadkar, K., *et al.* (1993). Complete functional C1q deficiency associated with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **94**, 267.
90. Bowness, P., Davies, K. A., Norsworthy, P. J., *et al.* (1994). Hereditary C1q deficiency and systemic lupus erythematosus. *Q. J. Med.* **87**, 455.
91. Pickering, M. C., Botto, M., Taylor, P. R., *et al.* (2000). Systemic lupus erythematosus, complement deficiency, and apoptosis. *Adv. Immunol.* **76**, 227.
92. Pickering, R. J., Michael, A. F., Jr., Herdman, R. C., *et al.* (1971). The complement system in chronic glomerulonephritis: Three newly associated aberrations. *J. Pediatr.* **78**, 30.
93. Moncada, B., Noorbibi, K., Day, B., *et al.* (1972). Lupus erythematosus-like syndrome with a familial defect of complement. *N. Engl. J. Med.* **286**, 689.
94. Rich, K. C., Jr., Hurley, J., and Gewurz, H. (1979). Inborn C1r deficiency with a mild lupus-like syndrome. *Clin. Immunol. Immunopathol.* **13**, 77.
95. Lee, S. L., Wallace, S. L., Barone, R., *et al.* (1978). Familial deficiency of two subunits of the first component of complement. *Arthritis Rheum.* **21**, 958.
96. Schaller, J. G., Gilliland, B. G., Ochs, H. D., *et al.* (1977). Severe systemic lupus erythematosus with nephritis in a boy with deficiency of the fourth component of complement. *Arthritis Rheum.* **20**, 1519.
97. Tappeiner, G., Scholz, S., Linert, J., *et al.* (1978). Hereditary deficiency of the fourth component of complement (C4): Study of a family. *Inserm* **800**, 399.
98. Ballow, M., McLean, R. H., Einarson, M., *et al.* (1979). Hereditary C4 deficiencyCgenetic studies and linkage to HLA. *Transplant Proc.* **11**, 1710.
99. Tappeiner, G., Hintner, H., Scholz, S., *et al.* (1982). Systemic lupus erythematosus in hereditary deficiency of the fourth component of complement. *J. Am. Acad. Dermatol.* **7**, 66.
100. Tappeiner, G. (1982). Disease states in genetic complement deficiencies. *Int. J. Dermatol.* **21**, 175.
101. Urowitz, M. B., Gladman, D. D., and Minta, J. O. (1981). Systemic lupus erythematosus in a patient with C4 deficiency. *J. Rheumatol.* **8**, 741.
102. Kjellman, M., Laurell, A. B., Low, B., *et al.* (1982). Homozygous deficiency of C4 in a child with a lupus erythematosus syndrome. *Clin. Genet.* **22**, 331.
103. Mascart-Lemone, F., Hauptmann, G., Goetz, J., *et al.* (1983). Genetic deficiency of the fourth component of complement presenting with recurrent infections and a SLE-like disease: Genetical and immunological studies. *Am. J. Med.* **75**, 295.
104. Lhotta, K., Kronig, P., Hinter, H., *et al.* (1990). Renal disease in a patient with hereditary complement deficiency of the fourth complement component of complement. *Nephron* **56**, 206.
105. Berliner, S., Weinberg, A., Zamir, R., *et al.* (1984). Familial cryoglobulinemia and C4 deficiency. *Scand. J. Rheumatol.* **13**, 151.
106. Sturfelt, G., Truedsson, L., Johansen, P., *et al.* (1990). Homozygous C4A deficiency in systemic lupus erythematosus: Analysis of patients from a defined population. *Clin. Genet.* **38**, 427.
107. Petri, M., Watson, R., Winkelstein, J. A., and McLean, R. H. (1993). Clinical expression of systemic lupus erythematosus in patients with C4A deficiency. *Medicine (Baltimore)* **72**, 236.
108. Steinsson, K., Arnason, A., Erlendsson, K., *et al.* (1995). A study of the major histocompatibility complex in a Caucasian family with multiple cases of systemic lupus erythematosus: Association with the C4AQ0 phenotype. *J. Rheumatol.* **22**, 1862.
109. McLean, R. H., Wyatt, R. H., and Julian, B. A. (1984). Complement phenotypes in glomerulonephritis, increased frequency of homozygous null C4 phenotypes in IgA nephropathy and Henoch-Schonlein purpura. *Kidney Int.* **26**, 855.
110. Gell, J., Tye, M. J., and Agnello, V. (1983). Selective depression of the C4 component of complement: Evidence for an association with genetic deficiency of C4 and dermatologic diseases. *Diagn. Immunol.* **1**, 49.
111. Hauptmann, G. (1983). Observations presented in discussions at Symposium: Clinical Aspects of Complement Mediated Diseases, Bellagio. Italy, May.
112. Provost, T. T., Arnett, F. C., and Reichlin, M. (1983). Homozygous C2 deficiency, lupus erythematosus and anti-Ro (SSA) antibodies. *Arthritis Rheum.* **26**, 1279.
113. Whaley, K., and Schweble, W. (1997). Complement and complement deficiencies. *Semin. Liver Dis.* **17**, 197.
114. Agnello, V. (1986). Lupus diseases associated with hereditary and acquired deficiencies of complement. *Springer Semin. Immunopathol.* **9**, 161.
115. Maddison, P. J. (1982). ANA-negative SLE. *Clin. Rheum. Dis.* **8**, 105.
116. Sontheimer, R. D., Thomas, J. R., and Gilliam, J. N. (1979). Subacute cutaneous lupus erythematosus. *Arch. Dermatol.* **115**, 1409.
117. Sontheimer, R. D., Stastny, P., Maddison, P., *et al.* (1982). Serologic and HLA associations in subacute cutaneous lupus erythematosus (SCLE): A clinical subset of lupus erythematosus. *Ann. Intern. Med.* **97**, 664.
118. Glass, D., Raum, D., Gibson, D., *et al.* (1976). Inherited deficiency of the second component of complement. *J. Clin. Invest.* **58**, 853.

119. McCarty, D. J., Tan, E. M., Zvaifler, N. J., *et al.* (1981). Serologic studies in a family with heterozygous C2 deficiency. *Am. J. Med.* **6**, 945.
120. Davies, K. A., Erlendsson, K., Beynon, H. L., *et al.* (1993). Splenic uptake of immune complexes in man is complement-dependent. *J. Immunol.* **151**, 3866.
121. Osofsky, S. G., Thompson, B. H., Lint, L. F., *et al.* (1977). Hereditary deficiency of the third component of complement in a child with fever, skin rash, and arthralgias: Response to transfusion of whole blood. *J. Pediatr.* **90**, 180.
122. Sano, Y., Nishimukai, H., Kitamura, H., *et al.* (1981). Hereditary deficiency of the third component of complement in two sisters with systemic lupus erythematosus-like symptoms. *Arthritis Rheum.* **24**, 1255.
123. Rosenfeld, S. I., Kelly, M. R., and Leddy, J. P. (1976). Hereditary deficiency of the fifth component of complement in man. *J. Clin. Invest.* **57**, 1626.
124. Tedesco, F., Silvani, C. M., Agelli, M., *et al.* (1981). A lupus-like syndrome in a patient with deficiency of the sixth component of complement. *Arthritis Rheum.* **23**, 1438.
125. Trapp, R. G., Mooney, H., Husain, I., *et al.* (1980). Hereditary complement (C6) deficiency with discoid lupus/Sjögren's syndrome. *Arthritis Rheum.* **23**, 757.
126. Mooney, E. (1984). Complement factor 6 deficiency associated with lupus. *J. Am. Acad. Dermatol.* **11**, 896.
127. Zeitz, H. J., Miller, G. W., Lint, T. F., *et al.* (1981). Deficiency of C7 with systemic lupus erythematosus. *Arthritis Rheum.* **24**, 87.
128. Jasin, H. E. (1977). Absence of the eighth component of complement in association with systemic lupus erythematosus-like disease. *J. Clin. Invest.* **60**, 709.
129. Pickering, R. J., Rynes, R. I., LoCascio, N., *et al.* (1982). Identification of the subunit of the eight component of complement (C8) in a patient with systemic lupus erythematosus and absent C8 activity: Patient and family studies. *Clin. Immunol. Immunopathol.* **23**, 323.
130. Kawai, T., Kahoh, K., Narita, M., *et al.* (1989). Deficiency of the 9th component of complement (C9) in a patient with systemic lupus erythematosus. *J. Rheum.* **16**, 542.
131. Kawai, T., Katoh, K., Narita, M., Tani, K., and Okubo, T. (1989). Deficiency of the 9th component of complement (C9) in a patient with systemic lupus erythematosus. *J. Rheumatol.* **16**, 542.
132. Takeda, I., Igarashi, S., Nishimaki, T., and Kasukawa, R. (1994). A case of systemic lupus erythematosus in late component (C9) complement deficiency. *Ryumachi* **34**, 628.
133. Senaldi, G., Davies, E. T., Peakman, M., *et al.* (1995). Frequency of mannose-binding protein deficiency in patients with systemic lupus erythematosus. *Arthritis Rheum.* **38**, 1713.
134. Davies, E. J., Snowden, N., Hillarby, M. C., *et al.* (1995). Mannose-binding protein gene polymorphism in systemic lupus erythematosis. *Arthritis Rheum.* **38**, 110.
135. Lau, Y. L., Lau, C. S., Chan, S. Y., Karlberg, J., and Turner, M. W. (1996). Mannose-binding protein in Chinese patients with systemic lupus erythematosus. *Arthritis Rheum.* **39**, 706.
136. Sullivan, K. E., Wooten, C., Goldman, D., and Petri, M. (1996). Mannose-binding protein genetic polymorphisms in black patients with systemic lupus erythematosus. *Arthritis Rheum.* **39**, 2046.
137. Davies, E. J., The, L.-S., Ordi-Ros, J., *et al.* (1997). A dysfunctional allele of the mannose binding protein gene associates with systemic lupus erythematosus in a Spanish population. *J. Rheum.* **24**, 485.
138. Hartmann, D., Fremeaux-Bacchi, V., Weiss, L., *et al.* (1997). Combined heterozygous deficiency of the classical complement pathway proteins C2 and C4. *J. Clin. Immunol.* **17**, 176.
139. Segurado, O. G., Arnaiz-Villena, A. A., Iglesias-Casarrubios, P., *et al.* (1992). Combined total deficiency of C7 and C4B with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **87**, 410.
140. Agnello, V., Koffler, D., Eisenberg, J. W., *et al.* (1971). C1q precipitins in the sera of patients with systemic lupus erythematosus and other hypocomplementemic states: Characterization of high and low molecular weight types. *J. Exp. Med.* **134**, 118S.
141. Agnello, V., Winchester, R., Ruddy, S., *et al.* An unusual SLE related syndrome: Erythema multiforme, hypocomplementemia and circulating C1q precipitins. *In* "Proceedings of the 13th International Congress on Rheumatology, Kyota, Japan." Excerpta Medica, Amsterdam, International Congress Series No. 229.
142. Agnello, V., Ruddy, S., and Winchester, R. J. (1975). Hereditary C2 deficiency in systemic lupus erythematosus and acquired complement abnormalities in an unusual SLE-related syndrome. *Birth Defects* **11**, 312.
143. McDuffic, F. C., Sams, W. M., Maldonado, J. E., *et al.* (1973). Hypocomplementemia with cutaneous vasculitis and arthritis. *Mayo Clin. Proc.* **48**, 340.
144. Marder, R. J., Rent, R., Choi, E. Y., *et al.* (1976). C1q deficiency associated with urticarial-like lesions and cutaneous vasculitis. *Am. J. Med.* **61**, 560.
145. Marder, R. J., Burch, F. X., Schmid, F. R., *et al.* (1978). Low molecular weight C1q-precipitins in hypocomplementemic vasculitis-urticaria syndrome: Partial purification and characterization as immunoglobulin. *J. Immunol.* **121**, 613.
146. Ludivico, C. I., Myers, A. R., and Maurer, K. (1979). Hypocomplementemic urticarial vasculitis with glomerulonephritis and pseudotumor cerebri. *Arthritis Rheum.* **22**, 1024.
147. Zeiss, C. R., Burch, F. X., Marder, R. J., *et al.* (1980). A hypocomplementemic vasculitis urticarial syndrome. *Am. J. Med.* **68**, 867.
148. McLead, R. H., Weinstein, A., Chapitis, J., *et al.* (1980). Familial partial deficiency of the third component of complement (C3) and the hypocomplementemic cutaneous vasculitis syndrome. *Am. J. Med.* **68**, 549.
149. Schwartz, H. R., McDuffie, F. C., Black, L. F., *et al.* (1982). Hypocomplementemic urticarial vasculitis. *Mayo Clin. Proc.* **57**, 321.

150. Sissons, J. G. P., Peters, D. K., Williams, D. G., *et al.* (1974). Skin lesions, angioedema, and hypocomplementemia. *Lancet* **2**, 1350.
151. Tuffanelli, D. L. (1975). Cutaneous immunopathology: Recent observations. *J. Invest. Dermatol.* **65**, 143.
152. Oishi, M., Takano, M., Miyachi, K., *et al.* (1976). A case of unusual SLE-related syndrome characterized by erythema multiforme, angioneurotic edema, marked hypocomplementemia, and C1q precipitins of the low molecular weight type. *Int. Arch. Allergy Appl. Immunol.* **50**, 463.
153. Geha, R. S., and Akl, K. F. (1976). Skin lesions, angioedema, eosinophilia, and hypocomplementemia. *J. Pediatr.* **89**, 724.
154. Wara, D. W., Reiter, E. O., Doyle, N. E., *et al.* (1975). Persistent C1q deficiency in a patient with a systemic lupus erythematosus-like syndrome. *J. Pediatr.* **86**, 743.
155. Feig, P. U., Soter, N. A., Yager, H. M., *et al.* (1976). Vasculitis with urticaria, hypocomplementemia, and multiple system involvement. *JAMA* **236**, 2065.
156. Soter, N. A. (1977). Chronic urticaria as a manifestation of necrotizing vasculitis. *N. Engl. J. Med.* **25**, 1440.
157. Mathison, D. A., Arroyave, C. M., Bhat, K. N., *et al.* (1977). Hypocomplementemia in chronic idiopathic urticaria. *Ann. Intern. Med.* **86**, 534.
158. Curd, J. G., Milgrom, H., Stevenson, D. D., *et al.* (1979). Potassium iodide sensitivity in four patients with hypocomplementemic vasculitis. *Ann. Intern. Med.* **91**, 853.
159. Gammon, W. R., and Wheeler, C. E. (1979). Urticarial vasculitis. *Arch. Dermatol.* **115**, 76,
159a. Youniou, P., Dorval, J. C., Cledes, J., *et al.* (1983). A study of lupus erythematosus-like disease and hereditary angio-oedema treated with danazol. *Br. J. Dermatol.* **108**, 717.
160. Mannik, M., and Wener, M. H. (1997). Deposition of antibodies to the collagen-like region of C1q in renal glomeruli of patients with proliferative lupus glomerulonephritis. *Arthritis Rheum.* **40**, 1504.
161. Wisnieski, J. J., Baer, A. N., Christensen, J., *et al.* (1995). Hypocomplementemic urticarial vasculitis syndrome: Clinical and serologic findings in 18 patients. *Medicine* **74**, 24.
162. Wisnieski, J. J., and Naff, G. B. (1989). Serum IgG antibodies to C1q in hypocomplementemic urticarial vasculitis syndrome. *Arthritis Rheum.* **32**, 1119.
163. Fremeaux-Bacchi, V. I., Weiss, L., Demouchy, C., Blouin, J., and Kazatchkine, M. D. (1996). Autoantibodies to the collagen-like region of C1q are strongly associated with classical pathway-mediated hypocomplementemia in systemic lupus erythematosus. *Lupus* **5**, 216.
164. Martensson, U., Sjoholm, A. G., Sturfelt, G., Truedsson, L., and Laurell, A. B. (1992). Western blot analysis of human IgG reactive with the collagenous portion of C1q: Evidence of distinct binding specificities. *Scand. J. Immunol.* **35**, 735.
165. Sissons, J. G., West, R. J., Fallows, J., *et al.* (1976). The complement abnormalities of lipodystrophy. *N. Engl. J. Med.* **294**, 461.
166. Jasin, H. E. (1979). Systemic lupus erythematosus, partial lipodystrophy and hypocomplementemia. *J. Rheumatol.* **6**, 43.
167. Davis, A. E., and Cicardi, M. (1996). "C1 Inhibitor Autoantibodies," pp. 126B131. Elsevier, New York.
168. Hory, B., Panouse-Perrin, J., Suzuki, Y., *et al.* (1981). Immune complex nephropathy and hereditary deficiency of C1 esterase inhibitor. *Nouv. Presse. Med.* **10**, 2193.
169. Suzuki, Y., Nihei, H., Mimura, N., *et al.* (1986). A case of hereditary angioneurotic edema associated with systemic lupus erythematosus. *Jpn. J. Med.* **25**, 281.
170. Guillet, G., Sassolas, B., Plantin, P., *et al.* (1988). Anti-Ro positive lupus and hereditary angioneurotic edema: A 7-year follow-up with worsening of lupus under danazol treatment. *Dermatologica* **177**, 370.
171. Gudat, W., and Bork, K. (1989). Hereditary angioedema associated with subacute cutaneous lupus erythematosus. *Dermatologica* **179**, 211.
172. Horiuchi, S., Baba, T., Uyeno, K., *et al.* (1989). A case of hereditary angioneurotic edema associated with systemic lupus erythematosus. *Nippon Hifuka Gakkai Zasshi* **99**, 921.
173. Duhra, P., Holmes, J., and Porter, D. I. (1990). Discoid lupus erythematosus associated with hereditary angioneurotic edema. *Br. J. Dermatol.* **123**, 241.
174. Cox, N. H., West, N. C., Ive, F. A., *et al.* (1991). Lupus erythematosus and hereditary angio-oedema. *Br. J. Dermatol.* **125**, 82.
175. Perkins, W., Stables, G. I., and Lever, R. S. (1994). Protein S deficiency in lupus erythematosus secondary to hereditary angio-oedema. *Br. J. Dermatol.* **130**, 381.
176. Donaldson, V. H., Bissler, J. J., Welch, T. R., *et al.* (1996). Antibody to C1-inhibitor in a patient receiving C1-inhibitor infusions for treatment of hereditary angioneurotic edema with systemic lupus srythematosus reacts with a normal allotype of residue 458 of C1-inhibitor. *J. Lab. Clin. Med.* **128**, 438.
177. Youniou, P., Dorval, J. C., Cledes, J., *et al.* (1983). A study of lupus erythematosus-like disease and hereditary angio-oedema treated with danazol. *Br. J. Dermatol.* **108**, 717.
178. Kohler, P. F., Percy, J., and Campion, W. M. (1974). Hereditary angioedema "familial" lupus erythematosus in identical twin boys. *Am. J. Med.* **56**, 406.
179. Rosenfeld, G. B., Partridge, R. E., Bartholomew, W., *et al.* (1974). Hereditary angioneurotic edema (HANE) and systemic lupus erythematosus (SLE) in one of identical twin girls. *Am. Acad. Allergy* **53**, 68.
180. Donaldson, V. H., Hess, E. V., and McAdams, A. J. (1977). Lupus-erythematosus-like disease in three unrelated women with hereditary angioneurotic edema. *Ann. Intern. Med.* **86**, 312.
181. Tuffanelli, D. L. (1977). Discoid lupus erythematosus and the variant form of hereditary angioedema. *Arch. Dermatol.* **113**, 374.
182. Young, D. W., Thompson, R. A., and Mackie, P. H. (1980). Plasmapheresis in hereditary angioneurotic edema and systemic lupus erythematosus. *Arch. Intern. Med.* **140**, 127.

183. Masse, M. C., and Connolly, S. M. (1982). An association between C1 esterase inhibitor deficiency and lupus erythematosus: Report of two cases and review of the literature. *J. Am. Acad. Dermatol.* **7**, 255.
184. Jordan, R. E., and Provost, T. T. (1976). The complement system and the skin. *In* "Yearbook of Dermatology" F. D. Mackinson and R. W. Pearson (eds.), p. 7. Yearbook Medical Publishers, Chicago.
185. Shiraishi, S., Watanabe, N. Y., Matsuda, K., *et al.* (1982). C1 inhibitor deficiency simulating systemic lupus erythematosus. *Br. J. Dermatol.* **106**, 455.
186. Masse, R., Youinou, P., Dorval, J.-C., *et al.* (1980). Reversal of lupus erythematosus-like disease with danazol. *Lancet* **2**, 651.
187. Donaldson, V. H., and Hess, E. V. (1980). Effect of danazol on lupus erythematosus-like disease in hereditary, angioneurotic edema. *Lancet* **2**, 1145.
188. Moller Rasmussen, J., Jepsen, H. H., Teisner, B., *et al.* (1989). Quantification by ELISA of erythrocyte-bound C3 fragments expressing C3d and/or C3c epitopes in patients with factor I deficiency and with autoimmune diseases. *Vox San.* **56**, 262.
189. Marquart, H. V., Rasmussen, J. M., and Leslie, R. G. (1997). Complement-activating ability of leucocytes from patients with complement factor I deficiency. *Immunology* **91**, 486.
190. Fijen, C. A., Kuijper, E. J., Te Bulte, M., *et al.* (1996). Heterozygous and homozygous factor H deficiency states in a Dutch family. *Clin. Exp. Immunol.* **105**, 511.
191. Cornillet, P., Gready, P., Pennaforte, J. L., *et al.* (1992). Increased frequency of the long (S) allotype of CR1 (the C3b/C4b recoptor, CD35) in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **89**, 22.
192. Sato, H., Yokota, E., Tokiyama, K., Kawaguchi, T., and Niho, Y. (1991). Distribution of the Hind III restriction fragment length polymorphism among patients with systemic lupus erythematosus with different concentrations of CR1. *Ann. Rheum. Dis.* **50**, 765.
193. Tebib, J. G., Martinez, O., Granados, J., Alarcon-Segovia, D., and Schur, P. H. (1989). The frequency of complement receptor type 1 (CR1) gene polymorphism in nine families with multiple cases of systemic lupus erythematosus. *Arthritis Rheum.* **32**, 1465.
194. Kumar, A., Kumar, A., Sinha, S., *et al.* (1995). Hind III genomic polymorphism of the C3b receptor (CR1) in patients with SLE: Low erythrocyte/CR1 expression is an acquired phenomenon. *Immunol. Cell. Biol.* **73**, 457.
195. Moulds, J. M., Reveille, J. D., and Arnett, F. C. (1996). Structural polymorphisms of complement receptor 1 (CR1) in systemic lupus erythematosus (SLE) patients and normal controls of three ethnic groups. *Clin. Exp. Immunol.* **105**, 302.
196. Witte, T., Gessner, J. E., Gotze, O., Deicher, H., and Schmidt, R. E. (1992). Complement receptor 3 deficiency in systemic lupus erythematosus. *Immun. Infekt.* **20**, 60.
197. Merry, A. H., Rawlingson, V. I., Uchikawa, M., *et al.* (1989). Studies on the sensitivity to complement-mediated lysis of erythrocytes (Inab phenotype) with a deficiency of DAF (decay accelerating factor). *Br. J. Haematol.* **73**, 248.
198. Agnello, V., Gell, J., and Tye, M. (1983). Partial genetic deficiency of the C4 component of complement in discoid lupus erythematosus and urticaria/angioedema. *J. Am. Acad. Dermatol.* **9**, 814.
199. Fishelson, Z., Attali, G., and Mevorach, D. (2001). Complement and apoptosis. *Mol. Immunol.* **38**, 207.
200. Paul, E., and Carroll, M. C. (2003). Complement C4 participates in negative selection of autoreactive cells. *In* "Immune Mechanisms and Disease." *New York Acad. Sci.*
201. Casciola-Rosen, L. A., Anhalt, G., and Rosen, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317.
202. Korb, L. C., and Ahearn, J. M. (1997). C1q binds directly and specifically to surface blebs of apoptotic human keratinocytes: Complement deficiency and systemic lupus erythematosus revisited. *J. Immunol.* **158**, 4525.
203. Botto, M., Dell'Agnolla, C., Bygrave, A. E., *et al.* (1998). Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature Genet.* **19**, 56.
204. Taylor, P. R., Carugati, A., Fadok, V. A., *et al.* (2000). A hierarchical role for classical pathway complement protein in the clearance of apoptotic cells *in vivo*. *J. Exp. Med.* **192**, 359.
205. Mitchell, D. A., Taylor, P. R., Cook, H. T., *et al.* (1999). C1q protects against the development of glomerulonephritis independently of C3 activation. *J. Immunol.* **162**, 5676.
206. Agnello, V., Gell, J., and Tye, M. (1983). Partial genetic deficiency of the C4 component of complement in discoid lupus erythematosus and urticaria/angioedema. *J. Am. Acad. Dermatol.* **9**, 814.

7

GENDER AND AGE IN LUPUS

Robert G. Lahita

LATE-ONSET SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction

The clinical onset of systemic lupus erythematosus (SLE) and its clinical course are affected by age. The relationship between age and the morbidity and mortality of SLE is unknown, but evidence shows that older age in women may be good. A discussion of SLE in the neonatal and the prepubertal child can be found in Chapters 17 and 18, respectively. This chapter limits the discussion of the "older" patient, to people over age 50.

The ratio of males to females is 1:8 in the older patient instead of 1:15 [1, 2]. Patients with drug-induced lupus are distinctly older and are usually male [3] because commonly used drugs such as procainamide and hydralazine are widely used for conditions in people over age 50 years. There are more male patients taking drugs such as procainamide. The topic of drug-induced SLE is covered in Chapters 44 and 45.

SLE has no predilection for males who are older, but relative to younger male patients (<50 years), the number of men with SLE increases with age. About 25% of the males in one study were over age 50 years [4]. Most investigators report that the ratios of females to males with SLE after age 50 decrease from 10–15:1 to 8:1. The older male presentation of SLE was atypical [5] in one study. In the largest series of SLE patients studied who were 50 years of age or older, 79% were women [6]. Reasons for an increase of older males with SLE may relate to a variety of factors and are given as sexual senescence, environmental agents, and possibly an increase of prolactin [7].

Clinical Presentation of the Aged Patient with SLE

In almost all studies of aged people with SLE, the majority met four or more of the American College of Rheumatology (ACR) criteria for SLE. In another study, 81% of older patients met four or more criteria, whereas 19% met three or less [8]. Because of late age of onset, the initial diagnosis of SLE was incorrect in 55% of patients. This is not surprising as physicians are reluctant to diagnose SLE and because this age group presents with atypical signs and symptoms. Therefore, the diagnosis of older age SLE can be delayed for up to 2 years. While there is no question that age may modify the clinical presentation of the disease, it is imperative that physicians learn to include SLE as part of any differential diagnosis in patients of older age presenting with myalgia, arthralgia, unexplained weight loss, fatigue, and low-grade fever.

The presentation of SLE in the older patient (aged 50–80 years) is different. The older SLE patient presents clinically more like a patient with drug-induced SLE than one with idiopathic disease [9]. These older patients have a low to absent incidence of renal disease and primarily pleuropericardial and musculoskeletal symptoms [10]. Older patients rarely have low complement levels and native DNA antibodies, whereas anti-single-stranded (ss) DNA antibodies are common [2]. Serositis, interstitial pulmonary disease, anti-La anti-

TABLE 1 Clinical and Laboratory Features in 13 Late-Onset Patients with SLE Compared to a Meta-Analysis of Other Series[a,b]

	13 patients, present study (OR)	170 patients (pooled OR)
Clinical		
Fever	0.51	0.21 ↓
Butterfly rash	0.23 ↓	
Discoid rash	0.86	
Photosensitivity	0.83 ↓	1.10
Mouth ulcer	0.85	0.74
Alopecia	0.21 ↓	0.47 ↓
Raynaud's syndrome	0.38	0.48 ↓
Arthritis	0.41	
Pleural	7.32 ↑	
Pericarditis	3.51	
Serositis	5.32 ↑	1.63 ↑
Lung involvement	7.33	3.48 ↑
Cardiac involvement	0.87	
Nephritis	1.71	0.79
CNS involvement	0.71	0.52 ↓
Myositis	1.84	0.68
Lymphadenopathy	0.54	0.39 ↓
Laboratory		
Leukopenia	0.87	0.67
Thrombocytopenia	1.17	
Anti-daDNA	0.56	0.76
Anti-Ro antibody	0.66	
Anti-La antibody	3.44	4.24 ↑
Hypocomplementemia	1.18	0.54 ↓

[a] From Mak *et al*. with permission.
[b] ↑/↓: increase/decrease incidence of feature compared to patients with early-onset disease.

bodies, and Sjogren's syndrome symptoms (secondary sicca) are commonly associated with the disease of the older age group [1] as revealed by meta analysis. Alopecia, Raynaud's, phenomenon, fever, lymphadenopathy, hypocomplementemia, and neuropsychiatric disease are not common in this age group. As in all aspects of medicine, generalizations cannot be made because some older patients have native DNA antibody and severe clinical signs of renal disease [11, 12].

In a retrospective study of 102 patients with late-onset SLE, there was loss of the female preponderance, a more insidious onset of presentation, less skin manifestations, and more serositis. Table 1 shows 13 patients from a study compared to a meta-analysis of other studies. No differences in renal, neuropsychiatric, cardiac, pulmonary, or liver involvement were found when the older group was compared to the new [13].

Race

In most studies cited, Caucasians are the predominant ethnic group with late-onset SLE [14]. In one study [2], 59% of the younger SLE group were Black patients, whereas only 20% in the older age group (>50 years) were Black. In still another study, 87% of the patients in the older group were Caucasian and about 6.5% were Black [8]. Reasons for this unusual racial predilection for older age SLE patients need further investigation but may be related to a greater severity of the disease in Blacks. In fact, data from the National Institutes of Health suggest that the over all mortality of lupus in the young black female is five times greater than that found in Caucasians. It is clear that age and race influence both morbidity and mortality. A major study [15, 16] of survival in SLE in which follow-up was 75.6 months revealed that race and socioeconomic status had significant effects on survival. No diminished lupus-related mortality with age was documented but morbidity in women decreases with age. The authors suggested that race distribution between young and old patients with SLE might confound the results.

Course and Therapy in the Older Patient

Older SLE patients had a mean age of onset of disease of 59.5 years in one study (range 50–81 years). In that study the mean age at diagnosis was 62.4 years (range 52–83 years). Moreover, these patients can be maintained on lower doses of corticosteroid, and conservative therapy is usually the rule. Only 42% of these patients required high-dose corticosteroid (>25 mg/day × I month), whereas 23% could be maintained on 1–25 mg per day, and 35% never needed steroids [8]. Patients with late-onset disease require less medication and lower doses of immunosuppressive agents [17].

Survival in the older age group is good; the 5-year survival rate was 92.3 ± 5.3%, and 83.1 ± 10% survived 9 years at follow-up. One conclusion might be that older patients rarely die of their disease or the complications of therapy when they are treated conservatively. However, at least one study suggests that major causes of death in the older age groups are related to therapy [18]. Unlike younger patients, where the major mortality is atherosclerosis, infections and perforated peptic ulcers remain the most common cause of death in older patients [6, 19, 20].

Comments on the Elderly SLE Patient

There are many factors that hypothetically could predispose the older patient to SLE, including [8] liver and thyroid disease or exposure to certain exogenous

agents, such as hormones. None of these theories is proven at this time.

A popular leitmotiv about why the immune system becomes hyperactive at a time when a human ages is that there is a decline of immune surveillance. The appearance of autoantibodies in older people without disease has always suggested that tolerance might decline with age. Readers are referred to Chapter 1 on autoimmunity, Chapter 3 on etiology, and Chapter 2 on antigenic tolerance and are asked to draw their own conclusions regarding the possible pathogenetic mechanisms that are operable.

GENDER AND SLE

Sex steroids play an important role in the maturation of organ systems that affect animals throughout life [21] and are therefore important to the health of most vertebrates. Even though the most obvious effects occur at puberty in the development of secondary sexual characteristics, hormones affect major developmental changes even before parturition. Diverse biologic functions including behavior, intelligence, sexual preference, physical stature, and the immune system, are likely targets for these hormones. The effects of sex steroids on the immune system are profound and long lasting because they control the maturation of various cell systems and may influence susceptibility to disease through the modulation of immune cell populations throughout the life of the animal. This might happen through alteration of cytokine levels, control of cell populations through processes such as apoptosis, or alteration of very basic molecular mechanisms. Lupus is one disease that is very affected by hormones, as the skew toward females is significant for SLE.

Murine Models of Autoimmunity and the Sex Hormones

Although this book contains an entire chapter about murine models of lupus (Chapter 8), it is appropriate to briefly mention the background for the study of gender effects on experimental SLE. Several strains of mice are used as models of autoimmune disease [22]. The disease in some murine models predominates in females of the species, examples are the *New Zealand white/black (NZW/NZB)Fl hybrid* and the *MRL lpr* strains in which females die before males [23, 24]. In one specific strain of mice the disease favors males, the *BXSB* (derived from the *C57BI/6* and satin beige mouse), and this strain is unusual in that the males die of early SLE-like disease [25] . Unlike the female predisposed strains of mice, disease manifestations in the *BXSB* mouse are not mediated hormonally because neither administration of sex steroids nor gonadal extirpation improves or changes the disease manifestations. One strain of mice bred because the mouse develops polyarteritis nodosa, called the *Palmerston North* (PN) mouse [26, 27], also develops an SLE-like illness that favors females. Gender is not solely responsible for the disease lupus in these inbred strains, as all mice succumb to lupus, male and female; however, sex hormones affect both the severity and the onset of the illness in both sexes of any species.

NZB/NZW Strains

The effects of gonadal hormones were first noted to play a regulatory role with regard to SLE in the New Zealand (NZB/NZW) mouse. The F1 descendants of the parental strains were important to lupus because inbreeding resulted in accelerated disease and development of disease manifestations such as autoimmune hemolytic anemia [23, 24] and diffuse proliferative glomerulonephritis. Circulating DNA-containing immune complexes and a variety of autoantibodies like those to native DNA and ssDNA [28–30] also accompanied the clinical disease as in the human. Before the discovery of the NZB/W strain, the manifestations of autoimmune disease were unknown in any animal species except humans. The gender difference is not amazingly large in the parent strains as a high percentage of the NZB females develop severe proliferative glomerulonephritis and die by 15 months of age, whereas the mortality for males is 17 months. When NZB mice are mated with NZW mice, the offspring (F1 hybrids) begin to develop kidney disease at 9 months, and again the most severe lesions are found in females [24]. To again emphasize gender, renal lesions are more common to the female (NZB x NZW)F1 in the cross of an NZB female and NZW male. In the F1 hybrids, 90% of the females develop dsDNA antibodies at age 8.5 months and more than 50% of the females die as a result of renal disease [31].

An explanation of various immune functions in these lupus mice has not revealed much. Thymic functions in these mice, particularly T-cell-mediated responses, have been studied and are blunted [32]. After age 22 weeks, the phytohemagglutinin responsiveness of circulating lymphocytes is lost, and this loss of function is more pronounced in females [33, 34]. This finding, one that is distinctly and skewed toward the female, might be the result of the development of anti-T-cell antibodies [35].

Cellular changes found in the NZB/NZW mouse are covered elsewhere in detail (Chapter 8). These observations are found more commonly in females of the F1 generation, which have an apparent B-cell abnormality

that is present from birth that results in the overproduction of IgM [36]. Athymic NZB mice are known to have hyperactive B cells (consistent with polyclonal B-cell activation) [37]. Aberrant B-cell dysfunction in NZB mice is reflected by their resistance to the induction and maintenance of tolerance [38]. This last characteristic is part of the "female character" of most of the murine SLE strains. Sex hormones also influence the response of the NZB strain to thymus-dependent antigens, as high-dose testosterone therapy decreases and orchidectomy increases plaque-forming colonies (PFC) in NZB males if the mice are irradiated sublethally after treatment with sex hormones [39].

Sex steroids also adversely affect the course of disease in F1 female hybrids of the NZB/NZW cross. As the mice age, the illness becomes more profound, but prepubertal castration of the NZB female mouse does not decrease the death rate. Early estrogen therapy accelerates the disease in females, whereas ovariectomy or testosterone therapy prolongs the life of the mouse. In males of this strain, castration causes early death and estrogen produces a mortality rate that is similar to that of females [40, 41]. Overall, testosterone therapy of female mice prolongs life and decreases overall morbidity [42–45].

It is possible that sex steroids modulate the generation of antigen-specific suppressor cells and that true sex differences in the responses to T- and B-cell mitogens exist in this strain. Androgens increase suppressor cell activity in NZB/NZW mice [46, 47] and it may do this by increasing levels of interleukin 2. Androgens also suppress the development of the thymocytotoxic antibody [48–50].

It is not rewarding to study sex hormone receptors on the lymphocytes of these mice because there are no differences. The thymus, a target of sex steroids, is of great physiologic significance, but estrogen and testosterone receptors are not located on mouse peripheral blood lymphocytes [51]. However, estrogen-induced receptor binding is observed in thymic tissues from male NZB/NZW mice, and estrogens may act entirely on the thymus gland [52]. The role of sex steroids in the pathogenesis of lupus-like disease in the inbred mouse is based on simple observations that alteration of sex steroid levels or gonadectomy affects the progression of disease. Importantly, the mouse will succumb to lupus whether male or female, but the sex steroids regulate disease activity.

MRL Strain

The MRL (Ipr/lpr or n/n) strain of mice has a lupus-like disease that is closer serologically to the human disease for various reasons. This mouse produces a greater variety of antibodies to antigens such as DNA, RNP, SM, and rheumatoid factor [53]. Because of the rheumatoid factor, this strain is a useful model for the study of rheumatoid arthritis [54]. These mice are also models of Sjogren's disease because of serologic characteristics and the development of signs such as lymphadenopathy. The mice are homozygous for the lymphoproliferative gene lpr and a large number have profound lymphadenopathy. The congenic n/n or +/+ strain lacks the lpr gene [55].

Sex differences in these MRL mice are less marked than in the NZB/NZW strain. No lymphadenopathy is found in +/+ mice, although necrotizing arteritis and glomerulonephritis are common and about 50% of the +/+ mice die with neoplasms. Again the female gender predominates, consequently, the mean ages for death are 17 months for females and 22 months for males [56].

Hormone therapy of these mice using high doses of the androgens testosterone and 5β-dihydrotestosterone delayed the occurrence of both lymphadenopathy and autoantibody production [57, 58]. Increased prolactin levels accelerate autoimmune diseases in NZB/W F1 mice.

BXSB Strain

BXSB mice are unique in that spontaneous autoimmune disease is more common in the males. This is distinctly different from the female predilection in most murine lupus strains. The males have a mean life span of 5 months, and the female life span averages 15 months. Examinations of cell populations of this strain have shown that both B- and T-cell functions are normal [25]. The accelerated autoimmune disease in these mice is linked to the Y chromosome, and prepubertal castration will not alter the course of the disease. The transfer of bone marrow cells from diseased males to nondiseased males leads to the development of the autoimmune disease [59, 60].

General Remarks about Murine Lupus and Sex Steroids

All of the evidence cited earlier indicates that sex hormones play a major role in the modulation of autoimmune diseases of some lupus mouse strains. There are certainly many other aspects to consider in the morbidity of murine SLE, and the major one is genetics and possibly the presence of a transmissible agent. It is also apparent that sex steroids have an adverse effect on the disease as in the human.

The disease manifestations wax and wane in a fashion commensurate with fluctuating steroid levels,

which vary with the estrus cycle in females. Fundamental questions, such as the susceptibility to SLE and the "window," if any, during which sex steroids exert their most profound influence, are the subjects of current investigation.

Sex Chromosomal Effects

Chromosomal effects are important because at least one murine strain, the BXSB, depends on the Y chromosome for the character of its immune responses. There are many other examples. Immune responses to DNA are linked to the presence of the X chromosome in the NZB/NZW mouse. NZB or NZW males mated to BXSB females result in female offspring that die of SLE nephritis at an early age. This suggests a role for the recessive X chromosome in the male parent [61].

Steroid Effects

Some research on the effects of androgens on immune function suggest that they act on immune tolerance [62]. Mice that develop autoimmune diseases have defective tolerance to a variety of antigens, and androgen levels could be at the heart of the problem. Graft-vs-host disease can induce a SLE-like syndrome in some mouse strains, and tolerance probably has a major position in the schema of autoimmunity [63]. Because androgens modulate the T-cell phenotype in transition from marrow to the thymus [46, 47], the target of androgen action is likely to be the thymocyte. Using 20α-hydroxysteroid dehydrogenase as a T-cell marker, investigators noted a decrease in thymocytes and a rise in T suppressor cells after androgen administration [64]. The hypothesis that androgens act in this manner is attractive because androgens afford a method of altering the absolute numbers of T cells.

Gonadotropin Effects

There is great interest in the effects of prolactin on immune function in mice and humans [65]. There are diverse effects of hyperprolactinemia in both male and female mice, and multiple studies show that prolactin stimulates the appearance of murine disease. Early studies found that the male NZW/NZB mouse autoimmune disease was accelerated and mortality was worsened by prolactin [66, 67]. Murine lupus, as noted earlier, is similar to that found in the human in that prolactin has a role to play in the exacerbation and possibly the initiation of lupus in the human (see later). Estrogens stimulate prolactin and may be the overriding mechanism. Bromocriptine inhibits the development of murine lupus [68]. Murine studies even demonstrate that estrogen itself might depend on the stimulation of prolactin for activity [69].

Sex Steroids and Human SLE

In human SLE there is little consistency of disease manifestation from patient to patient even within the same family. A patient or sibling from one family may present with autoimmune hemolytic anemia, whereas another will present with encephalopathy. Moreover, siblings from the same family who are of different gender might be equally affected.

Therefore, while gender plays a major role in the expression of SLE, it is not likely to be part of the etiology but rather an expression modifier. The vast majority of patients who present with SLE after puberty are females. Present evidence suggests that estrogens or those steroids that are exceptionally feminizing exacerbate SLE [70]. Among such compounds are synthetic estrogens, such as birth control agents [71, 72]. There are data to suggest that previous estrogen use is a predisposing factor for lupus and data to show that it is not.

The times in a woman's life that are important to SLE activity are the menstrual period [73, 74] and pregnancy, both before and after parturition. These periods may worsen the activity of SLE and depend on the levels of gonadal steroids. Because of the large numbers of women with lupus, the X chromosome could play a role [61]. A major concern regarding women with SLE is the fact that they are 50 times more likely to have a myocardial infarction than men (35 to 44 age groups). In those who had a cardiovascular event, an older age at diagnosis, longer disease duration, more steroid use, postmenopausal status, and high cholesterol levels were all associated with this increase in SLE cardiac disease [75].

Patients with Klinefelter's syndrome (XXY) are described who have clinical SLE and disordered estrogen metabolism [76]. Study of such males provoked the initial series of experiments designed to look at the patterns of estrogen and androgen metabolism in SLE. These studies revealed that patients with SLE have altered sex steroid metabolism. Because sex steroids regulate immune function, there can only be differences between the way the immune systems of males and females respond to sex steroids and gonadotropins. The only gonadotropin shown to have a major effect on the SLE of mice and humans at this time is prolactin.

Estrogens

Females are likely to have more aberrations of the immune system than males. Male and female patients with SLE have normal levels of estrogen; however, the

overall metabolism of such compounds favors more feminizing compounds such as estriol and 16β-OH estrone [70]. Specifically, the pattern of hydroxylation of estrone (Fig. 1) favors the 16-hydroxylated compounds over the cathechol estrogens [77]. Patients of both sexes have increased levels of 16α-hydroxyestrone (Table 2), whereas only SLE females and Klinefelter (XXY) males had elevated levels of estriol [78]. The compound 16α-hydroxyestrone and its elevation in SLE patients could have some special significance, as later studies demonstrated that this hormone had unique qualities [79].

One estrogen, 16α-hydroxyestrone, is feminizing, highly uterotropic, and modestly bound to cytosol receptors and testosterone–estradiol-binding globulin in contradistinction to compounds such as 17β-estradiol [80]. A radioimmunoassay, however, failed to show

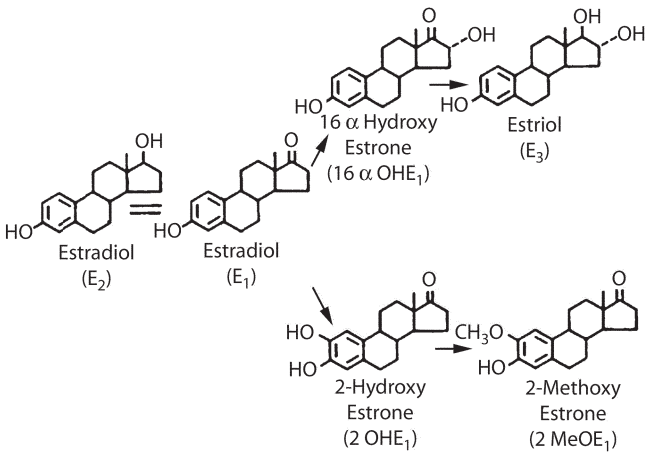


FIGURE 1 The metabolism of estradiol. Estradiol is converted to estrone that can go in one of two directions: toward the 16-feminizing metabolites or the catechol estrogens that are the 2-hydroxylated metabolites. In patients with lupus the preferential direction of estrone metabolism is in the 16-hydroxylated direction.

uniformly elevated levels of 16α-hydroxyestrone in active SLE patients [81]. This suggested either that a conjugated form of this steroid was active or that there were many metabolites of importance from this proposed scheme. Enzymatic systems in certain animals might favor the formation of such compounds, although nothing is known about lupus mice with regard to this metabolism. Clinical studies on the steroid 16α-hydroxyestrone showed that it had interesting properties *in vivo* that might explain its possible role in disease, including covalent binding of this steroid to erythrocytes and lymphocytes via a Heyn's rearrangement *in vivo* and the possibility that such covalent binding might occur at the level of the estrogen receptor or the T-cell receptor and result in an alteration of immune function [82, 83]. Studies of family members of SLE patients indicated that elevated hydroxylation of estradiol was commonly observed in nonaffected first-degree relatives as well as patients [84].

Enhanced binding of l6α-hydroxyestrone to various cell proteins was found in normal women ingesting oral contraceptives. Of particular interest, specific antiestrogen–protein adduct immunoglobulins were isolated from normal and SLE patients ingesting oral contraceptives, which means that these adducts are common. This finding suggested a common pathway to adduct formation in all women who ingest large amounts of estradiol or for one reason or another have an endogenous source of high estrogen level [79]. Males with SLE were also reported who had hormone protein adduct-specific IgG in their sera.

Androgens

There have been several studies of androgen levels in plasma of men and women with SLE and of androgen metabolism in men and women with SLE [70]. Some investigators have said that the reasons for SLE in the male are the result of too little androgen and too

TABLE 2 Rate of Hydroxylation in SLE

		Rate	
	Number studied	2-Hydroxylation[a]	16-Hydroxylation[a]
Males			
Normal	13	21.3 (±8.7) (7.4–34.2)	7.5 (±2.1) (3.2–11.1)
SLE	6	21.9 (±13.4) (8.6–35.0)	12.3 (±2.7) (8.8–16.8)[b]
Females			
Normal	14	40.4 (±5.9) (17.7–54.7)	9.5 (±2.2) (5.9–14.1)[b]
	15	25.6 (±17.4) (6.0–67.1)	15.7 (±5.0) (9.0–30.0)

[a] Mean, standard deviation, and range.
[b] $P < 0.01$.

much estrogen [85, 86]. These studies are exceptions, however, as most studies indicate that young men with SLE are hormonally normal and that estrogen:androgen ratios might be minimally elevated (if at all). Furthermore, data from studies of males with SLE do not help explain the large numbers of females who predominate with the disease. Studies of androgen metabolism in SLE females indicate that a difference in the overall metabolism of androgens can be found (Fig. 2). The oxidation of testosterone at C17 in SLE females is increased in comparison with males, who have both normal oxidation of testosterone and normal plasma levels of androgenic steroids [87]. Several studies of females with active SLE who never took corticosteroids had decreased plasma levels of androgen [88–90]. This observation was also found in Klinefelter patients, as well as in women with lupus. Low plasma androgens in women with SLE form the basis for androgen replacement therapy in this disease. Clinical studies involving the use of DHEA as a therapy for lupus result from this observation.

Gonadotropins

Prolactin is an immunomodulatory pituitary hormone and could be considered a cytokine itself. In the human, prolactin elevations have been observed in SLE juveniles and correlated with both disease activity and central nervous system (CNS) manifestations [91]. This finding is supported by *in vitro* work showing IgG and IgM induced anti-DNA antibodies by both normal and SLE lymphocytes in the presence of high levels of prolactin. Lectins did not produce this effect [92]. One study correlates elevated prolactin levels with elevated cortisol levels [93, 94].

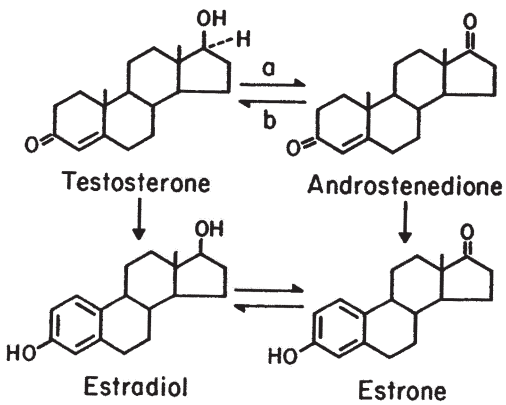


FIGURE 2 The oxidation of testosterone. Testosterone has several possible fates in the human. Testosterone can be converted to the weak androgen androstenedione that can then become estrone or testosterone can be aromatized to estradiol directly. In lupus patients the oxidation of testosterone at C17 (reaction a) is increased in women with lupus and not in men.

Women who are pregnant have higher serum prolactin levels if they have SLE [95]. Moreover, some investigators have associated the decline in serum testosterone during pregnancy in SLE patients to hyperprolactinemia. Perhaps, however, the most significant descriptions of hyperprolactinemia have been in men with SLE [96]. These descriptions of hyperprolactinemia are of particular interest as hyperprolactinemia is treated readily with bromocriptine. Studies using bromocriptine to treat SLE are routine in some clinics around the world.

Many studies refute the significance of prolactin in human SLE [97–99]. In a detailed study of a Chinese cohort of patients with SLE, Mok *et al*. [100] found no correlation of clinical activity with levels of prolactin in 72 lupus patients. A look at autoantibodies in patients with lupus found no association of prolactin levels with specific lupus autoantibodies [101]. One particular study suggests that prolactin is complexed with IgG but remains biologically active in the lupus patient [102, 103]. This binding of prolactin (a specific 23-kD a nonglycosylated form) to IgG is not covalent. Delayed clearance of this complex due to its high molecular weight is the proposed reason for the activity of the prolactin in most patients with lupus.

Many investigators associate prolactin with disease activity [104]. In one study, 61.9% of patients with hyperprolactinemia had active disease. The SLEDAI correlated with the levels of prolactin in these patients as well when prolactin levels were measured by both tests of immunoradioactivity and biological activity. Another series noted an association of hyperprolactinemia in the serum and urine of patients with severe renal disease [105]. The statistical power of most of these prolactin studies could explain the lack of consistency among research laboratories. One study suggests that the published results are contradictory due to the statistical power of the studies. In fact, the authors reviewed five studies and found that two of the studies did not have the statistical power to conclude an association with lupus activity and prolactin levels, whereas the other three studies did suggest an association [106]. High prolactin levels are found in 20–30% of patients with SLE in some clinics [107]. In addition to the suggestion that prolactin is itself a cytokine that provokes the synthesis of immunoglobulins by lymphocytes [108], German investigators found that using lymphocytes from SLE patients and measuring clinical activity with the European activity measure (ECLAM) that SLE patients have lymphocytes that are sensitive to levels of prolactin and are likely to be activated by physiological concentrations [109]. These data are supported by the

findings that T lymphocytes from SLE patients secrete more prolactin than controls, suggesting a difference in the regulation of genes responsible for control of this cytokine. In fact, single nucleotide polymorphisms in the upstream promoter regions of both pituitary and non-pituitary prolactin secretion exist. Such polymorphisms that are specific can affect prolactin transcription and possibly disease association in a cohort of lupus patients. This was the case in a study where patients had an increased frequency of the prolactin-1149G allele when compared to control subjects [110]. Whether prolactin is a cytokine or is itself increased by cytokines common in certain active lupus patients remains the subject of major investigation.

Finally, prolactin levels of mothers who were pregnant or breast-feeding and had lupus were studied as reproductive risk factors for the development of disease [111]. Surprisingly, breast-feeding was associated with a decreasing risk of developing SLE. In addition, the numbers of pregnancies or live births with lupus activity showed no relationship to levels of prolactin. These authors found no association of elevated prolactin levels with an increased risk of lupus.

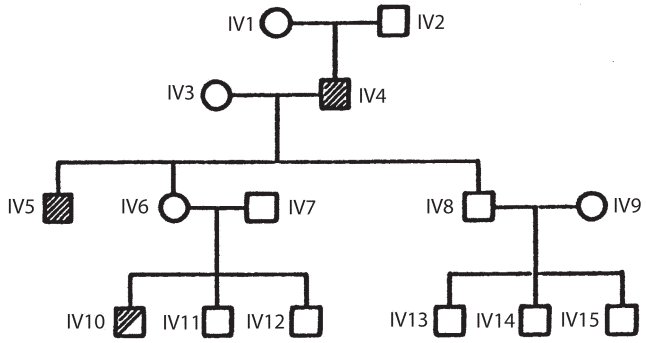


FIGURE 3 This pedigree shows one of three families where lupus occurs in males of each family. No females have acquired lupus in any of these families, whereas three generations of males are affected.

Males with SLE

The age of onset of the disease is distributed more evenly in males, as one-fourth are diagnosed after age 50 years [112]. Hormonal metabolic study data suggest that an increase in feminizing 16-hydroxylated estrogenic metabolites is found in SLE males, although no phenotypic evidence of hyperestrogenism is found [77, 84, 113–115]. The BXSB mouse develops SLE-like disease in a nonhormone-dependent fashion. The presence of the Y chromosome is most important in this strain. A group of human male relatives has been described who resemble the mouse strain BXSB in that "male-predominant" families exist in which SLE occurs in men in preference to females (Fig. 3) [116]. In one interesting study of males with lupus, females that have a male relative with lupus were more likely to have renal disease [117].

Males with SLE are in some series reported to be clinically different [118–120]. While several male studies show no clinical difference in severity of disease between women and men, others have suggested that males have a more severe form of the disease [121]. For example, increased pleuropericardial disease and peripheral neuropathy are said to be more common in males. Men were found to have more discoid lupus erythematosus and papular nodular mucinosis [122]. In a Spanish series of 261 SLE patients, 11.5% were males and they had less arthritis, more serositis, and a greater propensity for discoid rashes. A database on males from Malta also found more cardiorespiratory problems in males [123], and a Taiwan study suggested that males have a significantly lower FcγR distribution on monocytes and neutrophils and high prolactin levels that might have a role in the pathogenesis of lupus in this sex [124]. All of these data are collected from small numbers of men and such things as statistical bias might be significant.

Sex hormone profiles indicate that men with lupus have significantly higher levels of gonadotrophins like follicle-stimulating hormone (FSH) or leutinizing hormone (LH) than controls. A small percentage of patients (14%) in one study had low testosterone and elevated LH levels [125]. Patients with low androgens had more CNS disease and serositis when compared to controls. Finally, the prolactin to testosterone levels correlated with the SLEDAI scores in these men.

Studies of Russian male lupus patients are perhaps the most insightful [126, 127]. Investigators described elevated LH and FSH in SLE males; a lower trochanteric index (1.89 vs 2.00 for normal men), which is indicative of a lack of androgen effect on bone growth; severe aortic insufficiency and sacroileitis (12% of all men); and overall a greater incidence of severe vascular diseases, such as Raynaud's phenomenon and digital vasculitis [128].

Russian investigators found more severe disease in men, with 63% dying from end stage renal disease. Table 3 illustrates the vascular manifestations of male patients with SLE. Table 4 shows the frequencies of various clinical manifestations in both male and female patients. Note that the only significant increases in Russian males with SLE are the incidences of nephritis, Raynaud's phenomenon, and malar rash.

Finally, the Russian study also included male SLE patients with profound impotence. The causes of such impotence in young SLE males remain unknown

TABEL 3 Vascular Manifestations in Male Patients with SLE[a]

Feature	%
Digital vasculitis	33
Livedo vasculitis	41
Ulcers	10
Raynaud's phenomenon	29
Vascular involvement of	
Lungs	19
CNS	12
Heart	15
Avascular necrosis of bones	15

[a] Adapted from Folomeev *et al*. [127], with permission.

TABLE 4 Frequency (%) of SLE Manifestations in Male and Female Patients[a]

Symptom	Females ($n = 200$)	Males ($n = 170$)	$P <$
Skin involvement	100	94	—
Malar rash	42.5	65	0.02
Discoid lupus erythematosus	20	25	—
Digital vasculitis	18.2	33.3	0.02
Articular involvement	99.7	92.5	—
Cardiac involvement	87	79.9	0.05
Pneumonitis	22.3	17	0.05
Pleuritis	72	65.2	—
Pericarditis	51	41	—
Nephritis	58.3	75	0.02
CNS involvement	47.3	34.8	0.02
Peripheral nerve system involvement	36.1	8.7	0.01
Raynaud's phenomenon	16.6	29	0.02
Sacroiliitis	n.d.	49	—

[a] Adapted from Folomeev *et al*. [127], with permission.

(Table 5). Elderly males who present with SLE are also found to have low androgen levels and are hypogonadal [12]. Such males might respond to androgen therapy.

Males with Klinefelter's Syndrome

Patients with Klinefelter's syndrome can also have a variety of rheumatic diseases such as SLE and scleroderma [129–134]. Such males commonly have gynecomastia, infertility, a female fat phenotype, and the usual sequelae of hypogonadism. These Klinefelter males have met ARA criteria for SLE both serologically and clinically (Fig. 4). The incidence of SLE or any other autoimmune disease is not increased in patients with

TABLE 5 Results of Studies of Impotence in Systemic Lupus Erythematosus[a]

1. Thirty-six percent of males with SLE have sexual disturbances
2. Impotence develops before SLE onset
3. Decreased libido and satisfactory effect of androgen therapy suggest an imbalance of sex hormone levels
4. Impotent patients with SLE have a twofold decrease in testosterone levels

[a] These are male SLE patients with mild and moderate disease activity who did not receive cytotoxic or corticosteroid drugs.

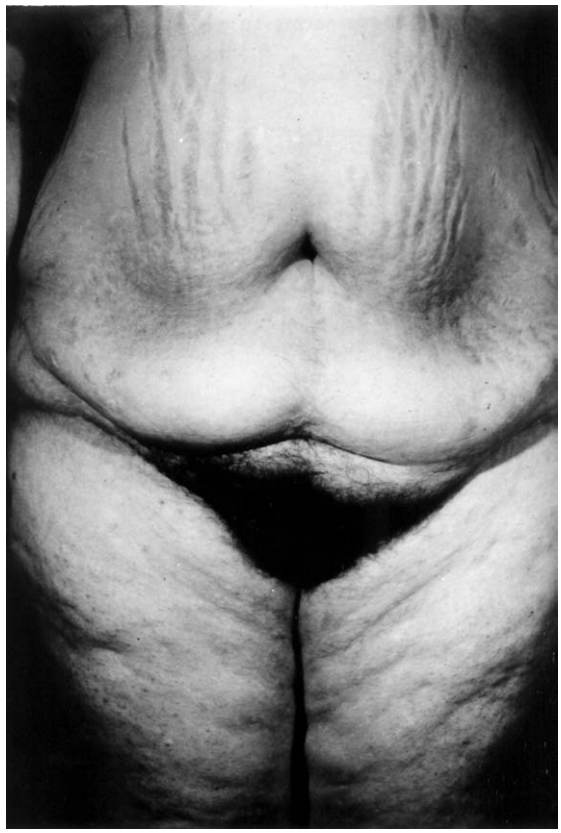


FIGURE 4 This is the midsection of a Klinefelter male with SLE. Note the ambiguous genitalia and the female distribution of fat.

Klinefelter's syndrome, even though this is frequently stated. Patients with SLE and Klinefelter syndrome together have the estrogen and androgen metabolism of females with SLE [76], but low levels of both sex steroid classes. This is the reason that the Klinefelter syndrome is a hypergonadotrophin state. When SLE does occur in young Klinefelter males, it can be treated with the synthetic androgens usually given to such males. Androgens such as methyltestosterone as a tablet, androgen patch,

or gel can alleviate the symptoms of the disease in Klinefelter males but do not alter the amount or type of autoantibody. The male with Klinefelter's syndrome and SLE oxidizes testosterone in an exaggerated fashion like females with SLE. This increased oxidation is not found in the SLE male with a normal XY karyotype. The hypergonadotropic state of men with Klinefelter's syndrome and SLE has not been investigated adequately at this time and may have some role in the etiopathogenesis of the disease.

Hyperestrogenism

Hyperestrogenic conditions are associated with antoantibodies in both normal males and females. In the human female, periods of hormonal change such as pregnancy and normal menses are associated with changes in immune function. These changes are also observed in mice. Clinical syndromes such as insulin-resistant diabetes mellitus, hirsutism, and cystic ovaries are found in patients with autoantibodies [52, 135]. Polycystic ovary disease may be considered autoimmune, and autoantibodies could result from the unopposed actions of plasma estrogen [136]. More recently, autoantibodies have been described in patients with endometriosis, and patients have been referred as those with a lupus-like illness [137, 138]. This may be of particular interest, as the uterus and the ovaries of humans and animals are a source of cytokines [139].

Estrogens and Lupus

Data from mice and humans have shown that unopposed estrogens exacerbate SLE. Data from human studies, however, are largely *in vitro* or are derived from anecdotal studies of patients on oral contraceptives. Most studies involving oral contraceptives and SLE or hormone replacement therapy in postmenopausal women with SLE suggest that the disease worsens with these agents. However, these studies are inconclusive and difficult because of the variability of the human illness, the designate end points of therapy, the steroids chosen, and the lack of a double-blinded study. Data strongly suggest that the use of hormone replacement therapy (HRT) predisposes women to the development of SLE [140]. Results of the contraceptive/hormone replacement trial, a double-blinded control study to look at oral contraceptive use and HRT in lupus women, are in progress and data should be available shortly. Current epidemiological studies do not support the idea that female hormones increase the risk of developing SLE [111, 141]. Sex steroids have been considered for use in inflammatory diseases such as gout and osteoarthritis because estrogens suppress neutrophil function [142], which is why [143] gout is uncommon in the premenopausal female [144]. The overall gender-related mechanisms for the selectivity of one sex for a disease over another continues to evade explanation and there is debate about this from some quarters [145]. Estrogens may be effective in the treatment of rheumatoid arthritis, and several clinical studies have argued both for [146, 147] and against their use [148]. The mechanism of suppression of inflammation in cases of rheumatoid arthritis may give some significant insight into the pathogenesis of this common disease and is based on two observations: (1) patients improve when pregnant and (2) oral contraceptives may have a protective effect. Menopause is an interesting time for most lupus patients. For many years, patients who reached menopause were known by most clinicians to improve. These conclusions were without data until recently when menopause has been conclusively associated with a remission of illness [149]. Most authors conclude that a "modest" decrease of activity occurs in those women reaching menopause [150].

Androgens and Lupus

Therapy of SLE with anabolic steroids such as Danazol or cyproterone acetate is not effective therapy for either murine or human SLE [151–154]. However, the use of hormones such as Danazol in idiopathic thrombocytopenic purpura increases platelet numbers and coagulation factors such as factor VIII. This suggests a role for certain patients with SLE [155, 156]. Other androgens under study, such as 19-nortestosterone, have also had limited use in the treatment of SLE in women. Doses used were 100 mg/ml per week intramuscularly. While the overall condition of female patients improved and patients admitted increased energy, loss of joint pains, and resolution of svstemic abnormalities such as skin rashes or anemia (Fig. 5), no significant change of serologies was noted. Anti-DNA and ANA titers remained elevated. Most curious in this treatment group was an overall worsening of lupus symptoms in male patients [157]. These findings in males on 19-nortestosterone correlated with lowered endogenous testosterone levels and elevated estradiol levels.

Another steroid, cyproterone acetate, a potent antigonadotrophic agent [158], resulted in resolution of some systemic abnormalities such as oral ulcers after 50 mg daily for a mean of 63 months. As with other androgens, no improvement in serologic features was observed.

Another approach to the treatment of lupus involves the use of gonadotropic hormone-releasing hormone agonists. These agents are used effectively to treat endometriosis and prostate carcinoma. Data about their

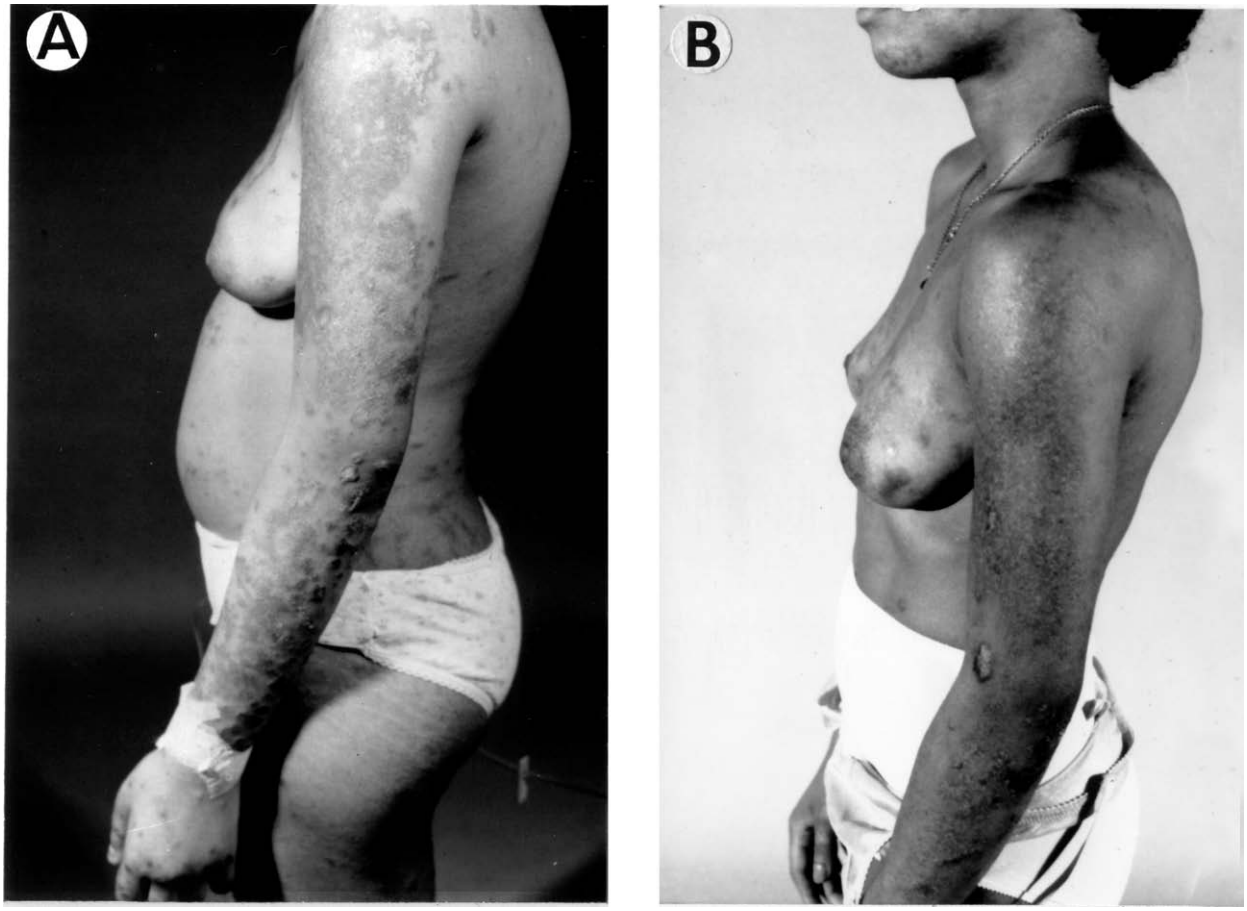


FIGURE 5 (**A**) A young woman prior to the institution of 19-nortestosterone therapy. (**B**) The same woman 2 years later after intramuscular injection of 100 mg/week of 19-nortestosterone.

use in SLE are inconclusive because of the small numbers studied [159, 160].

One androgen, studied extensively in women, is dehyroepiandrosterone (DHEA) [161, 162]. This drug at doses of 200 mg per day in tablet form results in clinical improvement as measured by standard indices and is steroid sparing, i.e., the total dose of prednisone might be lowered with its use. This drug suppresses cytokine levels such as IL-4, -5, and -6 and increases IL-2 during treatment. Natural killer cell activity also increases with this agent [163–165]. This drug is discussed in more detail in Chapter 48.

Sex Hormones, Behavior, and Autoimmune Diseases

Sex steroids are important to the development of various organs, and a major one is the brain. Attention has been directed to cerebral development, cerebral dominance, and the incidence of autoimmune disease [166, 167]. Some studies say that patients with antoimmune disease are predominantly left handed, indicating dominance of the right cerebral hemisphere. This variation is reported in patients with SLE [168–170] and other diseases of the immune system. Neuronal migration is under the influence of steroids such as testosterone, and new data suggest that SLE mice have aberrant neuronal migration patterns consistent with those observed in humans with learning disabilities such as dyslexia. Handedness in patients with SLE is more directed to the left than in the normal population. The finding of increased learning disabilities such as developmental dyslexia in patients with SLE, their unaffected male offspring, and unaffected male siblings has been confirmed by several groups and is a subject of considerable interest.

Sex Hormones and Immune Function

Androgens

Androgens have many effects on immune function. They are immunosuppressive because of *in vitro* observations in normal lymphocytes and because of their

effects on the disease manifestations of inbred autoimmune mice [171]. Testosterone suppresses anti-DNA antibody production in peripheral blood mononuclear cells from patients with SLE [122]. This occurs through the downregulation of IL-6 and the inhibition of B-cell activity. Graft rejection in rodents is delayed by the injection of testosterone. Resistance to certain viral infections is reduced, whereas in some cases resistance to certain infections is enhanced when androgens are given at certain doses [172, 173]. A consistent effect of androgens in animals is the immunosuppression of chickens through the retardation of the function and development of the *bursa of fabricius* [174–176]. Androgens also have an effect on the pluripotential stem cells of the bone marrow: namely the accelerated proliferation and differentiation of such stem cells into compartments of cells that number among them lymphoid elements. The important effects of androgens on immune maturation are reflected by the discovery of receptors for estrogen and dihydrotestosterone, a 5α reduced metabolite of testosterone in the thymus [177–179]. Androgen receptors are found on lymphocytes, but data are inconsistent. Androgens inhibit B- and T-cell maturation, reduce B-cell synthesis of immunoglobulins, and suppress the phytohemagglutinin-induced blast transformation of lymphocytes. Androgens are implicated as modifiers of regulatory genes that influence the function of structural genes [180]. For example, guinea pig mammary epithelial Ia antigens are increased in number through the effects of estrogens and prolactin and are decreased by testosterone [181]. The wide range of effects that this steroid displays might be due to variable androgen sensitivity of certain cell groups or the *in vivo* conversion of androgens to estrogens through well-recognized pathways.

Estrogens

Female mice make more antibody to foreign antigens than males, and this difference is reported for a variety of antigens [182]. Depending on the dose, estrogens have been both immunosuppressors and immunostimulants. The steroid 17β-estradiol prolongs first and second set skin grafts in mice after X irradiation and inhibits corneal graft acceptance in preimmunized rabbits [183]. In general, however, skin allograft rejection is naturally better in females than in males [184]. Estrogens regulate immunity by way of the thymus in rodents [185] and decrease the overall thymic population of small lymphocytes. Estrogen given prior to bone marrow transplantation results in increased graft failure. Estradiol and diethylstilbestrol (in concentrations of 10–50 mgm/ml) are known to reduce the phytohemagglutinin and concanavalin A response of lymphocytes *in vitro* [186]. The mixed lymphocyte reaction is enhanced by estradiol. Enhanced lymphocyte activity is observed at 200 ng/ml, whereas the effects are inhibited at 2000 ng/ml (thymidine incorporation). Fluctuant lymphocyte responses are observed during normal menses [74], during pregnancy, and during the use of oral contraceptives [187]. Castrated male and certain female mice display hyperplastic spleens and thymuses after challenge with thymic-dependent antigens, indicating an effect on thymic cell activity [188, 189]. Castration of males leads to accelerated allograft rejection. Syngeneic grafts of ovaries in males and grafts of testes in females have no significant effect on allograft rejection [190]. Sustained levels of estrogens in mice led to a marked reduction in natural killer cell activity. In other studies, estrogens have been found to depress cell-mediated immunity, natural killer cell function, and cancer cell immune surveillance. Estrogens also deplete thymic hormones and are known to produce a relative lymphopenia.

Estrogens are detrimental to animals with SLE and this refers not only to mice with SLE, but also other animals, such as dogs. In fact, tamoxifen and the antiestradiol antibody have beneficial effects on experimental SLE. This benefit occurs via cytokine regulation [191]. Men with prostatic cancer who are given diethylstilbestrol have markedly depressed cell-mediated immunity. Using normal lymphocytes, estradiol treatment of pokeweed mitogen-treated B cells shows an increase in plaque-forming cells (*in vitro*) [192, 193]. Estrogen receptors are found on $CD8^+$ T lymphocytes in some studies [194, 195], although data derived from more sensitive estrogen receptor CDNA probes have not confirmed these findings. Studies have shown that $CD4^+$ T helper cells increase after estrogen therapy [192]. By some studies, estrogens are thought to act by inhibiting $CD8^+$ suppressor T cells. Consequently, helper T cells would be enhanced with the resulting polyclonal B-cell immunoglobulin production. An exciting new direction is the inhibition of apoptosis *in vitro* using peripheral blood mononuclear cells from women with normal menses. The estradiol decreases tumor necrosis factor-α in SLE cells but not those from normal people.

Progestogens

Other sex steroids such as progesterone have been considered as effective therapeutic agents in diseases such as SLE. Progesterone is an immunosuppressive agent [41, 196], and levels of this substance rise during pregnancy when the placenta assumes an active role in its synthesis and secretion. At concentrations of 10–15 mgm/ml, this steroid reduces lymphocyte responses to phytohemagglutinin and to concanavalin A *in vitro*.

Other analogs (20α-hydroxyprogesterone) have similar effects [197].

Progesterone has been known to increase the relative amounts of $CD8^+$ suppressor cells and to decrease them in mice. In addition, progesterone has been invoked to explain many of the suppressive effects found in the sera of pregnant females.

SUMMARY

Sex steroids are potent modulators of immunity in all animal systems. They are very important in patients with SLE and may help explain the fluctuant activity in the disease lupus. Their role in the maturation of the immune system and their effect on the development of organs such as the brain could explain some of the abnormalities found in these systems.

References

1. Maddison, P. J., Isenberg, D. A., Goulding, N. J., Leddy, J., and Skinner, R. P. (1988). Anti La(SSB) identifies a distinctive subgroup of systemic lupus erythematosus. *Br. J. Rheumatol.* **27**, 27–31.
2. Ballou, S. P., Khan, M. A., and Kushner, I. (1982). Clinical features of SLE: Differences related to race and age of onset. *Arthritis Rheum.* **25**, 55.
3. Hess, E. V. (1986). Drug related lupus: The same or different? "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), pp. 869–880, Wiley, New York.
4. Urowitz, M. B., Stevens, M. B., and Shulman, L. E. (1967). The influence of age on the clinical pattern of SLE. *Arthritis Rheum.* **10**, 319.
5. Maragou, M., Siotsiou, F., Sfondouris, H., Nicolia, Z., Vayopoulos, G., and Dantis, P. (1989). Late-onset systemic lupus erythematosus presenting as polymyalgia rheumatica. *Clin. Rheumatol.* **8**, 91–97.
6. Kellum, R. E., and Haserick, J. R. (1964). Systemic lupus erythematosus, a statistical evaluation of mortality based on a conservative series of 299 patients. *Arch. Int. Med.* **113**, 200.
7. Mok, C. C., Lau, C. S., Lee, K. W., and Wong, R. W. (1998). Hyperprolactinemia in males with systemic lupus erythematosus. *J. Rheumatol.* **25**(12), 2357–2363.
8. Baker, S. B., Rovira, J. R., Campion, E. W., and Mills, J. A. (1979). Late onset SLE. *Am. J. Med.* **66**, 737.
9. Wilson, H. A., Hamilton, M. E., and Spyker, D. A. (1981). Age influences the clinical and serologic expression of SLE. *Arthritis Rheum.* **24**, 1230.
10. Foadd, B. S. I., Sheon, R. P., and Kirsner, A. B. (1972). Systemic lupus erythematosus in the elderly. *Arch. Int. Med.* **130**, 743.
11. Adu, D., Williams, D. G., and Taube, D. (1983). Late onset lupus-like disease in patients with apparent idiopathic glomerulonephritis. *Q. J. Med.* **52**, 471.
12. Baer, A., and Pincus, T. (1983). Occult systemic lupus erythematosus in elderly men. *JAMA* **249**, 3350.
13. McMurray, R. W. (1996). Prolactin and systemic lupus erythematosus. *Ann. Med. Interne* (*Paris*) **147**(4), 253–258.
14. Liu, H. W., Lin, S. F., Chen, J. R., Chuang, W. L., Yen, J. H., and Tsai, J. H. (1988). Subset of systemic lupus erythematosus with late onset. *Kao Hsiung I Hsueh Ko Hsueh Tsa Chih* **4**, 547–552.
15. Studenski, S., Allen, N. B., Caldwell, D. S., Rice, J. R., and Polisson, R. P. (1987). Survival in systemic lupus erythematosus: A multivariate analysis of demographic factors. *Arthritis Rheum.* **30**, 1326–1332.
16. Ward, M. M., and Studenski, S. (1990). Age associated clinical manifestations of systemic lupus erythematosus: A multivariate regression analysis. *J. Rheum.* **17**, 476–481.
17. Rashkov, R., and Kunev, K. (1990). The evolution of systemic lupus erythematosus. *Vutr. Boles* **29**, 91–96.
18. Hashimoto, H., Tsuda, H., Hirano, T., Takasaki, Y., Matsumoto, T., and Hirose, S. (1987). Differences in clinical and immunological findings of systemic lupus erythematosus related to age. *J. Rheumatol.* **14**, 497–501.
19. Hochberg, M. C. (1989). Mortality from systemic lupus erythematosus in England and Wales, 1974–1983. *Br. J. Rheumatol.* **26**, 437–441.
20. Harisdangkul, V., Nilganuwonge, S., and Rockhold, L. (1987). Cause of death in systemic lupus erythematosus: A pattern based on age at onset. *South Med. J.* **80**, 1249–1253.
21. Austin, H. A., Muenz, L. R., Joyce, K. M., Antonovych, T. A., Kullick, M. E., Klippel, J. H., *et al*. (1983). Prognostic factors in lupus nephritis: Contribution of renal histologic data. *Am. J. Med.* **75**, 382–391.
22. Bielschowski, M., and Goodall, C. M. (1970). Origin of the inbred New Zealand mouse strains. *Cancer Res.* **30**, 834.
23. Howie, J. B., and Helyer, B. J. (1965). Autoimmune diseases in mice. *Ann. N.Y. Acad. Sci.* **124**, 167–177.
24. Howie, J. B., and Helyer, B. J. (1968). The immunology and pathology of NZB mice. *Adv. Immunol.* **9**, 215–266.
25. Murphy, E. D., and Roths, J. B. (1979). A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. *Arthritis Rheum.* **22**, 1188–1193.
26. Walker, S. E., Gray, R. H., and Fulton, M. (1978). Palmerston north mice, a new animal model of systemic lupus erythematosus. *J. Lab. Clin. Med.* **92**, 932.
27. Walker, S. E., Kier, A. B., Siegfied, E. C., Harris, B. G., and Schultz, J. S. (1986). Accelerated autoimmune disease and lymphoreticular neoplasms in F1 hybrid PN/NZB and NZB/PN mice. *Clin. Immunol. Immunopathol.* **39**, 81–92.
28. Lambert, P. H., and Dixon, F. J. (1968). Pathogenesis of the glomerulonephritis of NZB/W mice. *J. Exp. Med.* **127**, 507.
29. Teague, P. O., Irion, G. J., and Myers, J. J. (1968). Antinuclear antibodies in mice. I. Influence of age and possible genetic factors on spontaneous and induced responses. *J. Immunol.* **101**, 791.

30. Tonietti, G., Oldstone, M. B. A., and Dixon, F. J. (1970). The effect of induced chronic viraql infections on the immunological lesions of New Zealand white mice. *J. Exp. Med.* **132**, 89.
31. Walker, S. E., Besch-Williford, C. L., and Keisler, D. H. (1994). Accelerated deaths from systemic lupus erythematosus in NZB × NZW F1 mice treated with the testosterone-blocking drug flutamide. *J. Lab. Clin. Med.* **124**, 401–407.
32. Fernandez, G., Yunis, E. J., and Good, R. A. (1976). Age and genetic influences on immunity in NZB and autoimmune resistant mice. *Clin. Immunol. Immunopathol.* **6**, 318.
33. Gottlieb, A., Lahita, R. G., Chiorazzi, N., and Kunkel, H. G. (1979). Immune function in systemic lupus erythematosus. *J. Clin. Invest.* **63**, 885.
34. Gelfand, M. C., and Steinberg, A. D. (1973). Basis of impaired graft rejection of ageing NZB/W mice. *Trans. Proc.* **5**, 145.
35. Shirai, T., Yashiki, T., and Mellors, R. C. (1972). Age decrease of cells sensitive to an autoantibody-specific for thymocytes and thymus dependent lymphocytes in NZB mice. *Clin. Exp. Immunol.* **12**, 455.
36. Motsopoulos, H. M., Boehm-Truitt, M., and Kassan, S. S. (1977). Demonstration of activation of B lymphocytes in New Zealand black mice at birth by an immunoradioactive assay for murine IgM. *J. Immunol.* **119**, 1639.
37. Ohsugi, Y., and Gershwin, M. E. (1979). Studies of congenitally immunologic mutant New Zealand mice. III. Growth of B lymphocyte clones in congenitally athymic (nude) and hereditary asplenic (Dh/+)NZB mice: A primary B cell defect. *J. Immunol.* **123**, 1260.
38. Staples, P. J., Steinberg, A. D., and Talal, N. (1980). Induction of immunological tolerance in older New Zealnd mice repopoulated with young spleen, bone marrow, or thymus. *J. Exp. Med.* **131**, 1223.
39. Morton, J. I., Weyant, D. A., Seigel, B. V., and Golding, B. (1981). Androgen sensitivity and autoimmune disease. I. Influence of sex and testosterone on the humoral immune responses of autoimmune and nonautoimmune mouse strains to SRBC. *Immunology* **44**, 661.
40. Siiteri, P. K., Jones, L. A., Roubinian, J., and Talal, N. (1980). Sex steroids and the immune system. I. Sex difference in autoimmune disease in NZB/NZW hybrid mice. *J. Steroid. Biochem.* **12**, 425–432.
41. Stites, D. P., and Siiteri, P. (1983). Steroids as immunosuppressants in pregnancy. *Immunol. Rev.* **75**, 117–138.
42. Carlsten, H., Holmdahl, R., Tarkowski, A., and Nilsson, L. A. (1989). Oestradiol- and testosterone-mediated effects on the immune system in normal and autoimmune mice are genetically linked and inherited as dominant traits. *Immunology* **68**, 209–214.
43. Roubinian, J. R., Talal, N., and Greenspan, J. S. (1979). Delayed androgen treatment survival in murine lupus. *J. Clin. Invest.* **63**, 902–911.
44. Roubinian, J. R., Talal, N., and Greenspan, J. S. (1979). Sex hormone modulation of autoimmunity in NZB/NZW mice. *Arthritis Rheum.* **22**, 1162–1169.
45. Roubinian, J. R., Talal, N., Greenspan, J. S., Goodman, J. R., and Siiteri, P. K. (1978). Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F1 mice. *J. Exp. Med.* **147**, 1568.
46. Weinstein, Y., and Berkovich, Z. (1981). Testosterone effect on bone marrow, thymus and suppressor T cells in NZB x NZW F1 mice: Its relevance to autoimmunity. *J. Immunol.* **126**, 998–1002.
47. Weinstein, Y., and Isakov, Y. (1983). Effects of testosterone metabolites and of anabolic androgens on the bone marrow and thymus in castrated female mice. *Immunopharmacology* **5**, 229–237.
48. Novotny, E. A., Raveche, E., and Sharrow, S. (1983). Analysis of thymocyte subpopulations following treatment with sex hormones. *Clin. Immunol. Immunopathol.* **28**, 205–217.
49. Raveche, E., Tijo, J. H., and Steinberg, A. D. (1980). Genetic studies in NZB mice. IV. The effects of sex hormones on the spontaneous production of anti-T cell antibodies. *Arthritis Rheum.* **23**, 48.
50. Raveche, E. S., Vigersky, R. A., Rice, M. K., and Steinberg, A. D. (1980). Murine thymic androgen receptors. *J. Immunopharmacol.* **2**, 425.
51. Detelfsen, M. A., Smith, B. C., and Dickerman, H. W. (1979). A high affinity, low capacity receptor for estradiol in normal and anemic mouse spleen cytosols. *Biochem. Biophys. Res. Commun.* **76**, 1151.
52. Golsteyn, E. J., and Fritzler, M. J. (1987). The role of the thymus-hypothalamus-pituitary-gonadal axis in normal immune processes and autoimmunity. *J. Rheumatol.* **14**, 982–990.
53. Theofilopoulos, A. N., and Dixon, F. (1986). Experimental murine systemic lupus erythematosus. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.). pp. 121–202. Wiley, New York.
54. Hang, L., Theofilopoulos, A. N., and Dixon, F. J. (1982). A spontaneous rheumatoid arthritis-like disease in MRL/l mice. *J. Exp. Med.* **155**, 1690.
55. Theofilopoulos, A. N., Shawler, D. R., and Eisenberg, R. A. (1980). Splenic immunoglobulin secreting cells and their regulation in autoimmune mice. *J. Exp. Med.* **151**, 445.
56. Andrews, B. S., Eisenberg, R. A., and Theofilopoulos, A. N. (1978). Spontaneous murine lupus-like syndromes: Clinical and immunopathological manifestations in several strains. *J. Exp. Med.* **148**, 1198.
57. Steinberg, A. D., Melez, K. A., Raveche, E. S., Reeves, J. P., Boegel, W. A., Smathers, P. A., *et al*. (1979). Approach to the study of the role of sex hormones in autoimmunity. *Arthritis Rheum.* **22**, 1170.
58. Steinberg, A. D., Roths, J. B., and Murphy, E. D. (1980). Effects of thymectomy and androgen administration upon the autoimmune disease of MRL/Mo-lpr/lpr mice. *J. Immunol.* **125**, 871.
59. Eisenberg, R. A., and Dixon, F. J. (1980). Effect of castration on male determined acceleration of autoimmune disease in BXSB mice. *J. Immunol.* **125**, 1939–1963.

60. Eisenberg, R. A., Izui, S., and McConahey, P. J. (1980). Male determined accelerated autoimmune disease in BXSB mice: Transfer by bone marrow and spleen cells. *J. Immunol.* **125**, 1032.
61. Mazes, E., and Fuchs, S. (1974). Linkage between immune response potential to DNA and X chromosome. *Nature* **249**, 167–168.
62. Laskin, C. A., Taurog, J. D., Smathers, P. A., and Steinberg, A. D. (1981). Studies of defective tolerance on murine lupus. *J. Immunol.* **127**, 1743.
63. Kuppers, R. C., Suiter, T., Gleichman, E., and Rose, N. R. (1988). The induction of organ-specific antibodies during the graft-vs-host reaction. *Eur. J. Immunol.* **18**, 161–166.
64. Fuks, A. S., and Weinstein, Y. (1979). 20 alpha-hydroxysteroid dehydrogenase (20 alpha SDH) activity in New Zealand mice: T lymphocytes and bone marrow cells. *J. Immunol.* **213**, 1266.
65. Jara, L. J., Lavalle, C., Fraga, A., Gomez-Sanchez, C., Silveira, L. H., Maritnez-Osuna, P., *et al*. (1991). Prolactin, immunoregulation, and autoimmune diseases. *Semin Arthritis Rheum.* **20**, 273–284.
66. Walker, S. E. (1993). Prolactin: An immune-stimulating peptide that regulates other immune-modulating hormones. *Lupus* **2**, 67–69.
67. Walker, S. E., Allen, S. H., Hoffman, R. W., and McMurray, R. W. (1995). Prolactin: A stimulator of disease activity in systemic lupus erythematosus. *Lupus* **4**, 3–9.
68. McMurray, R. W. (2001). Prolactin in murine systemic lupus erythematosus. *Lupus* **10**(10), 742–747.
69. Elbourne, K. B., Keisler, D., and McMurray, R. W. (1998). Differential effects of estrogen and prolactin on autoimmune disease in the NZB/NZW F1 mouse model of systemic lupus erythematosus. *Lupus* **7**(6), 420–427.
70. Lahita, R. G. (1992). Sex steroids and SLE: Metabolism of androgens to estrogens. *Lupus* **1**, 125–127.
71. Chapel, T. A., and Burns, R. E. (1971). Oral contraceptives and exacerbations of SLE. *Am. J. Obst. Gynec.* **110**, 366.
72. Petri, M., and Robinson, C. (1997). Oral contraceptives and systemic lupus erythematosus. *Arthritis Rheum.* **40**(5), 797–803.
73. Rose, E., and Pillsbury, D. M. (1944). Lupus erythematosus (erythematodes) and ovarian function: Observations on a possible relationship with a report of six cases. *Ann. Int. Med.* **21**, 1022.
74. Bjune, G. (1979). In vitro lymphocyte responses to PHA show co-variation with the menstrual cycle. *Immunol. Abstracts* 51.
75. Manzi, S., Meilahn, E. N., Rairie, J. E., Conte, C. G., Medsger, T. A., Jansen-McWilliams, L., *et al*. (1997). Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: Comparison with the Framingham study. *Am. J. Epidemiol.* **145**(5), 408–415.
76. Lahita, R. G., and Bradlow, H. L. (1987). Klinefelter's syndrome: Hormone metabolism in hypogonadal males with systemic lupus erythematosus. *J. Rheumatol.* **14**(Suppl. 13), 154–157.
77. Lahita, R. G. (1992). The importance of estrogens in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **63**, 17–18.
78. Lahita, R. G. (1986). The influence of sex hormones on the disease systemic lupus erythematosus. *Springer Semin. Immunopathol* **9**, 305–314.
79. Bucala, R., Lahita, R. G., Fishman, J., and Cerami, A. (1987). Anti-estrogen antibodies in users of oral contraceptives and in patients with systemic lupus erythematosus. *Clini. Exp. Immunol.* **67**, 167–175. [Abstract]
80. Fishman, J., and Martucci, C. (1980). Biological properties of 16-alpha hydroxyestrone: Implicationso in estrogen physiology and pathophysiology. *J. Clin. Endo. Metab.* **51**, 611–615.
81. Ikegawa, S., Lahita, R. G., and Fishman, J. (1983). Concentration of 16 alpha hydroxyestrone in human plasma as measured by a specific RIA. *J. Steroid Biochem.* **18**, 329–332.
82. Bucala, R., Fishman, J., and Cerami, A. (1982). Formation of co-valent adducts between cortisol and 16-alpha-hydroxyestrone and protein, possible role in pathogenesis of cortisol topxicity and SLE. *Proc. Nat. Acad. Sci.* **79**, 3320.
83. Bucala, R., Fishman, J., and Cerami, A. (1984). The reaction of 16-a hydroxyestrone with erythrocytes in vitro and in vivo. *Eur. J. Biochem.* **140**, 593–598.
84. Lahita, R. G., Bradlow, H. L., Fishman, J., and Kunkel, H. G. (1982). Estrogen metabolism in systemic lupus erythematosus: Patients and family members. *Arthritis Rheum.* **25**, 843–846.
85. Inman, R. D. (1978). Immunologic sex differences and the female preponderance in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 849–852.
86. Inman, R. D., Jovanovic, L., and Markenson, J. A. (1982). Systemic lupus erythematosus in men: Genetic and endocrine features. *Arch. Int. Med.* **142**, 1813–1815.
87. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1983). Increased oxidation of testosterone in systemic lupus erythematosus. *Arthritis Rheum.* **26**, 1517–1521.
88. Lahita, R. G., Bradlow, H. L., Ginzler, E., Pang, S., and New, M. (1987). Low plasma androgens in women with systemic lupus erythematosus. *Arthritis Rheum.* **30**, 241–248.
89. Jungers, P., Nahoul, K., and Pelissier, C. (1982). Low plasma androgens in women with active or quiescent SLE. *Arthritis Rheum.* **25**, 454–457.
90. Jungers, P., Pelissier, C., Bach, J. F., and Nahoul, K. (1980). Les androgenes plasmatiques chez les femmes atteintes de lupus erythemateux dissemine (LED). *Pathol. Biol.* **28**, 391–392.
91. El-Garf, A., Salah, S., Shaarawy, M., Zaki, S., and Anwer, S. (1996). Prolactin hormone in juvenile systemic lupus erythematosus: A possible relationship to disease activity and CNS manifestations. *J. Rheumatol.* **23**(2), 374–377.
92. Gutierrez, M. A., Molina, J. F., Jara, L. J., Garcia, C., Gutierrez-Urena, S., Cuellar, M. L., *et al*. (1996). Prolactin-induced immunoglobulin and autoantibody

production by peripheral blood mononuclear cells from systemic lupus erythematosus and normal individuals. *Int. Arch. Allergy Immunol.* **109**(3), 229–235.
93. Lash, A. A. (1993). Why so many women? 1. Systemic lupus erythematosus. *Medsurg. Nurs.* **2**(4), 259–264.
94. Folomeev, M., Dougados, M., Beaune, J., Kouyoumdjian, J. C., Nahoul, K., Amor, B., *et al*. (1992). Plasma sex hormones and aromatase activity in tissues of patients with systemic lupus erythematosus. *Lupus* **1**(3), 191–195.
95. Jara-Quezada, L., Graef, A., and Lavalle, C. (1991). Prolactin and gonadal hormones during pregnancy in systemic lupus erythematosus. *J. Rheum.* **18**, 349–353.
96. Lavalle, C., Loyo, E., Paniagua, R., Bermudez, J. A., Herrera, J., Graef, A., *et al*. (1987). Correlation study between prolactin and androgens in male patients with systemic lupus erythematosus. *J. Rheumatol.* **14**, 268–272.
97. Mok, C. C., and Lau, C. S. (1994). Lack of association between prolactin levels and clinical activity in patients with systemic lupus erythematosus. *J. Rheumatol.* **23**(12), 2185–2186.
98. Ostendorf, B., Fischer, R., Santen, R., Schmitz-Linneweber, B., Specker, C., and Schneider, M. (1996). Hyperprolactinemia in systemic lupus erythematosus? *Scand. J. Rheumatol.* **25**(2), 97–102.
99. Buskila, D., Lorber, M., Neumann, L., Flusser, D., and Shoenfeld, Y. (1996). No correlation between prolactin levels and clinical activity in patients with systemic lupus erythematosus. *J. Rheumatol.* **23**(4), 629–632.
100. Mok, C. C., Lau, C. S., and Tam, S. C. (1997). Prolactin profile in a cohort of Chinese systemic lupus erythematosus patients. *Br. J. Rheumatol.* **36**(9), 986–989.
101. Kozakova, D., Rovensky, J., Cebecauer, L., Bosak, V., Jahnova, E., and Vigas, M. (2000). Prolactin levels and autoantibodies in female patients with systemic lupus erythematosus. *Z. Rheumatol.* **59**(Suppl. 2), II/80–II/84.
102. Leanos-Miranda, A., Chavez-Rueda, K. A., and Blanco-Favela, F. (2001). Biologic activity and plasma clearance of prolactin-IgG complex in patients with systemic lupus erythematosus. *Arthritis Rheum.* **44**(4), 866–875.
103. Leanos-Miranda, A., Pascoe-Lira, D., Chavez-Rueda, K. A., and Blanco-Favela, F. (2001). Antiprolactin autoantibodies in systemic lupus erythematosus: Frequency and correlation with prolactinemia and disease activity. *J. Rheumatol.* **28**(7), 1546–1553.
104. Pacilio, M., Migliaresi, S., Meli, R., Ambrosone, L., Bigliardo, B., and Di Carlo, R. (2001). Elevated bioactive prolactin levels in systemic lupus erythematosus: Association with disease activity. *J. Rheumatol.* **28**(10), 2216–2221.
105. Miranda, J. M., Prieto, R. E., Paniagua, R., Garcia, G., Amato, D., Barile, L., *et al*. (1998). Clinical significance of serum and urine prolactin levels in lupus glomerulonephritis. *Lupus* **7**(6), 387–391.
106. Blanco-Favela, F., Quintal-Alvarez, G., and Leanos-Miranda, A. (1999). Association between prolactin and disease activity in systemic lupus erythematosus: Influence of statistical power. *J. Rheumatol.* **26**(1), 55–59.
107. Jara, L. J., Vera-Lastra, O., Miranda, J. M., Alcala, M., and Alvarez-Nemegyei, J. (2001). Prolactin in human systemic lupus erythematosus. *Lupus* **10**(10), 748–756.
108. Jacobi, A. M., Rohde, W., Volk, H. D., Dorner, T., Burmester, G. R., and Hiepe, F. (2001). Prolactin enhances the in vitro production of IgG in peripheral blood mononuclear cells from patients with systemic lupus erythematosus but not from healthy controls. *Ann. Rheum. Dis.* **60**(3), 242–247.
109. Jacobi, A. M., Rohde, W., Ventz, M., Riemekasten, G., Burmester, G. R., and Hiepe, F. (2001). Enhanced serum prolactin (PRL) in patients with systemic lupus erythematosus: PRL levels are related to the disease activity. *Lupus* **10**(8), 554–561.
110. Stevens, A., Ray, D. W., Worthington, J., and Davis, J. R. (2001). Polymorphisms of the human prolactin gene: Implications for production of lymphocyte prolactin and systemic lupus erythematosus. *Lupus* **10**(10), 676–683.
111. Cooper, G. S., Dooley, M. A., Treadwell, E. L., St. Clair, E. W., and Gilkeson, G. S. (2002). Hormonal and reproductive risk factors for development of systemic lupus erythematosus: Results of a population-based, case-control study. *Arthritis Rheum.* **46**(7), 1830–1839.
112. Stahl, N., and Decker, J. (1978). Androgenic status of males with SLE. *Arthritis Rheum.* **21**, 665–668.
113. Lahita, R. G. (1993). Sex hormones as immunomodulators of disease. *Ann. N. Y. Acad. Sci.* **685**, 278–287.
114. Lahita, R. G. (1985). Sex steroids and the rheumatic diseases. *Arthritis Rheum.* **28**, 121–126.
115. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1981). Increased 16 alpha hydroxylation of estradiol in systemic lupus erythematosus. *J. Clin. Endo. Metab.* **53**, 174–178.
116. Lahita, R. G., Chiorazzi, N., and Gibofsky, A. (1983). Familial systemic lupus erythematosus in males. *Arthritis Rheum.* **26**, 39.
117. Stein, C. M., Olson, J. M., Gray-McGuire, C., Bruner, G. R., Harley, J. B., and Moser, K. L. (2002). Increased prevalence of renal disease in systemic lupus erythematosus families with affected male relatives. *Arthritis Rheum.* **46**(2), 428–435.
118. Ansar Ahmed, S., Penhale, W. J., and Talal, N. (1985). Sex hormones, immune responses, and autoimmune diseases: Mechanisms of sex hormone action. *Am. J. Pathol.* **121**, 531–551.
119. Blank, M., Mendlovic, S., Fricke, H., Mozes, E., Talal, N., and Shoenfeld, Y. (1990). Sex hormone involvement in the induction of experimental systemic lupus erythematosus by a pathogenic anti-DNA idiotype in naive mice. *J. Rheumatol.* **17**, 311–317.
120. Hughes, G. R. (1984). Current understanding of systemic lupus erythematosus. *Inflammation* **8**(Suppl.), S75–S79.
121. Sthoeger, Z. M., Geltner, D., Rider, A., and Bentwich, Z. (1987). Systemic lupus erythematosus in 49 Israeli males: A retrospective study. *Clin. Exp. Rheum.* **5**, 233–240.
122. Anisman, H., Baines, M. G., Berczi, I., Bernstein, C. N., Blennerhassett, M. G., Gorczynski, R. M., *et al*. (1996). Neuroimmune mechanisms in health and disease. 2. Disease. *Can. Med. Assoc. J.* **155**(8), 1075–1082.

123. Camilleri, F., and Mallia, C. (1999). Male SLE patients in Malta. *Adv. Exp. Med. Biol.* **455**, 173–179.
124. Chang, D. M., Chang, C. C., Kuo, S. Y., Chu, S. J., and Chang, M. L. (1999). Hormonal profiles and immunological studies of male lupus in Taiwan. *Clin. Rheumatol.* **18**(2), 158–162.
125. Mok, C. C., and Lau, C. S. (2000). Profile of sex hormones in male patients with systemic lupus erythematosus. *Lupus* **9**(4), 252–257.
126. Alekberova, Z. S., and Folomeev, M. I. (1985). Sexual dimorphism in rheumatic diseases. *Revmatologiia (Mosk)* 58–61.
127. Folomeev, M., Alekberova, Z., and Polyntsev, J. (1990). The role of estrogen androgen imbalance in rheumatic diseases. *Terapeuticheskii Arkhiv* **62**(5), 17–21.
128. Alekberova, Z., Kotelnikova, G., and Folomeev, M. (1989). Aortic defects in systemic lupus erythematosus. *Terapeuticheskii Arkhiv* **61**(5), 35–38.
129. Bosmansky, K., and Kopecky, S. (1979). Progressive polyarthritis and Klinefelter's syndrome. *Fysiatr Revmatol Vestn* **57**, 160–163.
130. Fam, A., Izsak, M., and Saiphoo, C. (1980). SLE and Klinefelter's syndrome. *Arthritis Rheum.* **23**, 124.
131. French, M. A. H., and Hughes, P. (1983). Systemic lupus erythematosus and Klinefelter's syndrome. *Ann. Rheum. Dis.* **42**, 471–473.
132. Harris, F. (1980). Congenital hypothyroidism and Klinefelter's syndrome. *J. Med. Genet.* **17**, 326–327.
133. Kobayashi, S., Shimamoto, T., Taniguchi, O., Hashimoto, H., and Hirose, S. (1989). A case report of Klinefelter's syndrome associated with progressive systemic sclerosis. *Nippon Naika Gakkai Zasshi* **78**, 854–855.
134. Kobayashi, S., Shimamoto, T., Taniguchi, O., Hashimoto, H., and Hirose, S. (1991). Klinefelter's syndrome associated with progressive systemic sclerosis: Report of a case and review of the literature. *Clin. Rheumatol.* **10**, 84–86.
135. Vallotton, M. B., and Forbes, A. P. (1967). Autoimmunity in gonadal dysgenesis and Klinefelter's syndrome. *Lancet* **1**, 648–651. [Abstract]
136. Harrison, L. C., Dean, B., Peluso, I., Clark, S., and Ward, G. (1985). Insulin resistance, acanthosis nigricans, and polycystic ovaries associated with a circulating inhibitor of postbinding insulin action. *J. Clin. Endo. Metab.* **60**, 1047–1052.
137. Dmowski, W. P., Gebel, H. M., and Rawlins, R. G. (1989). Immunological aspects of endometriosis. *Obstetr. Gynecol. Clin. North Am.* 93–103.
138. Dmowski, W. P., Steele, R. W., and Baker, G. F. (1981). Deficient cellular immunity in endometriosis. *Am. J. Obstet. Gynecol.* **141**, 377.
139. Gutierez, M. A., Anaya, J. M., Cabrera, G. E., Vindrola, O., and Espinoza, L. R. (1994). Prolactin, a link between the neuroendocrine and immune systems: Role in the pathogenesis of rheumatic diseases. *Rev. Rhum. Ed. Fr.* **61**(4), 278–285.
140. Sanchez-Guerrero, J., Karlson, E., Liang, M. H., Hunter, D. J., Speizer, F. E., and Colditz, G. A. (1997). Past use of oral contraceptives and the risk of developing systemic lupus erythematosus. *Arthritis Rheum.* **40**(5), 804–808.
141. Liang, M. H., and Karlson, E. W. (1996). Female hormone therapy and the risk of developing or exacerbating systemic lupus erythematosus or rheumatoid arthritis. *Proc. Assoc. Am. Phys.* **108**(1), 25–28.
142. Buyon, J., Korchack, H. M., and Rutherford, L. E. (1984). Female hormones reduce neutrophil responsiveness *in vitro*. *Arthritis Rheum.* **27**, 623–630.
143. Bodel, P., Dillard, G. M., Kaplan, S. S., and Malawista, S. E. (1972). Antiinflammatory effects of estradiol on human blood leukocytes. *J. Lab. Clin. Med.* **80**, 373–384.
144. Mikkelson, W. M., Dodge, N. J., and Vlakenberg, H. (1965). The distribution of serum uric acid values in a population unselected as to gout or hyperuricemia. *Am. J. Med.* **39**, 242.
145. Lockshin, M. D. (2001). Invited review: Sex ratio and rheumatic disease. *J. Appl. Physiol.* **91**(5), 2366–2373.
146. Linos, A., O'Fallon, W. M., Worthington, J. W., and Kurland, L. T. (1983). Case control study of rheumatoid arthritis and prior use of oral contraceptives. *Lancet* **1**, 1299.
147. Linos, A., Worthing, J. W., O'Fallon, W. M., and Kurland, L. T. (1989). The epidemiology of rheumatoid arthritis in Rochester, Minnesota: A study of its incidence, prevalence, and mortality. *Am. J. Epidem.* **111**, 87–98.
148. Oka, M., and Vainio, U. (1966). Effect of pregnancy on the prognosis and serology of rheumatoid arthritis. *Acta. Rheum. Scand.* **12**, 47.
149. Mok, C. C., Lau, C. S., Ho, C. T., and Wong, R. W. (1999). Do flares of systemic lupus erythematosus decline after menopause? *Scand. J. Rheumatol.* **28**(6), 357–362.
150. Sanchez-Guerrero, J., Villegas, A., Mendoza-Fuentes, A., Romero-Diaz, J., Moreno-Coutino, G., and Cravioto, M. C. (2001). Disease activity during the premenopausal and postmenopausal periods in women with systemic lupus erythematosus. *Am. J. Med.* **111**(6), 464–468.
151. Agnello, V., Pariser, K., and Gell, J. (1983). Preliminary observation on Danazol therapy of systemic lupus: Effects on DNA antibodies, thrombocytopenia and complement. *J. Rheumatol.* **10**, 682–687.
152. Donaldson, V. H., and Hess, E. V. (1980). Effect of danazol on lupus erythematosus like disease in hereditary angioneurotic edema. *Lancet* **2**, 1143.
153. Dougados, M., Job-deselandre, C., Amor, B., and Menkes, J. (1987). Danazol therapy in systemic lupus erythematosus. *Clin. Trials. J.* **24**, 191–200.
154. Fretwell, M., and Altman, L. O. (1982). Exacerbation of a lupus erythematosus-like syndrome during treatment of non-C1 esterase inhibitor dependent angioedema with danazol. *J. Allergy. Clin. Immunol.* **69**, 306–310.
155. Ahn, Y. S., Mylvaganam, R., Garcia, R. O., Kim, C. I., Palow, D., and Harrington, W. J. (1987). Low-dose danazol therapy in idiopathic thrombocytopenic purpura. *Ann. Intern. Med.* **107**, 177–181.
156. Mylvaganam, R., Ahn, Y. S., Garcia, R. O., Kim, C. I., and Harrington, W. J. (1989). Very low dose danazol in idiopathic thrombocytopenic purpura and its role as an immune modulator. *Am. J. Med. Sci.* **298**, 215–220.
157. Lahita, R. G., Cheng, C. Y., Monder, C., and Bardin, C. W. (1992). Experience with 19-nortestosterone in the

therapy of systemic lupus erythematosus: Worsened disease after treatment with 19-nortestosterone in men and lack of improvement in women. *J. Rheumatol.* **19**, 547–555.
158. Liote, F., Dehaine, V., Pelissier, C., Kuttenn, F., and Jungers, P. (1991). Hormonal modulation with cyproterone acetate in systemic lupus erythematosus: A long term follow-up study. *Arthritis Rheum.* [Abstract]
159. El-Roeiy, A., Dmowski, W. P., and Gleicher, N. (1988). Effect of danazol and GnRH agonists (GnRH-a) on the immune system in endometriosis. *Soc. Gynecol. Invest.* 283. [Abstract]
160. Sudo, K., Shiota, K., Masaki, T., and Fujita, T. (1991). Effects of TAP-144-SR, a sustained-release formulation of a potent GnRH agonist, on experimental endometriosis in the rat. *Endocrinol. Jpn.* **38**, 39–45.
161. Khorram, O., Vu, L., and Yen, S. C. C. (1997). Activation of immune function by dehydroepiandrosterone (DHEA) in age-advanced men. *J. Gerontol* **52A**(1), 1–7.
162. Spencer, N. F. L., Poynter, M. E., Hennebold, J. D., Mu, H. H., and Daynes, R. A. (1996). Does DHEAS restore immune competence in aged animals through its capacity to function as a natural modulator of peroxisome activities? *In* "Dehydroepiandrosterone (DHEA) and Aging" (F. L. Bellino, R. D. Daynes, P. J. Hornsby, D. H. Lavren, and J. E. Nestler, eds.), pp. 200–216. New York Academy of Sciences, New York.
163. Araneo, B. A., and Daynes, R. A. (1995). Dehydroepiandrosterone functions as more than an antiglucocorticoid in preserving immunocompetence after thermal injury. *Endo* **136**(2), 393–401.
164. Daynes, R. A., and Araneo, B. A. (1992). Programming of lymphocyte responses to activation: Extrinsic factors, provided microenvironementally, confer fllexibility and compartmentalization to T cell function. *Chem. Immunol.* **54**, 1–20.
165. Daynes, R. A., Dudley, D. J., and Araneo, B. A. (1990). Regulation of murine lymphokine production *in vivo*. II. Dehydroepiandrosterone is a natural enhancer of interleukin 2 synthesis by helper T cells. *J. Immunol.* **20**, 793–802.
166. Nandy, K., Harbous, L., Bennet, D., and Bennet, M. (1983). Correlation between learning disorder and elevated brain reactive antibodies in aged C57B1/6 and young NZB mice. *Life Sci.* **33**, 1499.
167. Geschwind, N., and Behan, P. (1972). Left handedness: Association with immune disease, migraine and developmental learning disorders. *Proc. Nat. Acad. Sci. USA* **74**, 5097.
168. Lahita, R. G. (1988). Systemic lupus erythematosus: Learning disability in the male offspring of female patients and relationship to laterality. *Psychoneuroendocrinology* **13**, 385–396.
169. Sherman, G. F., Galaburda, A. M., and Geschwind, N. (1983). Ectopic neurons in the brain of the autoimmune mouse: Aneuropathophysiologic model of dyslexia? *Neuropathol. Proc.* **275**, 6.
170. Wood, L. C., and Cooper, D. S. (1989). Autoimmune thyroid disease, left-handedness, and developmental dyslexia. Unpublished work.
171. Ahmed, S. A., Dauphinee, M., and Talal, N. (1985). Effects of short term administration of sex hormones on normal and autoimmune mice. *J. Immunol.* **134**, 204–210.
172. Ablin, R. J., Bruns, G. R., Guinan, P., and Bush, I. M. (1974). Antiandrogenic suppression of lymphocytic blastogenesis: *In vitro* and in vivo observations. *Experientia* **30**, 1351.
173. Dorner, G., Eckert, R., and Hinz, G. (1980). Androgen dependent sexual dimorphism of the immune system. *Endocrinologie* **76**, 112.
174. Hirota, Y., Suzuki, T., and Bito, Y. (1980). The B-cell development independent of bursa of Fabricius but dependent upon the thymus in chickens treated with testosterone propionate. *Immunology* **39**, 37–46.
175. Hirota, Y., Suzuki, T., and Bito, Y. (1980). The development of unusual B-cell functions in the testosterone-propionate treated chicken. *Immunology* **39**, 29.
176. Hirota, Y., Suzuki, T., Chazono, Y., and Bito, Y. (1976). Humoral immune responses characteristics of testosterone propionate treated chickens. *Immunology* **30**, 341.
177. Grossman, C. (1989). Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *J. Steroid Biochem.* **34**, 241–251.
178. Grossman, C. J. (1984). Regulation of the immune system by sex steroids. *Endocr. Rev.* **5**, 435–455.
179. Grossman, C. J. (1988). The importance of hormones in the regulation of the immune system. *Immunol. Allergy Pract.* **10**, 104–106.
180. Lubahn, D. B., Joseph, D. R., Sar, M., Tan, J., Higgs, H. N., Larson, R. E., *et al*. (1988). The human androgen receptor: complementary deoxyribonucleic acid cloning, sequence analysis and gene expression in prostate. *Mol. Endocrinol.* **2**, 1265–1275.
181. Klareskog, L., Forsum, U., and Peterson, P. A. (1980). Hormonal regulation of the expression of Ia antigens on mammary gland epithelia. *Eur. J. Immunol.* **10**, 958–963.
182. Terres, G., Morrison, S. L., and Habicht, G. L. (1968). A quantitative difference in the immune response between male and female mice. *Proc. Exp. Biol. Med.* **27**, 664.
183. Thompson, J. S., Crawford, M. K., Reilly, R., and Stevenson, C. (1957). Estrogenic hormones in immune responses in normal and X irradiated mice. *J. Immunol.* **98**, 331.
184. Waltman, S. R., Brude, R. M., and Benios, J. (1971). Prevention of corneal rejection by estrogens. *Transplantation* **11**, 194.
185. Brodie, J. Y., Hunter, I. C., Stimson, W. H., and Green, B. (1980). Specific estradiol binding in cytosols from the thymus glands of normal and hormone-treated male rats. *Thymus* **1**, 337.
186. Wyle, F. A., and Kent, J. R. (1977). Immunosuppression by sex steroid hormones. I. The effect upon PHA- and PPD-stimulated lymphocytes. *Clin. Exp. Immunol.* **27**, 407–415.

187. Satoh, P. S., Fleming, W. E., Johnstone, K. A., and Ozmun, J. M. (1977). Active E rosette formation in women taking oral contraceptives. *N. Eng. J. Med.* **296**, 54.
188. Olsen, N. J., Watson, M. B., Henderson, G. S., and Kovacs, W. J. (1991). Androgen deprivation induces phenotypic and functional changes in the thymus of adult male mice. *Endo* **129**, 2471–2476.
189. Sasson, S., and Mayer, M. (1981). Effect of androgenic steroids on rat thymus and thymocytes in suspension. *J. Steroid Biochem.* **14**, 509–518.
190. Graff, R. J., Lappe, M. A., and Snell, G. D. (1969). The influence of the gonads and the adrenal glands on the immune response to skin grafts. *Transplantation* **7**, 105.
191. Buskila, D., Berezin, M., Gur, H., Lin, H. C., Alosachie, I., Terryberry, J. W., *et al*. (1995). Autoantibody profile in the sera of women with hyperprolactinemia. *J. Autoimmun.* **8**(3), 415–424.
192. Paavonen, T., Aronen, H., Pyrhonen, S., Hajba, A., and Andersson, L. C. (1991). The effects of anti-estrogen therapy on lymphocyte functions in breast cancer patients. *APMIS* **99**, 163–170.
193. Sthoeger, Z. M., Chiorazzi, N., and Lahita, R. G. (1988). Regulation of the immune response by sex hormones. I. In vivo effects of estradiol and testosterone on pokeweed mitogen-induced B-cell differentiation. *J. Immunol.* **141**, 91.
194. Cohen, J. H. M., Danel, L., Cordier, G., Saez, S., and Revillard, J. (1983). Sex steroid receptors in peripheral T cells: Absence of androgen receptors and restriction of estrogen receptors to OKT8 positive cells. *J. Immunol.* **131**, 2767–2771. [Abstract]
195. Danel, L., Sovweine, G., Monier, J. C., and Saez, S. (1983). Specific estrogen binding sites in human lymphoid cells and thymic cells. *J. Steroid Biochem.* **18**, 559.
196. Jo, T., Terada, N., Saji, F., and Tanizawa, O. (1993). Inhibitory effects of estrogen, progesterone, androgen and glucocorticoid on death of neonatal mouse uterine epithelial cells induced to proliferate by estrogen. *J. Steroid Biochem. Mol. Biol.* **46**(1), 25–32.
197. Mori, T., Kobayashi, H., and Nishimoto, H. (1977). Inhibitory effect of progesterone and 20-hydroxy-pregn-4-en-3-one on the phytohemagglutinin-induced transformation of human lymphocytes. *Am. J. Obstet Gynecol.* **127**, 151.

8

GENES AND GENETICS OF MURINE LUPUS

Dwight H. Kono
Roberto Baccala
Argyrios N. Theofilopoulos

The genetic basis of systemic autoimmunity has now been clearly documented, and this progress has, ironically, also revealed the gaps in our understanding of this complex disease entity. Although the etiology of systemic lupus erythematosus (SLE) is still unknown, elucidation of the genes and their contributions to the development of lupus should reveal many of the fundamental events underlying the initiation and maintenance of systemic autoimmunity. Identification of the genes involved in the immunological and inflammatory processes that promote end organ damage will reveal the components crucial for disease manifestations, which can provide targets for therapeutic intervention. Delineating the genetic alteration that enhances susceptibility will define the causative mechanisms that lead to immune system-mediated self-destruction. The specific genes and pathways participating in immune system activation and homeostasis, along with gene manipulation approaches, have been investigated, and many new potential avenues for loss of tolerance and development of systemic autoimmunity have been identified. This chapter summarizes our current view of this rapidly evolving field.

MOUSE MODELS OF LUPUS

Spontaneous and induced mouse models of SLE have provided insights into the genetics and immunopathology of this disease (Table 1). The most commonly studied spontaneous models are the (NZB×NZW)F_1 (BWF_1) hybrid, MRL-*Fas*^{lpr}, and BXSB mice, strains that share characteristics, such as hypergammaglobulinemia, antinuclear antibodies (ANA), and glomerulonephritis (GN), and also possess unique features, such as arthritis and expanded $CD4^-CD8^-$ (double-negative, DN) T cells in MRL-*Fas*^{lpr} mice and hemolytic anemia in NZB mice. Details on the clinical manifestations and immunopathology for these and other spontaneous and induced models have been reviewed previously [1, 2].

Two new induced models of lupus-like disease have been added since the previous edition (Table 1). Nonautoimmune-prone galactose-α1-3-galactose-deficient mice exposed topically to commercial bovine thrombin preparations commonly used in human surgical procedures developed antibodies to the xenogeneic galactose-α1-3-galactose and, in a few cases, autoantibodies to clotting factors. Unexpectedly, they also produced anti-double-stranded (ds)DNA and anticardiolipin autoantibodies and developed immune complex-mediated GN with a higher frequency of disease in females [3]. The other model, type I diabetes mellitus-susceptible NOD mice, were given intravenous *Mycobacterium bovis* (bacillus Calmette–Guerin), which prevented diabetes but, surprisingly, induced systemic autoimmunity manifested by accelerated hemolytic anemia, antinuclear antibodies, exacerbation of sialadenitis, and immune complex-mediated GN [4]. Although it was initially hypothesized that susceptibil-

TABLE 1 Spontaneous and Induced Mouse Models of Lupus

Spontaneous disease models
- NZ and related strains
  - NZB
  - NZW
  - (NZB×NZW)F_1
  - (NZB×SWR)F_1
  - (NZB×NZW) recombinant inbred (RI) lines "NZM/Aeg" lines [328]
  - (NZB×SM)RI lines "(NXSM)RI"
  - (NZB×C58)RI lines "(NX8)RI"
- MRL (*Fas*^{lpr} and wild-type) and related strains
  - MRL-*Fas*^{lpr}.*ll* (long-lived substrain) [329]
  - MRL-*Fas*^{lpr}, *Yaa* [69]
  - SCG/Kj-*Fas*^{lpr} (BXSB×MRL-*lpr*)RI [330]
- BXSB and related strains
  - BXSB-*ll* (long-lived substrain) [331]
  - (NZW×BXSB)F_1
  - (NZB×BXSB)F_1
- (SJL×SWR)F_1 [332]
- Palmerston North [333]
- Motheaten strains [56, 57, 334]

Induced disease models
- Heavy metal-induced autoimmunity [335]
- Drug-induced lupus [336]
- Pristane induced [337]
- Anti-idiotypic [338]
- Graft-versus-host disease
- BCG-injected NOD [4, 5]
- Bovine thrombin-exposed galactose-α1-3-galactose-deficient mice [3]

ity to two different types of autoimmune diseases might be due to common autoimmune-predisposing genes, later mapping studies revealed no colocalization of loci predisposing to lupus or diabetes other than the MHC region [5]. These models provide striking examples of the complex interactions between genetic susceptibility and environmental factors that influence predisposition to SLE and other autoimmune diseases and demonstrate the utility of mouse models to help address this issue.

PREDISPOSING GENES IN SPONTANEOUS LUPUS MOUSE MODELS

The MHC genes, Fas receptor and ligand, and *Hcph* (hemopoietic cell phosphatase, SHP-1) (Table 2) are among the few genes identified as predisposing to spontaneous lupus. The Y chromosome accelerator of autoautoimmunity and lymphoproliferation (*Yaa*) gene, responsible for the male predisposition to lupus-like disease in the BXSB strain, has also been implicated using consomic mice (transfer of the Y chromosome), but the gene is not known.

MHC Region

In most lupus-prone strains, particularly BWF_1 [3, 4] and BXSB [5], the MHC complex has been strongly linked to the development of autoimmunity, but the contribution of MHC is highly dependent on other background genes, e.g., the specific haplotype that confers susceptibility depends on the lupus background, and autoimmunity does not develop when the predisposing MHC haplotypes are on normal backgrounds [2]. Although the actual gene(s) within the MHC complex that promotes lupus susceptibility still remains to be identified, class II molecules are strong candidates based on H-2 congenic studies [6–8] and their central roles in both repertoire shaping and antigen recognition. This does not exclude the participation of other, closely linked, immunologically relevant genes, which are numerous in this region. One of the strongest associations with lupus disease is the heterozygous H-2^z haplotype in combination with one of several different H-2 haplotypes, including d, b, and v [9–13]. Studies have attempted to directly implicate class II molecules by expressing I-A^z or I-E^z transgenes in the NZB × C57BL/6 background, but no increased susceptibility was found in mice expressing either of these transgenes [14, 15]. Although this suggests that class II molecules are not responsible, it is also possible that the transgene failed to adequately recapitulate the expression patterns required to promote autoimmunity, as it is known that slight changes in class II expression substantially affect disease susceptibility, e.g., homozygous versus heterozygous expression [16], or the presence or absence of I-E [17]. In this regard, transgenic mice would not have the same levels of class II molecules as wild-type mice as they would have normal levels of class II in addition to the transgene.

The role of I-E in lupus-prone mice has been investigated by expressing the Eα transgene in H-2^b BXSB mice [8, 17, 18]. Interestingly, high levels and, to a lesser extent, lower levels of I-Eα transgene expression suppressed lupus development, apparently because of competitive inhibition of autoantigen presentation by peptide fragments from processed I-Eα that bound effectively to H-2^b class II molecules.

Other potential lupus-predisposing genes within the H-2 complex include a polymorphic NZW tumor necrosis factor (TNF)-α gene that appears to promote lupus in BWF_1 hybrids [19, 20], complement components C2 and C4 [21], IEX-1 [22], and a recessive NZW locus (*Sles1*) that appears to suppress autoimmunity in NZW mice [23]. The relationship of *Sles1*, however, to the

known class II and TNF-α polymorphisms in the NZW strain has yet to be determined.

Fas and Fas Ligand

Fas (APO-1 or CD95) is a 306 amino acid, 45-kDa, cell surface membrane protein related to the TNF receptor superfamily of type I membrane glycoproteins. Fas is expressed on actively proliferating cells in the thymus, liver, ovary, heart, skin, and gut epithelium, with particularly high levels on $CD4^+CD8^+$ thymocytes, activated T and B cells, and some neoplastic cells [24, 25]. Following binding to its ligand, Fas transduces signals leading, in most instances, to apoptotic cell death [26, 27]. The ligand for Fas, FasL, is a 40-kDa type II transmembrane glycoprotein belonging to the TNF ligand family of proteins. It is expressed almost exclusively on T-cell lineages, primarily upon activation [28] and in certain immunologically sequestered sites, such as the testis, eye, and placenta [28–31]. In these areas, FasL may contribute to the maintenance of immune privilege by inducing Fas-mediated apoptosis in invading inflammatory cells [32], and in the case of the retina, may also control the growth of new vision-damaging subretinal blood vessels [33].

Two spontaneous loss-of-function mutations in Fas (*Fas*^{lpr} and *Fas*$^{lpr\text{-}cg}$) and one in FasL (*Fasl*^{gld}) result in similar autoimmune manifestations (Table 2). The *Fas*^{lpr} (lymphoproliferative) defect is caused by an early retroviral transposon (ETn) insertion within the second intron between exons 2 and 3, which causes aberrant RNA splicing, a frame shift, and premature termination of the mRNA at the long terminal repeat region of the ETn [34–38]. Low levels (~10%) of wild-type Fas mRNA and surface protein, however, are detectable [36–40], and recombinant *Fas* knockout mice, but not *lpr* mice, develop liver hyperplasia in addition to lymphoproliferation [41]. The *Fas*$^{lpr\text{-}cg}$ mutation (*lpr* complementing *gld*) [41] is caused by a single point mutation (T→A at nucleotide 786; isoleucine→asparagine; this residue is valine in humans) within the intracytoplasmic domain of Fas that modifies an amino acid in the so-called "death domain" essential for signal transduction [35]. Finally, the *Fasl*^{gld} allele (generalized lymphoproliferative disease) [42] is a point mutation in the carboxy-terminal extracellular domain (T→C at nucleotide 847, phenylalanine→leucine) of FasL on chromosome 1, which abolishes the binding of Fas to the FasL [43–45].

Homozygosity for these mutations promotes accumulation of $CD4^-CD8^-$ (double negative, DN), $B220^+$, $TCR\alpha\beta^+$ T cells and the induction or acceleration of systemic autoimmunity [1, 46], but both severity of the autoimmune disease and degree of lymphoproliferation depend on other background genes [1]. *Fas*^{lpr} does not complement *Fasl*^{gld}, whereas the *Fas*$^{lpr\text{-}cg}$ allele, as suggested by its name, can complement both the *Fas*^{lpr} allele and, to a slightly lesser degree, the *Fasl*^{gld} allele. Furthermore, in contrast to the recessive *Fas*^{lpr} mutation, autoimmunity and lymphoproliferation are observed in heterozygous MRL-*Fas*$^{lpr\text{-}cg/+}$ mice, although less severe and without the characteristic expansion of DN T cells [47]. In humans, defects in Fas have been identified as a cause of the rare autoimmune lymphoproliferative syndrome (ALPS or Canale–Smith syndrome) [48–50]. This syndrome has also been described with loss-of-function mutations in caspase 10, a cysteine protease involved in the downstream apoptosis-promoting pathway of Fas [51]. The majority of SLE patients, however, do not appear to have deficiencies or mutations in *Fas* or *Fasl* [52–55].

SHP-1

SHP-1 is a protein tyrosine phosphatase expressed ubiquitously in hematopoietic lineage cells. Two spontaneous recessive mutations, the motheaten (*me*) and motheaten viable (*me*^{v}), both lead to similar early lethal phenotypes, which differ slightly in severity because of more complete gene deletion in the *me* variant. Although increased immunoglobulin levels and autoantibodies are detected, the major disease manifestations arising from severe abnormalities in virtually all hematopoietic cell lineages are not similar to spontaneous lupus, nor are they mediated by autoantibodies or T and B lymphocytes [56–60]. The basis for the motheaten phenotype rests largely on the requirement for SHP-1 in inhibiting cell activation following its recruitment by negative regulatory molecules containing immunoreceptor tyrosine-based inhibitory motifs (ITIM) [61]. Autoantibody production is likely due to the inability of CD22, and possibly FcγRIIB, to negatively regulate the B-cell antigen receptor in the absence of SHP-1 [62].

Yaa Gene

BXSB mice have a marked male predilection to systemic autoimmunity in contrast to the female predominance in humans and other susceptible mouse strains. This striking sexual dichotomy is not due to hormonal factors, but to a gene on the Y chromosome, designated *Yaa* [63–65]. Because conventional genetic mapping approaches are not possible for the nonrecombining Y chromosome, *Yaa* has yet to be cloned. Nevertheless there is some information about its contribution to systemic autoimmunity.

TABLE 2 Susceptibility Genes Predisposing to Lupus-Related Traits[a]

Name	Chr	cM	Best association marker	Cross[b]	Phenotype[c]	Parental allele	Ref.
Bxs4	1	7.7	D1Mit3	B10×(B10×BXSB)F1	LN	BXSB	108
Bxs1	1	32.8	D1Mit5	BXSB×(B10×BXSB)F1	GN/ANA/spleen	BXSB	107
—	1	54.0	D1Mit48	(W×Ba)F1×W	IgM ssDNA/IgM histone	BALB/c	339
Bana3	1	60.9	D1Mit396	(NOD×Ba)×NODBC	ANA (*M.bovis*)	NOD	5
Bxs2	1	63.1	D1Mit12	BXSB×(B10×BXSB)F1	GN/ANA/spleen	BXSB	107
—	1	65.0	D1Mit494	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Sialadenitis	MRL	104
Swrl1	1	87.9	D1Mit15	B×(SWR×B)F1	dsDNA/histone	SWR	340
Sle1	1	87.9	D1Mit15	(NZM×B6)×NZM	GN	NZM (NZW)	12
				(NZM×B6)F2	dsDNA/GN/spleen	NZM (NZW)	88
Hmr1	1	87.9	D1Mit15	(SJL×DBA/2)F2	Glomerular IgG deposits (HgIA resistance)	DBA/2	341
		92.3	D1Mit36	(B×DBA/2)F2	Glomerular IgG deposits (HgIA resistance)	DBA/2	341
Cgnz1	1	92.3	D1Mit36	(NZM2328×C57L)F1×NZM2328	Chronic GN	NZM2328 (NZW)	342
Lbw7	1	92.3	D1Mit36	BWF2	chr/spleen	NZB	78
Nba2	1	92.3	D1Mit111	(B×SM)×W	GN	NZB	13
		92.3	D1Mit148	(B×SM)×W/(B6.H2^z×B)×B	ANA/gp701C/GN	NZB	343
		94.2	Crp/Sap	[(B6.H2^z & Ba.H2^z)×B]F1×B	GN	NZB	344
Bxs3	1	100.0	D1Mit403	BXSB×(B10×BXSB)F1	dsDNA	BXSB	107
Agnz1	1	101.0	D1Mit37	(NZM2328×C57L)F1×NZM2328	Acute GN	NZM2328 (NZW)	342
—	1	106.3	D1Mit17	(W×Ba)F1×W	ssDNA	NZW	339
—	2		D2Mit12	(MRL-lpr×Ba)F2	ssDNA/dsDNA	MRL +/+, lpr/+	345
Wbw1	2	86.0	D2Mit285	(W×PL)F1×B	Mortality/GN	NZW	346
Sles2	3	35.2	D3Mit137	(B6.NZMc1×W)F1×W	dsDNA/GN (resistance)	NZW	23
Bxs5	3	39.7	D3Mit40	B10×(B10×BXSB)F1	ANA/IgG3	BXSB	108
Lprm2	3	66.2	D3Mit16	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Vasculitis (resistance)	MRL	102
Arvm1	4	19.8	D4Mit89	(MRL-lpr×C3H-lpr)BC and F_2	Vasculitis	MRL	106
Lprm1	4	32.5	D4Mit82	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Vasculitis	MRL	102
Acla2	4	40.0	D4Mit79	W×(W×BXSB)F1	CL	BXSB	109
Sle2	4	44.5	D4Mit9	(NZM×B6)×NZM	GN	NZM (NZW)	12
Spm1	4	45.9	D4Mit58	(B6×NZB)F_1×NZB	Spleen	NZB	347
Adaz1	4	49.6	D1Mit36	(NZM2328×C57L)F1×NZM2328	dsDNA	NZM2328	342
Lbw2	4	55.6	D4Nds2	BWF2	Mortality/GN/spleen	NZB	78
Sles2	4	57.6	D4Mit12	(B6.NZMc1×W)F1×W	dsDNA/GN (resistance)	NZW	23
—	4	62.3	D4Mit70	(B×SM)×W	GN	NZB	13
Arvm2	4	57.6	D4Mit147	(MRL-lpr×C3H-lpr)BC and F_2	Vasculitis	MRL	106
Asm2	4	65.0	D4Mit199	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Sialadenitis	MRL female	104
nba1	4	65.7	Epb4.1 (elp-1)	BWF1×W	GN	NZB	348
Lmb1	4	69.8	D4Mit12	(B6-lpr×MRL-lpr)F2	Lprn/dsDNA	B6	101
Imh1/Mott	4	69/69.8	D4Mit66/48	BWF1×W	Hyper-IgM/GN/dsDNA	NZB	349, 350
Sle6	5	20.0	D5Mit4	(B6.NZMc1×NZW)F1×NZW	GN	NZW	23
Lmb2	5	41.0	D5Mit356	(B6-lpr×MRL-lpr)F2	Lprn/dsDNA	MRL	101
Lprm4	5	54.0	D5Mit23	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Spleen	MRL	102
Lbw3	5	84.0	D5Mit101	BWF2	Mortality	NZW	78
—	6	35.0	D6Mit8	MRL-lpr×(MRL-lpr×C3H-lpr)F1	GN (resistance)	MRL	351
Lbw4	6	64.0	D6Mit25	BWF2	Mortality	NZB	78
—	6	74.0	D6Mit374	(NZM×B6)F2	dsDNA	B6	88
Sle5	7	0.5	D7Mit178	(NZM×B6)F2	dsDNA	NZM (NZW)	88
Lrdm1	7	6.0	Pou2f2(Otf-2)	(MRL-lpr×CAST)F1×MRL-lpr	GN	MRL	103
Sle3	7	16.0	D7Mit25	(NZM×B6)F2	GN	NZM (NZW)	88
Lbw5	7	23.0	D7Nds5(Ngfg)	BWF2	Mortality	NZW	78
Lmb3	7	27.0	D7Mit211	(B6-lpr×MRL-lpr)F2	Lprn/dsDNA	MRL	101
Sle3	7	28.0	p	(NZM×B6)×NZM	GN	NZM (NZW)	12
Aem2	7	28.4	D7Mit30	(B6×B)F_1×B	RBC	NZB	347
—	7	51.5	D7Mit17	(B×SM)×W	GN	NZB	13
—	7	56.5	D7Mit7	(B×W)F1×W	dsDNA	NZB	352
Myo1	7	69.0	D7Mit14	W×(W×BXSB)F1	MI	BXSB	109
Pbat2	8	11	D8Mit96	W×(W×BXSB)F1	Platelet	BXSB	109

(*continues*)

TABLE 2 (*continued*)

Name	Chr	cM	Best association marker	Cross[b]	Phenotype[c]	Parental allele	Ref.
sbb1	9	17.0	D9Mit67	(B6×Ba)F2-FcγRIIb$^{-/-}$	Spleen(FcγRIIb ko)	BALB/c	125
baa1	9	28.0	D9Mit22	(W×Ba)F1×W	IgM ssDNA/IgM histone	BALB/c	339
Gp1	9	57.9	D9Mit53	BXSB×(B10×BXSB)F1	gp70IC	BXSB	353
Bana2	10	0.0	D10Mit213	(NOD×Ba)×NODBC	ANA (*M.bovis*)	BALB/c	5
Asm1	10	38/40	D10Mit115/259	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Sialadenitis	MRL	104
Aem3	10	41.5	D10Mit42	(B6×B)F_1×B	RBC	NZB	347
Lmb4	10	51.0	D10Mit11	(B6-lpr×MRL-lpr)F2	Lprn/GN	MRL	101
—	10	69.0	D10Mit35	(NZM×B6)F2	GN	NZM & B6[d]	88
—	10	70.0	D10Mit297	(B10.A^z×B)F1×B	chr	B10	354
—	11	2.0/17.0	D11Mit2/84	(Ba.H2z×B)F1×B	GN	NZB	344
—	11	20.0	D11Mit20	(NZM×B6)F2	GN/dsDNA	NZM	88
Lbw8	11	28.0	IL4	BWF2	chr	NZB	78
—	11	28.5	D11Mit207	(W×Ba)F1×W	ssDNA	NZW	339
—	11	54.0	D11Mit70	(MRL-lpr×Ba)F2	dsDNA/ssDNA/CL	MRL	345
ssb2	12	6.0	D12Mit12	(B6×Ba)F2-FcγRIIb$^{-/-}$	ANA (FcγRIIb ko)	B6	125
Lrdm2	12	27.0	D12Nyu3	(MRL-lpr×CAST)F1×MRL-lpr	GN	MRL	103
Bxs6	13	24.0	D13Mit253	BXSB×(B10×BXSB)F1 and B10×(B10×BXSB)F1	gp70/gp70IC	BXSB	353
Yaa1	13	35.0 + 6	D13Mit250	B6×(W×B6-Yaa)F1	gp70IC	NZW	355
—	13	59.0	D13Mit226	(B10.A^z×B)F1×B	gp70IC/GN	B10	354
—	13	71.0	D13Mit31	(NZM×B6)×NZM	dsDNA	NZM	12
—	13	71.0	D13Mit150	(B×SM)×W	GN	NZB	13
—	14	19.5	D14Nds4	(W×Ba)F1×W	Histone	NZW	339
Swrl2	14	27.5	D14Mit37	B×(SWR×B)F1	GN/dsDNA	SWR	340
Myo2	14	39.0	D14Mit68	W×(W×BXSB)F1	MI	BXSB	109
	14	42.5/40.0	D14Nki41/Mit34	[(B6.H2z & Ba.H2z)×B]F1×B	GN	NZB	344
Lprm3	14	44.0	D14Mit195	MRL-lpr×(MRL-lpr×C3H-lpr)F1	GN (resistance)	MRL	102
Paam1	15	17.8	D15Mit111	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Arthritis in males	MRL	356
Lprm5	16	21.0	D16Mit3	MRL-lpr×(MRL-lpr×C3H-lpr)F1	dsDNA	MRL	102
Bah2	16	34.6	D16Mit58	(NOD×Ba)×NODBC	RBC (*M.bovis*)	BALB/c	5
nwa1	16	38.0	D16Mit5	(W×Ba)F1×W	Histone	NZW	339
nwa1	16	38.0	D16Mit5	(B×W)F1×W	GN/dsDNA	NZW	352
Bana1/Bah1	17	0.9	D17Mit24	(NOD×Ba)×NODBC	ANA/RBC (*M.bovis*)	NOD	5
sbb3	17	16.0	D17Mit198	(B6×Ba)F2-FcγRIIb$^{-/-}$	ANA/spleen (FcγRIIb ko)	BALB/c	125
Acla1	17	18.2	D17Mit16	W×(W×BXSB)F1	CL	NZW/BXSB	109
Sles1	17	18.8	H2/D17Mit34	(B6.NZMc1×W)F1×W	GN/dsDNA (resistance)	NZW	23
Pbat1	17	18.9	D17Nds2	W×(W×BXSB)F1	Platelet	NZW/BXSB	109
Wbw2	17	24.0	D17Mit177	(W×PL)F1×B	Mortality/GN	NZW	346
Agnz2	17	55.7	D17Mit130	(NZM2328×C57L)F1×NZM2328	Acute GN	C57L	342
Swrl3	18	20	D18Mit17	B×(SWR×B)F1	dsDNA/histone	SWR	340
—	18	22.0	D18Mit227	MRL-lpr×(MRL-lpr×C3H-lpr)F1	Sialadenitis	MRL	104
Lbw6	18	47.0	D18Mit8	BWF2	Mortality/GN	NZW	78
nwa2	19	41.0	D19Mit11	(W×Ba)F1×W	ssDNA	NZW	339
—	19	50.0	D19Mit3	(NZM×B6)×NZM	dsDNA	NZM	12

[a] Includes only named loci with linkages $P < 0.01$ or lod > 1.9. Loci are listed by their approximate chromosomal locations based on the marker with the highest association. Chr, chromosome. cM distances are based on the Mouse Genome informatics (Jackson Laboratory).

[b] B, NZB; B6, C57BL/6; B10, C57BL/10; Ba, BALB/c; CAST, CAST/Ei; lpr, *Fas^{lpr}*; NOD, NOD/Lt; NZM, NZM/Aeg2410; PL, PL/J; W, NZW; (MRL-lpr×C3H-lpr)BC and F_2, both MRL-lpr×(MRL-lpr×C3H-lpr)F_1 and (MRL-lpr×C3H-lpr)F_2 crosses; (NOD×Ba)×NODBC, NOD backcrossed to (NOD×BALB/c)F1 in all four combinations.

[c] Original phenotypes that mapped to loci are shown; chr, antichromatin autoantibody; CL, anticardiolipin autoantibody; dsDNA, anti-dsDNA autoantibody; GN, glomerulonephritis; gp70IC, gp70 immune complexes; histone, antihistone autoantibody; LN, lymphadenopathy; Lprn, lymphoproliferation; MI, myocardial infarct; platelet, antiplatelet autoAb and thrombocytopenia; RBC, anti-RBC autoAb; spleen, splenomegaly. Autoantibodies are IgG unless otherwise specified. Does not include gp70 loci that were not linked to autoimmunity [357]. Induced or genetically modified models are indicated in parentheses; *M.bovis*, *M. bovis* iv; HgIA, mercury-induced autoimmunity.

[d] complex inheritance: either parental strain promotes GN, but heterozygosity protects.

Transfer of the *Yaa* gene (Y chromosome) to nonautoimmune and autoimmune mice has demonstrated that it contributes additively to lupus, but is dependent on other background genes. Nonautoimmune strains, such as CBA/J or C57BL/6, are largely unaffected by the *Yaa* gene, whereas all lupus-susceptible strains examined, including NZB, NZW, and MRL-*Fas*^{lpr}, have accelerated disease that generally maintains the clinical characteristics of the background strain [1, 66–68]. Mice with mild lupus appear affected more by the *Yaa* gene than strains with already accelerated disease [7]. This selective augmentation by *Yaa* contrasts with the induction of generalized autoimmunity by the *lpr* and *gld* mutations [68] and suggests that different mechanisms are involved. This is further supported by the findings that *lpr* and *Yaa* congenic mice with identical backgrounds have different phenotypes [1] and that the *lpr* and *Yaa* mutations are additive [69]. Interestingly, DBA/1.*Yaa* (consomic for the BXSB Y chromosome) were less susceptible to collagen-induced arthritis (CIA) than wild-type DBA/1 mice, suggesting that *Yaa* plays different roles in CIA and lupus [70].

Regarding the mechanisms responsible for the *Yaa* phenotype, double bone marrow chimera experiments using a mixture of *Yaa*$^+$ and *Yaa*$^-$ cells of different IgH allotypes revealed selective production of anti-DNA antibody and hypergammaglobulinemia by *Yaa*$^+$ B cells [71]. The antibody-promoting effect of the *Yaa* gene is applicable not only to self-antigens, but to foreign antigens [72], and, analogous to the effects in lupus mice, enhancement was observed mainly for antigens eliciting low, T-cell-dependent antibody responses and not for those eliciting high antibody responses [72]. Accordingly, the *Yaa* gene was postulated to increase the expression of an "intercellular adhesion molecule" on B cells, which promotes low-avidity, T helper–B-cell interactions [65]. Thus, nontolerant T helper cells normally quiescent in *Yaa*$^-$ animals because of insufficient antigen presentation become activated in *Yaa*$^+$ animals. Other mechanisms that increase antigen presentation, intracellular signaling, and coreceptor molecule expression can also be postulated and elucidation undoubtedly will require identification of the molecular defect. The *Yaa* gene has been found not to accelerate murine AIDS [73]. This was attributed to the already generalized activation of most lymphocytes, including B cells, that accompanies this disease.

Other mixed bone marrow chimera experiments with *Yaa*$^+$ and *Yaa*$^-$ T cells that express different Thy-1 allotypes revealed that autoimmunity developed regardless of whether *Yaa*$^+$ cells were present or eliminated by anti-Thy-1 antibodies [74]. Furthermore, similar degrees of homeostatic T cell proliferation and disease induction were observed when αβT-cell-deficient BXSB mice were reconstituted with either *Yaa*$^+$ or *Yaa*$^-$ T cells [75]. Thus, these findings indicate that the *Yaa* defect does not require expression in T cells. That B cells, but not T cells, from Yaa$^+$ mice have enhanced proliferative responses compared with cells from Yaa$^-$ mice is also consistent with this possibility [76].

LOCI PREDISPOSING TO SPONTANEOUS SLE IN LUPUS MOUSE MODELS

Identification of genes predisposing to quantitative traits, such as those predisposing to lupus, typically involve four major steps: (1) trait mapping performed by genome-wide scans using evenly distributed markers spanning the chromosomes. (2) Generation of interval-specific congenic strains containing an introgressed genomic fragment to confirm mapping and identify the major intermediate phenotypes; a relatively large region is generally taken to assure that the specific gene or genes are present within the interval. (3) Generation of panels of congenics with crossovers or smaller intervals to finely map the location of the susceptibility gene or genes; this may be achieved in one or two stages, i.e., localization to ~5 cM-sized fragments and then to <0.1 cM-sized fragments [77]. (4) The final step requires cloning, sequencing, and identification of genes within the fragment. Candidate genes can then be selected based on expression, structure, function, or other characteristics for screening the parental strains for polymorphisms. The completed sequence and annotation of the mouse genome should expedite this effort greatly.

Genome-wide scans to map lupus-related traits have utilized a variety of crosses that have included the three major lupus-prone strains and several induced or genetically manipulated models. To date, at least 70 named loci linked to one or more lupus traits distributed over all 19 autosomal chromosomes have been identified. These and additional unnamed loci are listed by chromosome and chromosomal location (distance in centimorgans from the centromere) in Table 2. Some of these loci, identified by different groups, appear to be identical, whereas others appear to represent unique loci. Overall, data suggest that susceptibility to spontaneous lupus in these strains is more likely due to different specific sets of a few major loci than to the presence of a large number of common predisposing loci.

Loci Derived from NZB and NZW Crosses

NZB and NZW mice have been the most extensively studied of the lupus-prone strains. Genome-wide scans

of intercrosses, backcrosses, and crosses to normal background strains have resulted in the identification of loci contributing to one or more lupus-related traits on 16 autosomal chromosomes (Table 2). Interestingly, lupus traits were also mapped to the nonautoimmune strain for 12 of the 57 loci reported and include regions derived from BALB/c, SWR, C57BL/6, C57BL/10, and C57L mice. Whether these loci are due to susceptibility alleles in the normal strain or suppressor genes in the lupus strains will need to be defined by allele distribution, type of mutation, and function of the alleles. Importantly, non-MHC loci confirmed in more than one cross include *Sle1/Cgnz1* (NZW derived) and *Lbw7/Nba2* (NZB derived) on chromosome 1, *Lbw2/Sle2/nba1/Imh1/Mott/Spm1* (NZB derived) on chromosome 4, *Lbw5/Sle3* (NZW derived) on chromosome 7, *Lbw8* (NZB derived) on proximal chromosome 8, and *nwa1* (NZW derived) on chromosome 16. According to mapping studies, inheritance of lupus traits is multiplicative and depends on the number and specific combination of susceptibility loci (epistasis). This indicates that different sets of loci contribute to different traits, i.e., lymphoid hyperplasia, autoantibody production, GN, and mortality [23, 78–80].

Interval-specific congenic C57BL/6 (B6) mice have been used to further define the roles of *Sle1*, *Sle2*, and *Sle3* in lupus pathogenesis. These mice contain introgressed genomic fragments of chromosomes 1, 4, or 7, respectively, from the NZB/NZW-derived recombinant inbred strain, NZM/Aeg2410 [81, 82]. *Sle1* congenic mice (B6.NZMc1) developed elevated IgG antinuclear antibodies (particularly targeting H2A/H2B/DNA subnucleosomes), but no GN [83]. Adoptive bone marrow transfer studies using combinations of B6.NZMc1, wild-type B6, B-cell-deficient, and αβT-cell-deficient mice revealed that *Sle1* is functionally expressed in both B and T cells [84]. In contrast, B6.NZMc4 congenic mice exhibited generalized B-cell hyperactivity, B1 cell expansion, and elevated levels of polyclonal IgM, but no increased IgG antinuclear antibodies or GN [85]. It was hypothesized that the expanded B1-cell population, which expressed higher levels of costimulatory molecules such as B7, might promote autoimmunity by facilitating the presentation of self-antigens to T cells [86]. B6.NZMc7 congenic mice developed elevated, but low, levels of antinuclear antibodies and a low incidence of GN. That *Sle3* might promote generalized T-cell activation was suggested by the observation of a marked increase in activated T cells, elevated CD4:CD8 ratios, and resistance to activation-induced cell death [87]. Based on these initial findings, a simple additive model was proposed wherein specific gene alterations resulting in a loss of tolerance to nucleosome components (*Sle1*) combined with a B- and a T-cell defect (*Sle2*, *Sle3*, respectively) lead to the development of lupus in NZM/Aeg2410 mice [80]. A more complex inheritance of lupus traits, however, has been indicated by studies involving crosses of these congenic mice that demonstrate epistatic interactions and the presence of NZW-derived suppressor loci present in the NZM/Aeg2410 strain [23, 88–90]. For example, bicongenic B6.*Sle1/Sle3* mice develop severe lupus with accelerated GN and early mortality, whereas B6.*Sle2/Sle3* mice exhibit much milder lupus manifestations [90]. Furthermore, when combinations of the three *Sle* loci were examined, *Sle1* appeared necessary for the development of early mortality.

Fine mapping revealed that *Sle1* is composed of a cluster of at least three (*Sle1a-c*) subloci [91], each of which independently caused a loss of tolerance to chromatin and evoked distinct serologic and cellular characteristics. However, these subloci alone do not induce severe nephritis when combined with other *Sle* loci, suggesting the presence of yet another major locus within the *Sle1* interval (*Sle1d* inferred). Overall, these findings demonstrate the complexity within a single locus and raise the possibility that complex trait loci may represent the sum of effects arising from clusters of closely situated lupus-predisposing allelic variants.

The complement receptor 2 (*Cr2*) gene has been identified as a candidate gene for *Sle1c* [92]. In mice, the *Cr2* gene encodes both CR1 and CR2 glycoproteins through alternative splicing (reviewed in Carroll [21] and Fearon and Carroll [93]). CR1/CR2 are expressed predominantly on the surface of mature B cells and follicular dendritic cells and bind C3 and C4 degradation products that have bound to antigen or immune complexes. CR2 reduces the threshold of B-cell activation and has been implicated in B-cell apoptosis and antigen processing and presentation, particularly in the germinal center. *Cr2*-deficient mice are defective in IgG responses to T-cell-dependent antigens, the generation of memory B cells, and the formation of germinal centers. CR2 also plays a significant role in the presentation of self-antigens to establish tolerance and, in fact, *Cr2* deficiency combined with the Fas^{lpr} mutation results in accelerated and more severe disease [94, 95]. Lower levels of CR1 and CR2 have also been reported in SLE [96]. When the *Cr2* alleles of NZM/Aeg2410 (NZW) and B6 strains were compared, they were found to be highly polymorphic, with differences in 16 nucleotide residues, including 11 that resulted in amino acid changes and a 3-bp insertion [92]. Of interest was a C→A (His→Asn) mutation at residue 1342 located in the external domain short consensus repeat 7 (SCR7) of CR1 and SCR1 of CR2, which introduced a new functional N-linked glycosylation site within the ligand-binding domain. This mutation was associated with

reduced C3dg binding, diminished CR1/CR2-mediated signaling, and reduced IgG response to T-cell-dependent antigens. Based on the three-dimensional structure, it was hypothesized that glycosylation of the Asn at residue 1342 disrupted the function of CR2 by altering its ability to properly dimerize [92].

Another candidate gene, *Ifi202*, for the *Nba2* locus on chromosome 1 has also been identified by comparing microarray expression profiles of spleen cells from B6 and B6.*Nba2* congenic mice [97]. Remarkably, greater than a 10-fold increase in *Ifi202* and a decrease in *Ifi203* were the only differences out of 11,000 genes examined, and both are part of the Ifi200 cluster situated within the *Nba2* interval. Increased expression of Ifi202 at both RNA and protein levels was also documented in NZB spleen cells compared with NZW. Several polymorphisms were identified in the promotor region of the *Ifi202* gene; however, the specific alteration responsible for the expression difference was not determined. *Ifi202* is an interferon-inducible family of two genes (*Ifi202a* and *Ifi202b*) that has been implicated in the regulation of cell survival, proliferation, and differentiation [98]. Which Ifi202 gene is responsible for the increased expression in NZB mice, or whether both are involved, is not known, nor is how Ifi202 predisposes to the splenomegaly and autoantibody production observed in B6.*Nba2* mice [97].

Two other potential NZ candidate genes have been studied: FcγRIIb [99] (discussed in a later section) and CD22 [100]. The $CD22^a$ variant is present in NZW mice and is located within the *Sle5* and *Lbw5* intervals [100]. $CD22^a$ mRNAs have aberrant alternative splicing due to the insertion in the second intron of a 794-bp pair cluster of short interspersed nucleotide elements. This is associated with B-cell expression of CD22 being reduced after LPS stimulation to about half the level observed with the $CD22^b$ (B6 mice) allele. It should be noted that while CD22 is a candidate gene for *Sle5*, it is not a candidate for *Lbw5*, as NZB mice are also $CD22^a$.

Loci in MRL-*Fas*^{lpr} Crosses

It is known that the *Fas*^{lpr} mutation promotes loss of tolerance and autoimmunity, but the manifestations and severity of lupus-like disease are highly dependent on background susceptibility genes. Several groups have defined lupus-related quantitative trait loci (QTL) in crosses of MRL-*Fas*^{lpr} mice, a strain that develops particularly severe spontaneous accelerated systemic autoimmunity (Table 2). QTL for one or more lupus traits have been identified on 14 of the autosomal chromosomes. This large number of loci may be partially attributable to the different strains of crosses utilized, as well as the variety of traits assessed, such as sialadenitis, GN, and vasculitis, which are most likely caused by overlapping, but distinct, sets of susceptibility genes. Nevertheless, several loci on chromosomes 4 (Arvm1, Lprm1), 5 (*Lmb2*, *Lprm4*), 7 (*Lmb3*, *Ldrm1*), and 10 (*Lmb4*, *Asm1*) had overlapping intervals and may be identical [101–104]. Interestingly, another locus, *Lmb1* (chromosome 4), which mapped to the nonautoimmune B6 background, had an additive effect on lymphoproliferation equal to the other *Lmb* QTL [101], indicating that nonautoimmune mice can harbor *bona fide* susceptibility genes, but presumably in numbers and combinations insufficient for disease induction. Such genes undoubtedly account for the background effects observed when using different strain combinations to map QTLs.

CD72 is a C-type lectin superfamily member expressed on the surface of B cells [105]. It is a negative regulator of B-cell activation and plays a role in B-cell development. Of interest, the $CD72^c$ variant in MRL mice, derived from the LG/J strain, was suggested as a candidate for the *Arvm1* locus, particularly since the $CD72^c$ allele compared with the $CD72^b$ allele (C3H strain) has 13 amino acid substitutions that include acidic, basic, and neutral changes [106]. This, however, is very tentative, as functional studies were not done.

Loci in BXSB Crosses

It is known that the presence of the Y chromosome *Yaa* gene [43] in BXSB male mice leads to the development of severe accelerated lupus, but other background genes are also clearly important, as indicated by the lack of significant autoimmune responses in consomic nonautoimmune background CBA/J.BXSB-Y [66] or B6.BXSB-Y [67] mice. Genome-wide searches have identified 12 BXSB-derived loci encompassing eight chromosomes in backcrosses of BXSB to C57BL/10 (B10) or to NZW strains (Table 2). In reciprocal BXSB×B10 backcrosses, five QTL were found to be linked to antinuclear antibodies, lymphoproliferation, and/or GN, of which four were located in different regions of chromosome 1 and one on chromosome 3 [107, 108]. In contrast, a different set of BXSB-derived loci linked to other traits (anticardiolipin antibodies, antiplatelet antibodies, thrombocytopenia, and/or myocardial infarction) were identified on chromosomes 4, 7, 8, 14, and 17 in the BXSBxNZW backcross study [109]. These findings, taken together with studies in other crosses discussed earlier, clearly demonstrate that the clinical heterogeneity of lupus in spontaneous mouse models is primarily the result of specific combinations of susceptibility genes rather than being influenced by the environment.

Conclusions from Studies on Susceptibility Loci in Spontaneous Lupus Strains

A number of generalizations can be made in assessing the results of mapping studies using different configurations of crosses among the four different lupus-prone background strains. (1) Although each strain apparently contains a few major susceptibility loci, overlap of susceptibility genes among the various lupus-predisposed strains is minimal, indicating a potentially large pool of susceptibility genes. (2) Different sets of genes apparently govern predisposition to different traits within a strain, although some traits share common genes. (3) In general, the genetic contributions are additive, but can depend on specific combinations (epistasis). This suggests that some immunopathologic manifestations require the parallel engagement of multiple genetic defects at the same time, intimating incremental effects on a common pathway or mechanism to a threshold that must be exceeded before the development of autoimmunity. Therefore, therapeutic intervention aimed at only one of these altered alleles might have a more profound effect than expected from the total number of susceptibility genes. (4) The manifestation of highly penetrant component phenotypes in interval-specific congenic strains clearly provides the opportunity to clone the underlying genes.

SYSTEMIC AUTOIMMUNITY IN NORMAL BACKGROUND GENE KNOCKOUT/MUTATED AND TRANSGENIC MICE

The role of specific genes in the immune system and possible mechanisms of systemic autoimmunity are being defined through genetic manipulation of nonautoimmune background strains. Deletion or overexpression of single genes results in remarkable examples of tolerance loss and systemic autoimmunity that have yielded valuable new models to detail SLE immunopathogenesis. Although the relevance of many of these models to spontaneous disease is uncertain, they have been particularly important in molecular studies of potential mechanisms of autoimmunity. Thus far, these gene defects have been shown to enhance B- or T-cell activation, inhibit certain apoptotic pathways, alter antigen presentation, reduce complement clearance of apoptotic bodies or soluble self-antigen, modify cytokine milieu, alter cell signal transduction, reduce glycosylation, and enhance cell cycling, as well as other mechanisms (Tables 3 and 4). Common mechanisms derived independently from different molecular defects have also emerged. These studies involve only a fraction of the total number of immunologically relevant genes, but extrapolation of their results suggests the potential that a large number of genetic defects can result in systemic autoimmunity and also indicates that many of these defects may involve common pathways or mechanisms. The relevance of these gene defects induced in mice by genetic manipulation to spontaneous SLE remains to be determined and is an area of significant ongoing research.

B-Cell Activation Genes

The fate of B cells following antigen receptor (BCR) engagement is a complex process that involves direct or indirect interaction of the BCR with numerous molecules that can promote or inhibit cell activation. Among these are several tyrosine kinases (lyn, fyn, Btk, Blk, Syk), phosphatases (CD45, SHP-1, SHP-2, and SHIP), and accessory molecules (CD19, CD22, FcRγIIB). Genetic manipulation of many of these B-cell regulatory molecules has resulted in mice with lupus-like characteristics, e.g., gene knockouts of lyn [110, 111] or CD22 [112–114], and spontaneous mutations of SHP-1 in motheaten mice [56, 57]. Lyn is a nonreceptor scr-related tyrosine kinase required for CD22-mediated negative regulation of BCR signaling. Although lyn also plays a role in positive signaling, this function appears largely redundant, as lyn deficiency results in hypersensitivity to BCR-mediated triggering. Homozygous deletion of lyn leads to increased activation and higher turnover rates of B cells, splenomegaly, elevated IgM levels, autoantibodies, and GN. Homozygous deficiency of CD22, a B-cell-specific cell surface sialoadhesin that binds specificially to asialoglycoproteins, has similar results, i.e., hyperresponsiveness to BCR signaling, expansion of the peritoneal B1-cell population, and autoantibodies. This similarity between lyn and CD22 knockout phenotypes arises from the inhibitory action of CD22, which requires recruitment and phosphorylation of lyn, followed by subsequent recruitment of SHP-1 into close proximity to the BCR, where it can dephosphorylate the BCR and downregulate the response. Heterozygous deletions of CD22, lyn, and SHP-1 were shown to have additive effects on B-cell abnormalities, consistent with the contention that they are limiting elements to a common pathway [115].

The functional cell surface receptor complex for C3 fragments, which promotes BCR signal transduction and is crucial for B-cell development and tolerance, is composed of CD19, CD21, and Tapa-1 (CD81) on B cells. Mice overexpressing a CD19 transgene develop hyperresponsive B cells to BCR cross-linking, marked expansion of the B1-cell population, hypergammaglobulinemia, and autoantibodies [116]. Moreover, anti-

TABLE 3 Genes Associated with Lupus-Like Manifestations in Knockout/Mutated and Transgenic Normal Background Mice[a]

Name	Gene	Chr	cM	Major autoimmune manifestations[b]	Ref.
Knockout/mutated					
CTLA-4	*Cd152*	1	30.1	Multiorgan lymphoproliferative disease, myocarditis, pancreatitis	147–149
CD45 (protein tyrosine phosphatase, receptor type C	*Ptprc*	1	74.0	Lymphoproliferation, dsDNA, splenomegaly, GN	181
Fasl (spontaneous)	*Fasl*	1	85.0	Lymphoproliferation, DN T cells, auto-Abs, GN (gld mutation)	44
Serum amyloid P component	*Sap*	1	94.2	Antichromatin Ab, GN, female predominance	216
PD-1 (programmed cell death 1)	*Pdcd1*	1	ND[c]	Proliferative arthritis, GN, glomerular IgG3 deposits	184
Mannoside acetyl glucosaminyltransferase 5	*Mgat5*	1	ND	Proliferative GN, enhanced EAE	239
IL-2Rα	*Il2ra*	2	6.4	Lymphoproliferation, hyper-IgG, auto-Ab, anti-RBC Ab	157
Nrf2 (nuclear, factor, erythroid derived 2, like 2)	*Nfe2l2*	2	45.0	Hyper-IgG, anti-dsDNA Ab, GN, splenomegaly	250
TYRO3 protein tyrosine kinase 3 (Tyro 3 family)	*Tyro3*	2	67.1	Triple knockout (*Tyro3*, *Axl*, *Mer*): lymphoproliferation, increased activated T and B cells, auto-Ab, GN	208
c-mer protooncogene (Tyro 3 family)	*Mer*	2	ND	Auto-Ab (*Mer* knockout alone) (also see TYRO3)	212
Bim (Bcl2 interacting mediator of cell death, Bcl2-like 11)	*Bcl2l11*	2	ND	Lymphoid/myeloid cell accumulation, auto-Ab, GN, vasculitis	195
IL-2	*Il2*	3	19.2	Lymphoproliferation, hyper-IgG, auto-Ab, anti-RBC Ab	156
GADD45 (growth arrest and DNA damage-inducible 45 alpha)	*Gadd45a*	3	70.5	Auto-Ab, GN, mortality	187
lyn	*Lyn*	4	0.0	Enhanced B-cell activation, splenomegaly, hyper-IgM, auto-Ab, GN	110, 111
C1q	*C1qa*	4	66.1	Auto-Ab, GN	215, 358, 359
	C1qb	4	66.1		
	C1qc	4	66.1		
SHP-1 (spontaneous)	*Hcph*	6	60.22	Auto-Ab (*me* and *me*^{v} mutations)	56, 57
AXL receptor tyrosine kinase (Tyro3 family)	*Axl*	7	6.0	See TYRO3	
TGFβ1	*Tgfb1*	7	6.5	Multiorgan lymphocytic and monocytic infiltrates	164
CD22	*Cd22*	7	9.0	Enhanced B-cell activation, auto-Ab	112, 360
Zfp-36 (tristetraprolin)	*Zfp36*	7	10.2	Complex systemic disease: cachexia, dermatitis, arthritis	225, 227, 361
LAT (linker for activation of T cells)	*Lat*	7	ND (tel)	Mutation inhibits T-cell development, but induces lymphoproliferation	189
fyn (+lyn ko)	*Fyn*	10	25.0	synergizes with the *Lyn* ko to accelerate disease	362
Aiolos (zinc finger protein, subfamily 1A, 3)	*Znfn1a3*	11	ND	Activated B cells, increased IgG, auto-Ab	131
G protein-coupled receptor G2A	*G2a-pending*	12	ND	Lymphoid hyperplasia, hyper-IgG, auto-Ab, GN	191
Protein kinase Cδ	*Prkcd*	14	11.0	Splenomegaly, lymphadenopathy, , hyper-IgM/IgG1/IgG2a, dsDNA GN	139
IL-2Rβ	*Il2rb*	15	43.3	Lymphoproliferation, hyper-IgG, auto-Ab, anti-RBC Ab	158

(*continues*)

TABLE 3 (*continued*)

Name	Gene	Chr	cM	Major autoimmune manifestations[b]	Ref.
Dnase1	*Dnase1*	16	1.7	ANA, immune complex GN	242
Cbl-b	*Cblb*	16	ND	Multiorgan lymphoid infiltrates, anti-dsDNA Ab	175
p21 cyclin-dependent kinase inhibitor 1A	*Cdkn1a*	17	15.23	antichromatin Ab, GN, female predominance minimal to no disease	232 233, 234
Complement component 4	*C4*	17	18.8	Impaired immune complex clearance, ANA, GN, female predominance	363
α-Mannosidase II	*Man2a1*	17	ND	Hyper-IgG, auto-Ab, GN	238
Emk (ELKL motif kinase, Par-1)	*Emk*	19	3.0	Growth retardation, hypofertility, splenomegaly, lymphoid infiltrates, immune complex GN	246
Fas (spontaneous)	*Fas*	19	23.0	Lymphoproliferation, DN T cells, auto-Abs, GN, arthritis (*lpr* and *lpr^{cg}* mutations)	35
Pten (+/– mice)	*Pten*	19	24.5	Lymphadenopathy, auto-Ab, GN, decreased survival, female predominance	202
Transgenic					
FLIP (retrovirus-mediated expression)	*Cflar*	1	30.1	Hyper-IgG, auto-Abs, GN	206
Bcl-2 (B-cell promotor)	*Bcl2*	1	59.8	Lymphoid hyperplasia, hyper-IgG, auto-Ab, GN	196
CD19	*Cd19*	7	59.0	Increased B-cell activation, B1 cell population, IgG, auto-Ab	116
BAFF (α1-antitrypsin or β-actin promotor, BLyS, TALL-1, THANK)	*Tnfsf13b*	8	3.0	Auto-Ab (RF, CIC, dsDNA Ab), GN	137, 138
Fli-1 (class I promotor)	*Fli1*	9	16.0	Lymphoid hyperplasia, auto-Ab, GN	141
IFN-γ (keratin promotor)	*Ifng*	10	67.0	Auto-Ab, GN, female predominance	214
IL-4 (class I promotor)	*Il4*	11	29.0	Hyper-IgG1/IgE, auto-Ab, GN	218
IEX-1 (Ig μ enhancer)	*Ier3*	17	(Mhc)	Lymphoproliferation, auto-Ab, GN, skin lesions, arthritis	22
CD154/CD40L (B-cell or epidermis-specific promotor)	*Tnfsf5*	X	18.0	B-cell promotor: late-onset auto-Ab, GN epidermis promotor: dermatitis, lymphadenopathy, hyper-IgG, dsDNA, GN	142 213

[a] Genes are listed in order of their chromosomal locations. Gene names and chromosomal locations are from the Mouse Genome Informatics or Locus Link (www.ncbi.nlm.nih.gov/LocusLink/).
[b] Ab, antibodies; dsDNA, anti-dsDNA auto-Ab.
[c] Not determined.

HEL antibodies resulted from expression of the CD19 transgene in antihen egg lysozyme Ig (HEL-Ig)/soluble HEL double-transgenic mice, suggesting that the underlying mechanism might be a defect in anergy to certain soluble antigens [117]. Apparently, lowering the BCR signaling threshold alters the tolerance/immunity balance in both CD19 transgene and CD22/lyn/SHP-1 knockout mice, leading to the development of autoimmunity.

The FcγRIIb on B cells also inhibits B-cell antigen receptor signaling [118], primarily by recruiting, through its intracytoplasmic immunoreceptor tyrosine-based inhibitory motif, the inositol phosphatase SHIP rather than the phosphotyrosine phosphatase SHP-1 [119, 120]. Gene knockout of FcγRIIb amplifies humoral and anaphylactic responses [121] and promotes the development of lupus-like disease [122], as well as type II collagen-induced arthritis [123] and type IV collagen-induced Goodpasture's syndrome [124]. Interestingly, two deletions in the promotor region of FcγRIIb in NZB mice have been identified that are associated with lower levels of FcγRIIb expression in germinal centers and hypergammaglobulinemia [99]. This polymorphism maps to NZB loci on chromosome 1 linked to autoantibody production (*Lbw7* and *Nba2*, see later and Table 4), but whether this is the actual allele remains to be determined. Complementation studies revealed that the FcγRIIb knockout synergizes with both the *Yaa* gene and, to a lesser extent, the *Sle1* locus, but not with the *Fas^{lpr}* defect, which was actually protective [125]. Back-

TABLE 4 Mechanisms for Induction of Systemic Autoimmunity

Enhanced B-cell activation[a]
- Lyn, CD22 or SHP-1 knockout
- FcγRIIb knockout
- Aiolos knockout
- PKCδ knockout (tolerance pathway)
- CD19 transgenic
- Tnfsf13b (BAFF, BlyS, TALL-1, or THANK) transgenic
- Fli-1 transgenic
- CD40L transgenic (B-cell-specific expression)

Enchanced T-cell activation
- CTLA-4 knockout
- IL-2 or IL-2R knockout
- CD45 E613R knockin mutation (B cells as well)
- PD-1 knockout (probably B cells as well)
- TGF-β deficiency (knockout/dominant negative)
- Cbl-b knockout
- *Gadd45a* knockout
- LAT Y136F knockin mutation
- G protein-coupled receptor G2A knockout

Defective apoptosis
- Fas or FasL mutations (lpr and gld mice, ALPS in humans; also caspase 10)
- Bim knockout (not related to Fas)
- Bcl-2 transgenic
- Flip transgenic
- IEX-1 transgenic

Enhanced antigen presentation
- Tyro3 family (*Tyro3*, *Axl*, *Mer*) triple gene knockout
- CD40L transgenic (keratin-14 promotor)

Complement and related genes
- C1q knockout
- C4 knockout
- SAP knockout

Cytokine-mediated activation
- IL-4 transgenic
- IFN-γ transgenic
- TTP (Zfp-36) deficiency (excessive TNF-α)
- TNF-α transgenic

Defective signal transduction
- Cbl-b knockout (enhanced activation of T cells, possibly B cells)
- $Pten^{+/-}$ knockout (defective Fas from increased PIP-3 elevating Akt levels)

Unopposed cell cycling
- $p21^{cip1/waf1}$ knockout (cyclin kinase inhibitor, primarily T cells)

Glycosylation enzymes
- α-Mannosidase II knockout
- *Mgat5* knockout (enhanced T-cell activation)

Other Mechanisms
- Dnase1 knockout
- Emk knockout
- Nrf2 knockout (antioxidant)

[a] Genes are categorized according to the most likely or predominant mechanism.

ground genes are also important, as only FcγRIIb-deficient B6, and not BALB/c, mice develop lupus-like disease. Genome-wide analysis revealed three regions, designated *sbb1–3* on chromosomes 9, 12, and 17, that were linked to ANA and/or proteinuria [125] (Table 3). Interestingly, one of these loci, *sbb1*, was derived from the nonsusceptible BALB/c genome. Overall, these studies suggest that enhancement of BCR signaling, as shown by overexpression of CD19 and knockouts of CD22, lyn, SHP-1, or FcγRIIb, may be a potential pathogenic mechanism for lupus, and they further document the complex interactions among genetic variants that can influence disease susceptibility.

Aiolos, a zinc finger DNA-binding protein that shares a common GGGA core sequence-binding motif with the closely homologous nuclear factor Ikaros [126], is highly expressed in mature B cells and, to a lesser extent, in developing bone marrow B cells and thymocytes. Aiolos is mainly localized to the 2 MD chromatin remodeling complex in the T-cell nucleus that also contains Ikaros, the Mi-2 ATPase, histone deacetylases, and other components of the nucleosome remodeling histone deacetylase (NURD) complex [126]. NURD complexes have been suggested as participants in transcriptional repression by promoting DNA methylation and allowing repressors to gain access to chromatin [127–129]. Ikaros has also been found to interact with the mSin3 family of corepressors [130]. These findings have led to the hypothesis that Ikaros family members regulate gene expression during lymphocyte development by recruiting certain histone deacetylase complexes to specific promotors [130]. Mice with homozygous deletions of the Aiolos gene develop defects primarily in the B-cell compartment, and these defects include hyperreponsiveness to BCR and CD40 stimulation and increased numbers of conventional B cells, a marked reduction in B1 cells, increased proportion of B cells with activated phenotype, hypergammaglobulinemia (particularly of IgE and IgG1), a three-fold reduction in IgM, and positive ANAs in about half of the mice studied by 16 weeks of age [131]. T-cell abnormalities were limited to a slight increase in the proliferative capacity of thymocytes and mature T cells. Thus, Aiolos deficiency apparently facilitates B-cell entry into cell cycle and maturation to germinal center lymphocytes, as well as a breakdown of B-cell tolerance. Whether overt autoimmune disease develops in these mice is the subject of ongoing long-term studies [131].

Tnfsf13b (also called BAFF, BlyS, TALL-1, THANK, or zTNF4) is a newly identified member of the TNF ligand superfamily expressed primarily on cells of myeloid origin, such as monocytes and dendritic cells [132–135]. Both the transmembrane protein and a

secreted homotrimeric form, released by cleavage at a furin canonical motif in the stalk region, are active in the costimulation of B cells, the main cell type known to express its receptor. Two other members of the TNF family, TACI and BCMA, have been identified as receptors for Tnfsf13b [136]. Overexpression of *Tnfsf13b* in the liver (α_1-antitrypsin promotor with the APO E enhancer) [137] or in multiple tissues (β-actin promotor) [138] resulted in similarly elevated B-cell numbers and, to a lesser extent, T cells, as well as increases in activated bcl-2-expressing mature B cells, memory/effector phenotype T cells, and syndecan-1-positive plasma cells. B cells from transgenic mice survived longer in culture than wild-type controls, and these mice further developed a lupus-like disease characterized by elevated levels of all immunoglobulin subclasses, rheumatoid factor, anti-DNA antibodies, circulating immune complexes, and kidney immunoglobulin deposits with proteinuria and elevated blood urea nitrogen. Importantly, elevations in *Tnfsf13b* have been shown in both BWF_1 and MRL-*Fas*^{lpr} mice, and blocking *Tnfsf13b* function in the former with a soluble TACI-IgGFc fusion protein inhibited proteinuria and prolonged survival [136].

Protein kinase Cδ (PKC-δ) is expressed in its highest levels in developing pro- and pre-B cells and is involved in B-cell receptor signaling. Mice homozygous for the null mutation in the PKC-δ gene developed splenomegaly, lymphadenopathy, increased numbers of B2 cells, germinal center formation and IL-6 production, mild increases of IgM, IgG1, and Ig2a, and antinuclear and IgG1 anti-DNA autoantibodies, as well as GN [139, 140]. Further studies in PKC-δ-deleted mice transgenic for the hen egg lysozyme (HEL) Ig receptor exposed to soluble HEL (as artificial autoantigen) indicated that the absence of PKC-δ prevented B-cell tolerance and allowed maturation and terminal differentiation of self-reactive B cells [139]. Because a deficiency of PKC-δ did not affect BCR-mediated B-cell activation *in vitro* and *in vivo*, it was concluded that PKC-δ plays a selective and essential role in tolerogenic, but not immunogenic, B-cell responses.

Reported aberrant expression of a transgenic *ets* family protooncogene, Fli-1, under the control of the MHC class I promoter, resulted in a constellation of lupus-like manifestations that included lymphoid hyperplasia, hypergammaglobulinemia, elevated antinuclear antibodies, and severe immune complex GN [141]. Fli-$1^{-/-}$ B cells were hyperresponsive to a variety of stimuli, showed resistance to activation-induced cell death, and had prolonged survival compared to nontransgenic B cells. Although these findings suggest an important regulatory function for Fli-1 in B-cell response and homeostasis, a more limited role was indicated by the finding that immunological defects in Fli-1 knockout mice were confined to a mild generalized thymic hypocellularity.

Ectopic expression of a transgenic CD40L on B cells (VH promoter, IgH intron enhancer and Ig 3′ enhancer) was shown to result in enhanced polyclonal IgG, anti-dsDNA, and, in about half of mice, the development of immune complex GN [142]. CD40L is a member of the TNF ligand family, expressed mainly on activated T cells [143]. It interacts with CD40 on B cells and plays a pivotal role in promoting B-cell proliferation and survival. Of interest, previous studied have reported B cell expression of CD40L in patients with SLE [144, 145] and in BXSB mice [146].

T-Cell Activation Genes

Knockout deletion of specific genes that primarily effect T-cell function results in systemic autoimmunity (Tables 3 and 4). CTLA-4, a surface glycoprotein expressed exclusively on T cells, acts as an inhibitor of the CD28-B7.1/B7.2 costimulatory pathway in part by binding with higher affinity to B7.1 and B7.2. As a result, a multiorgan lymphoproliferative disease associated with an increased frequency of activated B and T cells, hypergammaglobulinemia, and early mortality develops in mice with homozygous deletion of CTLA-4 at 3–4 weeks of age, accompanied by severe myocarditis and pancreatitis [147–149]. Altered thymocyte development is not, however, the cause of this abnormal T-cell expansion and disease manifestations [150], which rather arises from a failure to maintain homeostasis in activated peripheral T cells, primarily the $CD4^+$ subset [151, 152]. The precise mechanism through which this occurs has yet to be determined [153].

A similar syndrome results in gene knockouts of IL-2 [154–156] or either of its high (IL-2Rα) [157] or low (IL-2Rβ) [158] affinity receptors and includes late immunosuppression with defective antibody and CTL responses, as well as lymphoproliferation, expansion of memory/effector phenotype T cells, polyclonal hypergammaglobulinemia, autoantibodies, and immune-mediated hemolytic anemia. Mice lacking IL-2 [159] or IL-2Rα [157], but not IL-2Rβ [88], develop inflammatory bowel disease similar to human ulcerative colitis. Autoantibody production depends on the expanded $CD4^+$ T cells [158] and CD40/CD40L interaction [154]. The resistance of IL-2-deficient T cells to activation-induced cell death, at least partly from decreased Fas-mediated apoptosis, is the cause of the accumulation of T lymphocytes [160]. Other studies in mice maintained under germ-free conditions demonstrated that colitis,

but not other autoimmune manifestations, requires exposure to environmental pathogens [161].

Soon after birth, TGF-β1 gene knockout mice rapidly develop massive necrotizing lymphocytic and monocytic infiltrates in multiple organs and die by 3 weeks of age [162, 163]. Serum IgG autoantibodies to nuclear antigens, as well as Ig glomerular deposits, are detectable, but they appear to play a minor role in overall disease severity [164]. Although deficiencies of either class II or class I (β2m) molecules combined with TGF-$\beta1^{-/-}$ reduced both tissue inflammation and autoimmunity, implicating both $CD4^+$ and $CD8^+$ T cells in these processes, in both instances there was only partial improvement in survival because of the remaining lethal myeloproliferative abnormalities [165, 166]. The pivotal role of TGF-β in maintaining T-cell homeostasis and suppressing autoimmunity was highlighted by studies directly inhibiting TGF-β1 in T cells by a dominant-negative TGFβ receptor type II transgene under the control of a modified CD4 promotor specific for $CD4^+$ and $CD8^+$ T cells. This resulted in sickness, wasting, and diarrhea around 3–4 months of age, monocytic infiltrates in multiple tissues, enlarged peripheral lymphoid organs, increased percentage of memory/effector phenotype T cells, hypergammaglobulinemia, autoantibodies, and glomerular immune complex deposits [167].

The cbl family of adaptor proteins functions predominantly to inhibit receptor and nonreceptor tyrosine kinases [168, 169]. Two members, cbl-b and cbl, share a complex structure consisting of an amino-terminal phosphotyrosine-binding (PTB) domain, a C3HC4 RING finger, a proline-rich region capable of binding proteins with SH3 domains, several phosphotyrosine residues for binding SH2 domains, a ubiquitin recognition sequence, and, at the carboxy terminal, a putative leucine zipper. A smaller third member (cbl-c or cbl-3) has been identified that contains the PTB, RING finger, and a truncated proline-rich region, but is missing the rest of the carboxy portion [170, 171]. It is thought that cbl proteins suppress signaling by directly inhibiting PTKs via several possible mechanisms: (1) binding to negative regulatory sequences or induction of conformational changes, (2) sequestering molecules critical to cell activation, by recruiting distinct negative regulators, or (3) ubiquitination, internalization, and degradation of receptor PTKs [168, 172, 173]. Cbl-b is expressed in normal and malignant mammary epithelial cells, a variety of normal tissues, and in hematopoietic tissues and cell lines. In accordance with their negative regulatory role, T cells from mice homozygous for the cbl-b gene knockout exhibit enhanced proliferation to antigen receptor signaling and do not require CD28 costimulation for IL-2 production or generation of T-dependent antibodies [174, 175]. Significantly, enhanced basal and activated levels of Vav, a guanine exchange factor for Rac-1/Rho/CDC42, was the only alteration in TCR signaling identified in these knockout mice. This was consistent with previous findings that cbl-b binds to Vav and, when overexpressed, inhibits Vav stimulation of the c-Jun terminal kinase [176]. Cbl-$b^{-/-}$ mice exhibit an increased susceptibility to experimental autoimmune encephalomyelitis [174] and to a spontanous generalized autoimmune disease [175] involving multiorgan lymphoaccumulation of polyclonal activated B and T cells with parenchymal damage, increased plasma cells, and antibodies to dsDNA by 6 months of age. Curiously, spontaneous autoimmunity occurred in only one [175] of the two Cbl-b knockout studies, suggesting that background strain, environment, or other factors are also important factors.

CD45 is a receptor-like transmembrane protein tyrosine phosphatase (RPTP) expressed on all nucleated hematopoietic cells and is required for signal transduction through antigen receptors [177–179]. The activity of this molecule can be regulated negatively subsequent to dimerization by binding of the catalytic site from one molecule to a structural wedge from the acidic residue containing the membane-proximal region of the other [180]. It was, therefore, predicted that mutation of the inhibitory structural wedge would lead to inappropriate RPTP activation under normal dimerizing conditions, thereby causing inappropriate src-kinase activation with potentially pathological consequences. Indeed, "knockin" mice in which a single critical glutamate (aa613) in the inhibitory structural wedge was mutated to arginine developed, later in life, a lymphoproliferative syndrome with polyclonal B- and T-cell activation, splenomegaly, lymphadenopathy, increased IL-10 and IFN-γ, and premature death [181].

PD-1 is a 55-kDa ITIM-containing transmembrane cell surface glycoprotein expressed on activated T and B lymphocyes and monocytic cells [182]. Upon engagement of its ligands PD-L1 or PD-L2, it transmits a negative signal. Clues to its function have been gained from gene knockout experiments in mice deficient for PD-1, which are found to develop moderate hyperplasia of lymphoid and myeloid cells, increases in several Ig isotypes (particularly IgG3), enhanced responses to IgM cross-linking, and alterations in peritoneal B1 cells [183]. Older (14-month-old) C57BL/6-PD-$1^{-/-}$ mice spontaneously develop GN and proliferative arthritis, but not elevated anti-dsDNA antibodies or rheumatoid factor [184]. Further acceleration of GN and arthritis occurs when the PD-1 deletion is combined with the *Fas*^{lpr} mutation. Interestingly, manifestations appear highly dependent on background genes, e.g., in contrast to C57BL/6 mice, BALB/c PD-$1^{-/-}$ mice reportedly

develop lethal pancreatitis with massive thrombosis, indicating that PD-1 deficiency may accelerate background predisposition to autoimmunity, similar to the *Fas*^{lpr} and Yaa mutations. While the mechanistic details have not yet been elucidated, it seems that PD-1 plays an important nonredundant role in maintaining lymphocyte and myeloid cell homeostasis following activation.

The Gadd45 (growth arrest and DNA damage-inducible gene) family is composed of three members, α, β, and γ, which play pivotal roles in replication, growth arrest, and apoptosis [185, 186]. Of these, Gadd45a is the only member regulated by p53 and is an important molecule in several processes, including maintenance of genomic stability, cell growth control, nucleotide excision repair, chromatin accessibility, and apoptosis. Several of these effects may be mediated by interactions of the Gadd45a with Cdc2, PCNA, and the MEKK4/MTK1 kinases [187]. Studies in Gadd45a-deficient mice have shown that this molecule is a negative regulator of T-cell proliferation induced by antigen receptor-mediated activation, and that deletion of the Gadd45a gene leads to the development of a lupus-like syndrome, particularly when coupled with deletion of the p21 cyclin-kinase inhibitor [187]. While disease in Gadd45a-deficient mice was more severe in females than males, equal severity was seen when mice were deficient for both Gadd45a and p21.

LAT (linker for activation of T cells) is a transmembrane scaffolding protein that, after TCR engagement, becomes tyrosine phosphorylated and recruits multiple signaling molecules important for T-cell activation [188]. The distal four tyrosine residues of LAT (Y132, Y171, Y191, and Y226) are required for its activity. "Knock-in" mutation of LAT in mice in which the Tyr^{136} position was mutated to phenylalanine showed a severe, but incomplete, block in T-cell development associated with a marked reduction in immature $CD4^+8^+$ and mature $CD4^+$ and $CD8^+$ thymocytes and splenocytes [189]. However, by 4 weeks of age, homozygous mutant mice exhibited lymphadenopathy and splenomegaly with elevated markers of activated polyclonal $CD4^+$ T cells, B cells, macrophages and eosinophils, lymphocytic infiltrates in various organs, high production of IL-4, hypergammaglobulinemia (especially IgE and IgG1), and autoantibodies to nuclear antigens [189, 190]. These phenomena were attributed to defective PLCγ1-calcium signaling pathway in early T-cell development, leading to inefficient negative selection of self-reactive T cells and their exportation to the periphery wherein activation of these cells may not depend on LAT or PLC-γ1 [189].

G2A is an orphan G protein-coupled receptor expressed in various tissues, including lymphoid tissues [191]. A variety of studies have suggested that G2A is a negative regulator of proliferation and integration of extracellular signals with cytoskeletal reorganization. Mice with targeted disruption of the gene encoding G2A showed a normal pattern of T and B lineage differentiation. As they aged, however, they developed secondary lymphoid organ enlargement, expansion of T and B cells, enhanced T-cell proliferation responses, and, when over 1 year of age, a progressive wasting syndrome, lymphocytic infiltration into various tissues, hypergammaglobulinemia, immune complex GN, and antinuclear autoantibodies [191]. Although the molecular basis underlying the hyperresponsiveness of $G2A^{-/-}$ lymphocytes to TCR stimulation is presently unknown, the results reinforce the concept that lower thresholds for TCR activation can lead to overt autoimmune disease. Such a lower threshold for activation coupled with defective Fas-mediated apoptosis has also been considered to be a major contributor to the MRL-*lpr* lymphadenopathy and spontaneous lupus-like disease [192].

Apoptosis Genes

Bim is a proapoptotic ligand of the Bcl-2 family that shares homology with other members in only the short (nine amino acid) BH3 motif [193]. Through this domain, Bim binds to antiapoptotic Bcl-2 molecules and blocks their function. Bim is largely bound to the cytoplasmic dynein light chain LC8 normally sequestered in the microtubule-associated dynein motor complex [194]. Certain apoptotic signals release LC8, allowing it, together with Bim, to translocate to Bcl-2 and inhibit its function. Homozygous knockout of Bim resulted in an incompletely penetrant embryonic-lethal phenotype, apparently for nonimmunologic reasons. In the surviving offspring, however, alterations in the homeostasis of multiple hematopoietic cell lineages developed [195]. As anticipated, Bim-deficient B and T lymphocytes were resistant to certain apoptosis-promoting signals, but not to FasL. The knockout mice were found to have lymphoid hyperplasia with increased naive T and B cells, altered thymocyte subset composition, and increased granulocytes and monocytes in the peripheral blood. With age, these mice developed systemic autoimmunity manifested by progressive lymphadenopathy and splenomegaly, dramatic expansion of plasma cells, hyper-IgM, -IgG, and -IgA, antinuclear antibodies, immune complex GN, and vasculitis; the survival rate at 1 year was 55%.

Transgenic expression of the bcl-2 gene in B cells under the immunoglobulin enhancer resulted in similar lymphoid hyperplasia, hypergammaglobulinemia, high titers of antinuclear antibodies, and immune complex

GN [196]. Current studies suggest that constitutive bcl-2 expression may promote autoimmunity by blocking the apoptosis of autoantibody-producing B cells that normally arise spontaneously in germinal centers during the primary response to foreign antigens [197–199].

Chromosome 10q23 is associated with a wide range of malignancies, and the protein/lipid phosphatase PTEN was initially identified as a tumor suppressor gene on this chromosome [200, 201]. Germline mutations of PTEN cause Cowden disease, Lhermitte–Duclos syndrome, and Bannayan–Zonana syndrome, all autosomal-dominant disorders. Homozygous knockouts of PTEN are embryonic lethal, but heterozygous mice develop an autoimmune disorder characterized by severe polyclonal lymphadenopathy, diffuse inflammatory cell infiltrates of most organs, hypergammaglobulinemia, anti-DNA antibodies, immune complex GN, and decreased survival [202]. Females were affected more severely (mortality at 12 months vs 15 months in males). Defective Fas-mediated activation-induced cell death of T and B lymphocytes was observed in PTEN-deficient mice due to impaired Fas signaling associated with increases in the survival factor Akt. This suggests that uninhibited increases in phosphatidylinositol (3,4,5)-triphosphate (PIP-3), the major substrate for PTEN [203], leads to the recruitment and activation of Akt and possibly other factors, which then inhibit Fas-mediated killing [202]. PTEN-deficient mice, however, in contrast to *Fas*^{lpr} mice, had no increases in either B220^{+} or CD4^{-}CD8^{-} DN T-cell populations and, further, PTEN heterozygous knockouts had more severe disease than nonautoimmune background Fas-deficient mice. Thus, the development of autoimmunity in PTEN-deficient mice cannot be completely accounted for by Fas deficiency.

FLIP (gene name *Cflar*, for caspase 8 and FADD-like apoptosis regulator, also termed I-FLICE, CASH, Casper, CLARP, FLAME, MRIT) is a death effector domain-containing protein that is structurally similar to the apoptosis-promoting CASP8 (FLICE), but lacks a caspase domain. In contrast to FLICE, FLIP inhibits death receptor-induced apoptosis by blocking CASP8 recruitment and activation [204, 205]. Bone marrow cells, transfected retrovirally with FLIP prior to transplantation, resulted in B and T cell resistance to activation-induced cell death and expansion of these cell populations. In 4- to 6-month-old animals, hypergammaglobulinemia, anti-dsDNA autoantibodies, and glomerular immunoglobulin deposits with histologic evidence of GN characterized by glomerular sclerosis and thickening of mesangium and basement membranes were observed [206]. There was no accumulation of B220^{+} or CD4^{-}CD8^{-} DN T cells, or activated T cells despite the fact that FLIP blocks Fas signaling of activation-induced cell death, suggesting that Fas and/or FLIP may affect other nonoverlapping pathways.

IEX-1 (immediate early response gene X-1), also named IER3, p22/PRG1, Dif-2, or mouse homology *gly96*, regulates cell growth and apoptosis. Its expression is regulated by a variety of factors, such as X-irradiation, ultraviolet radiation, steroids, growth factors, inflammatory stimuli, and various activators of Nf-κB/rel transcription factors [207]. Mice transgenic for IEX-1 [under an H-2k^{b} promoter at the 5′ end and an Ig heavy chain (μ) enhancer at the 3′ end for specific expression in lymphocytes] were found to exhibit decreased apoptosis of activated T cells, increased duration of an immune response effector phase, splenomegaly, lymphadenopathy, accumulation of activated T cells, increased polyclonal IgG2a and anti-dsDNA autoantibodies, alopecia, arthritis, and immune complex GN [22]. These findings are particularly intriguing considering that the *IEX-1* gene maps within the MHC locus of humans and mice.

Enhanced Antigen Presentation

Mutant triple knockout mice lacking a group of closely related receptor tyrosine kinases, Tyro3, Axl, and Mer (the Tyro3 family, TAM), have been reported to develop severe systemic autoimmunity characterized by splenomegaly, lymphadenopathy, increases in activated T and B cells, autoantibodies to phospholipids associated with thromboses and hemorrhage, autoantibodies to collagen and dsDNA, and deposition of immune complexes in tissues [208].

The Tyro3 family of proteins is not expressed by quiescent lymphocytes, but are expressed by many other cell types, including antigen-presenting cells (APCs) (macrophages, dendritic cells). Macrophages freshly isolated from TAM-deficient mice have an increased expression of MHC class II molecules and produce elevated amounts of IL-2 and IFN-γ, suggesting their activation *in vivo*. Expression of these molecules and of CD86 (B7.2) was increased by these cells as well as by CD11c^{+} cells subsequent to *in vitro* incubation with LPS. Moreover, CFSE-labeled B and T cells transferred to TAM-deficient mice hyperproliferated compared to cells transferred in the wild-type controls. Although Tyro-3 receptors are known to convey growth-promoting and prosurvival signals to cells [209], findings with mutant mice suggest that these receptors are also able to activate negative feedback loops that attenuate the positive signaling pathways that they have activated, and that the loss of negative signals from these receptors has more profound effects than the loss of the positive growth-promoting signals. Another means by which absence of these receptors may promote auto-

immunity is by the ineffective removal of apoptotic cells, which are increased in TAM-deficient mice [210]. This latter possibility has been supported by the finding that mice with a cytoplasmic truncation of *mer* have macrophages deficient in the clearance of apoptotic thymocytes and develop a mild form of lupus-like disease with antibodies to chromatin [211, 212].

Targeted expression of CD40L to basal keratinocytes of the epidermis of mice using the keratin-14 promoter was reported to lead to the activation of resident tissue APCs (Langerhans cells) associated with dermatitis, lymphadenopathy, hypergammaglobulinemia, anti-dsDNA autoantibodies, and immune complex GN [213]. These findings are reminiscent of those observed in mice transgenic for IFN-γ under the involurin promoter [214]. Overall, they indicate that *in situ* activation of APCs in the skin can lead not only to local but also systemic, autoimmune, and inflammatory responses, presumably due to the migration of activated APC to the secondary lymphoid organs, and the engagement of preexisting, quiescent, nontolerant self-reactive T cells.

Complement Genes

Deficiencies of early complement components (C1q-s, C2, or C4) in humans are known to result in predisposition to SLE, indicating an important regulatory role in suppressing autoimmunity. This loss of tolerance to nuclear antigens cannot be fully explained by the inadequate clearance of immune complexes, and gene knockout mice for C1q and C4 were therefore generated to study this issue. Homozygous C1q-deficient mice recapitulated the human disorder with the development of mild, but typical, features of lupus, including autoantibodies and a 25% incidence of immune complex GN [215]. Furthermore, an atypical accumulation of apoptotic bodies in the glomeruli of C1q-deficient mice was observed, suggesting that C1q plays an essential role in the clearance of apoptosis by-products.

In C4 deficiency, the contribution to lupus susceptibility was evidenced only on the *Fas*^{lpr} background [94], indicating that lack of C4 alone cannot induce autoimmunity. B cells from C4-deficient mice were shown to have a tolerance defect to soluble HEL in experiments using a HEL Ig transgenic model, giving rise to the hypothesis that maintaining self-tolerance is crucially affected by the presentation of tolerizing antigens to B cells by early complement components.

This mechanism may be applicable to the unexpected development of lupus-like autoimmunity in mice homozygous for the deletion of serum amyloid P component (SAP), a highly conserved plasma protein originally named for its presence in amyloid deposits [216, 217]. In these mice, lupus manifestations included a clear female predominance of disease, autoantibodies to chromatin and its components (although not to other nuclear, tissue, or organ antigens), immune complex GN, and low mortality. In contrast to C1q deficiency, no accumulation of apoptotic bodies was detected in glomeruli. SAP binds to DNA and chromatin and can displace H1 histones, thereby increasing solubility and reducing the rate of degradation and clearance of chromatin [216]. It was hypothesized that SAP promotes self-tolerance to chromatin and its subunits by preventing immunogenic antigen processing and/or by tolerizing chromatin-reactive lymphocytes.

Cytokine Ligand and Receptor Genes

Systemic autoimmunity can also develop in mice transgenic for the major Th1 and Th2 cytokines, IFN-γ and IL-4, respectively, given the appropriate circumstances. Expression of IFN-γ in the suprabasal layer of the epidermis under the control of the involucrin promotor resulted not only in a severe inflammatory skin disorder, but also in the production of autoantibodies to dsDNA and histone, and an immune complex proliferative GN, particularly in females [214]. This suggests that presentation of nuclear antigen by skin Langerhans cells, and perhaps keratinocytes, may be sufficient for the production of antinuclear autoantibodies, which may explain the ultraviolet sensitivity of SLE patients. Similarly, C3H mice transgenic for the IL-4 gene under the control of the MHC class I promotor also developed systemic autoimmunity characterized by elevated MHC class II molecules and CD23, enhanced responses to polyclonal stimuli *in vitro*, increased levels of IgG1 and IgE, anemia, antinuclear antibodies, and immune complex GN [218]. It is thought that these manifestations result from direct IL-4-induced polyclonal activation of B cells, as $CD4^+$ T cells were not required and there was no evidence of inefficient B-cell negative selection. The role of IL-4 in promoting lupus, however, is more complicated, as autoimmunity was not observed in other transgenics expressing IL-4 on B or T cells [219–222] and in a spontaneous model of lupus expression of an IL-4 transgene did not exacerbate disease, but was protective [223]. This would imply that a number of factors, such as the level and site of IL-4 production and background genetic susceptibility, can modify the effects of IL-4 on systemic autoimmunity.

Tristetraprolin (TTP or Zfp-36) is a widely expressed zinc-binding protein initially throught to function as a transcription factor, particularly in lymphoid tissues, where high levels are found [224]. Mice with a homozygous knockout of TTP develop a complex syndrome consisting of cachexia, patchy alopecia, dermatitis, conjunctivitis, erosive arthritis, myeloid hyperplasia,

glomerular mesangial thickening, and antinuclear antibodies [225]. These manifestations are primarily the result of excessive TNF-α production by macrophages and are almost entirely reversed by anti-TNF-α antibody [225, 226], suggesting that TTP functions as a nonredundant negative regulator of TNF-α. The mechanism was later discovered to be the result of TTP binding to an AU-rich element in the TNF-α mRNA, which destabilizes the mRNA [227]. In contrast to the autoimmune-promoting effects of elevated levels of TNF-α, physiological amounts may, under certain circumstances, play a role in suppressing systemic autoimmunity. In studies of TNFR1 (p55) knockout mice, nonautoimmune mice did not develop defects in apoptosis or autoimmunity [228, 229], yet the same knockout in lupus-prone C57BL/6-*Fas*^{lpr} mice resulted in accelerated lymphoproliferation and autoimmune disease [230]. These findings may be attributable to TNF compensation for the lack of Fas in *Fas*^{lpr} mice, as TNF can induce the death of activated peripheral T cells [231].

Cell Cycle-Related Genes

Systemic autoimmunity was also reported to occur in mixed background C57BL/6 (B6) × 129/Sv mice deficient for the cyclin inhibitor p21$^{cip1/waf1}$ [232]. Disease was characterized by lymphoid hyperplasia, elevated IgG1, IgG antinuclear antibodies, GN, and early mortality. *In vitro* T-cell proliferation was enhanced in these mice, as was the accumulation of effector/memory phenotype (CD44^{high}) CD4$^+$ T cells, although levels of other activation/effector/memory cell markers, such CD25, CD62L, CD69, and CD45RB, were similar to wild-type mice. An increased proportion of splenic B cells also expressed an activated (HSA^{low}, IgG^{low}) phenotype. Interestingly, in this initial study, females were reported to have more severe disease than males. Based on these findings, it was suggested that p21 negatively regulates T-cell proliferation following long-term stimulation, as is presumably the situation for autoantigen-reactive CD4$^+$ T cells.

In contrast, another study of p21-deficient mixed background B6 × 129/Sv mice found increased *in vitro* and *in vivo* T-cell cycling and activation, enhanced homeostatic proliferation, moderate hypergammaglobulinemia, and low penetrant antichromatin autoantibodies, but only mild lymphoid hyperplasia, minimal immune complex GN, and no gender differences [233]. Furthermore, transfer of the p21 null mutation onto the BXSB background did not lead to accelerated disease in the lupus-prone females [234]. Importantly and relevant to most of the studies in this section, use of the mixed background 129/Sv × B6 to study the effects of specific genetic alterations may be confounded by the genetic heterogeneity of the host. It is therefore imperative that adequate controls, such as littermate controls or congenic strains with late-life lupus predisposition, should be employed.

Of interest, other studies in BXSB mice found increased p21 and other cyclin inhibitors in the expanded population of memory/effector CD4$^+$ T cells that are predominantly arrested in G1 [235]. This finding led to the opposite hypothesis that accumulating CD4$^+$ T cells are rendered incapable of entering the cell cycle after successive rounds of division and are resistant to apoptosis because of the buildup of cyclin inhibitors, a state similar to replicative senescence. Although no longer cycling, such cells may nonetheless secrete cytokines and activate B cells. Indeed p21-deficient male BXSB mice have reduced numbers of memory/effector CD4$^+$ T cells along with decreased autoantibody levels, GN, and mortality (Lawson, *et al*. unpublished observations).

Glycosylation Enzymes

The α-mannosidase II enzyme is encoded by a single gene in mammals and resides in the Golgi apparatus, where it trims two mannose residues from hybrid N-linked oligosaccharides. This trimming of the mannose residues allows the subsequent addition of multiple glycan branches by glycosyltransferases required for the generation of complex N-glycans found on mammalian cell surfaces [236, 237].

Mice rendered deficient in α-mannosidase II were reported to develop a lupus-like syndrome characterized by various autoantibodies, including anti-dsDNA, anti-Sm, and anti-histone, as well as hypergammaglobulinemia and immune complex GN [238]. Nevertheless, lymphoid cell development, composition, and responses were normal. No clear explanation for the appearance of systemic autoimmunity was evident, but a possibility is that alterations in N-glycan branching among some glycoproteins and tissues may lead to the formation of neoepitopes for which tolerance has not been established.

A related finding with a more clear explanation was the appearance of proliferative GN in mice deleted of the β1,6 *N*-acetylglucosaminyltransferase V (Mgat5), an enzyme in the N-glycosylation pathway [239]. An important mechanistic finding in this work was that absence of this enzyme led to lowering of the T-cell activation threshold by enhancing TCR clustering. Thus, T cells of deficient mice showed increased recruitment of TCRs to anti-CD3ε antibody-coated polystyrene beads and required lower concentration of anti-CD3 plus anti-

CD28 for efficient proliferation compared to control cells. Additional experiments showed that Mgat5 initiates GlcNAc β1,6 branching on N-glycans, thereby increasing *N*-acetylactosamine, the ligand for galectins, which are proteins known to modulate T-cell proliferation and apoptosis [239–241]. The findings indicate that a galectin–glycoprotein lattice strengthened by Mgat5-modified glycans restricts TCR recruitment to the site of antigen presentation, and therefore dysregulation of Mgat5 increases T-cell activation and susceptibility to autoimmunity.

Miscellaneous

DNase1 is a 32- to 38-kDa protein that is the major nuclease present in the blood, urine, and secretions. Interestingly, knockout of the DNase1 gene in nonautoimmune background mice has been reported to increase the incidence of SLE manifestations, including positive ANA, anti-DNA antibodies, and immune complex GN [242]. Although this suggests that accumulation of self-DNA may trigger the loss of tolerance and lupus, the precise mechanism is not known and other possibilities, such as a reduced degradation of exogenous nonmethylated CpG-rich DNA from pathogens, might also play a role. This observation is somewhat unexpected, as mammalian DNA alone is a poor immunogen for generating anti-DNA antibodies, and cell transfer studies in mice have suggested that genetic susceptibility to lupus is expressed in bone marrow-derived cells [1]. Nevertheless, reduced DNase1 activity, as observed in sera of lupus patients [243], may contribute to overall SLE susceptibility. Interestingly, an identical heterozygous nonsense mutation in *DNASE1* was detected in two SLE patients; however, the significance of this has yet to be determined [243].

ELKL motif kinase (Emk) is a serine/threonine kinase with a conserved region of about 100 amino acids that terminates with the sequence glutamate-leucine-lysine/asparagine-leucine (a region referred to as the ELKL domain) [244]. Emk has also been called MARK2 (microtubule affinity-regulating kinase 2) and mPar-1 based on homology with the Par-1 protein kinase of *Caenorhabditis elegans* [245]. Evidence suggests that Emk regulates cell polarity, cell cycle progression, and microtubule dynamics. It is expressed in several tissues, including the thymus and mature T and B cells [246]. $Emk^{-/-}$ mice showed growth retardation and hypofertility [246, 247]. In addition, deficient mice, as they aged, showed splenomegaly, lymphadenopathy, increased activated phenotype T cells ($CD44^{hi}CD62^{low}$, but no increases in IL-2R or CD69), increased responses to thymus-dependent antigens, lymphoid infiltrates in lungs, salivary glands, and kidneys, and membranoproliferative GN with immune deposits and proteinuria [246].

The basic leucine zipper transcription factor Nrf2 (NF-E2-related factor 2, or bZip-type transcription factor or NF-E2, nuclear factor-erythroid 2) regulates an antioxidant response element (ARE) or electrophile response element (EpRE), whose consensus sequence is found in the 5′ regulatory region of a number of genes encoding detoxifying and antioxidant enzymes [248, 249]. Nrf-2-deficient female mice over 5 months of age reportedly developed severe GN with immune complex deposits together with higher serum IgG, anti-dsDNA antibody, and slight splenomegaly [250]. In this regard, it is of interest that reactive oxygen species have been considered to be potential participants in the pathogenesis of lupus nephritis [251]. Moreover, homozygosity for glutathione-*S*-transferase *GSTM1* null alleles correlated with autoantibodies and lupus predisposition in humans [251] and mice deficient in heme-oxygenase-1 (HO-1) (a gene containing ARE) were noted to develop GN resembling that of the Nrf-2-deficient mice [252].

GENE KNOCKOUT AND TRANSGENIC LUPUS BACKGROUND MICE

Many investigators have directly examined the role of deleted or overexpressed immune-related genes in systemic autoimmunity in congenic lupus background mice (Table 5, listed by chromosome and chromosomal location). The effects of gene manipulation on different stages of the disease pathogenesis have provided important information on the crucial molecules and pathways, as well as a deeper understanding of the molecular basis for the diverse manifestations.

B-Cell-Related Genes

Studies in MRL-Fas^{lpr} mice with deletion of the Jh locus (no B cells) [253] have clearly shown the crucial role of B cells in lupus disease manifestations; despite the presence of lymphoproliferation, there were no signs of nephritis or vasculitis, confirming the theory that autoantibodies are central to these processes. Similar observations were made in Fas-intact MRL-+/+ mice deficient for the Jh locus [254], indicating that B cells are important not only for autoantibody production, but also for the spontaneous activation of $CD4^+$ and $CD8^+$ T cells [255, 256]. Moreover, genetic manipulation of MRL-Fas^{lpr} mice resulting in B-cell expression of surface, but not secreted immunoglobulin,

TABLE 5 Effects of Genetic Manipulation of Lupus-Prone Mice

Name and alteration	Gene[a]	Chr	cM	Strain[b]	Result[c]	Ref.
CD28 ko	*Cd28*	1	30.1	MRL-*lpr*	Reduced GN	274
IL-10 ko	*Il10*	1	69.9	MRL-*lpr*	Enhanced lymphoproliferation, IgG2a Auto-Ab, GN, skin lesions, mortality	297
CD45 +/– ko	*Ptprc*	1	74.0	C3H/HeJ-*gld*	Reduced IgG, auto-Ab, DN T cells	306
FcR γ-chain ko	*Fcer1g*	1	93.3	BWF1	Same auto-Ab, glom. dep., but reduced GN, mortality	316
CD21/CD35 ko	*Cr2*	1	106.6	*lpr*	Accelerated disease	21, 94, 95
soluble Crry Tg (complement receptor-related protein)	*Crry*	1	106.6	MRL-*lpr*	Reduced GN, no effect on glom. dep., auto-Ab, lymphoproliferation	311
β2-Microglobulin ko (MHC class I ko)	*β2m*	2	69.0	MRL-*lpr* C3H-*lpr* and -*gld* NZB MRL-*lpr*	Reduced lymphoproliferation Reduced lymphoproliferation Reduced anti-RBC Ab Inhibits nephritis, accelerates skin disease	261, 262 260 266 264
CD1 ko	*Cd1*	3	48.0	MRL-*lpr*	No effect on disease	264
osteopontin ko (Eta-1, secreted phosphoprotein 1)	*Spp1*	5	56.0	B6-*lpr*	Reduced polyclonal and auto-Ab, delayed lymphoproliferation and kidney disease	303
CD8 ko	*Cd8a* *Cd8b*	6 6	30.5 30.5	MRL-*lpr*	Reduced lymphoproliferation	259
5-Lipoxygenase ko	*Alox5*	6	53.2	MRL-*lpr*	Slight accelerated mortality (males only)	364
CD4 ko	*Cd4*	6	60.18	MRL-*lpr*	Increased lymphoproliferation; decreased auto-Ab, GN, mortality	259, 365
ICAM-1 ko	*Icam1*	9	7.0	MRL-*lpr* MRL-*lpr*	Reduced mortality, auto-Ab, GN, vasculitis reduced pulmonary inflammation and mortality, but same auto-Ab, lymphoproliferation and GN	314 313
IFN-γR ko	*Ifngr* *Ifngr2*	10 16	15.0 65.0	MRL-*lpr* BWF1	Reduced GN Reduced GN, mortality	278, 282 280
fyn ko	*Fyn*	10	25.0	MRL-*lpr*	Reduced DN T cells, auto-Ab, GN	305
Perforin ko	*Pfp*	10	36.0	MRL-*lpr*	Accelerated disease	271
IFN-γ ko	*Ifng*	10	67.0	(MRL-*lpr*)F2 MRL-*lpr* HgIA	Reduced GN, mortality, auto-Ab Reduced GN, mortality, auto-Ab Reduced auto-Ab, GN	277 279 281
IL-12b (p40) Tg (antagonist)	*Il12b*	11	19.0	MRL-*lpr-cg*	Reduced auto-Ab, slightly reduced GN and survival	290
IL-4 ko	*Il4*	11	29.0	MRL-*lpr*F2 BXSB	Reduced GN No effect	277 293
Nitric oxid synthetase 2 ko	*Nos2*	11	45.6	MRL-*lpr* (N4)	Same auto-Ab, GN, arthritis Reduced vasculitis	315 366
MCP-1 ko (small inducible cytokine A2)	*Scya2*	11	46.5	MRL-*lpr*	Reduced GN, mortality; same auto-Ab and glom. dep.	318
T-box 21 ko (T-bet)	*Tbx21*	11	ND[d]	MRL-*lpr*	Reduced morality, GN, auto-Ab, hyper-IgG	285
Jh (no B cells)	*Igh-J*	12	58.0	MRL-*lpr*	No disease	253, 254
Jh, mIg Tg (B cells with mIg, no Abs)		12		MRL-*lpr*	Infiltrate, no GN	257
TCRαδ ko (no $\alpha\beta^+$ or $\gamma\delta^+$ T cells)		14	19		No IgG auto-Ab, GN	367
TCRδ ko (no $\gamma\delta^+$ cells)	*Tcrd*	14	19.5		Disease acceleration	368
TCRα ko (no $\alpha\beta^+$ cells)	*Tcra*	14	19.7	MRL-*lpr* BXSB	Major disease reduction Marked disease reduction	268, 369 75
B7.2 ko	*Cd86*	16	26.9	MRL-*lpr*	Reduced GN	275

(*continues*)

TABLE 5 (*continued*)

Name and alteration	Gene[a]	Chr	cM	Strain[b]	Result[c]	Ref.
B7.1 ko	*Cd80*	16	28.0	MRL-*lpr*	More severe, distinct GN	275
B7.1/B7.2 double ko	*Cd80/86*	16		MRL-*lpr*	Marked disease reduction	370
MHC class II ko	*H2-Aa* *H2-Ea*	17	18.65	MRL-*lpr*	Reduced auto-Ab, GN, same lymphoproliferation	258
Human DR Tg	*H-2*	17	18.65	NZM/Aeg2410	Auto-Ab repertoire alterations	371
Complement C4 ko	*C4*	17	18.8	MRL-*lpr*	Accelerated disease	94, 310
Complement factor B ko	*H2-Bf*	17	18.85	MRL-*lpr*	Reduced auto-Ab, GN, vasculitis	307
Tnf +/– ko	*Tnf*	17	19.07	NZBxB6.129$^{Tnf/o}$)F1	Enhanced disease	372
C3 ko	*C3*	17	34.3	MRL-*lpr*	Worse proteinuria and glom. dep., but same GN score, auto-Ab and CIC	309
				129 × MRL-*lpr/C4 ko*	No difference vs. 129×MRL-*lpr/C4 ko*	310
TdT ko	*Tdt*	19	39.5	BWF1	Same auto-Ab, reduced GN and mortality	373
				MRL-*lpr*	Reduced hyper-IgG auto-Ab, GN, mortality, vasculitis	374
CD40L ko	*Tnfsf5*	X	18.0	MRL-*lpr*	Reduced auto-Ab, GN	273
Protein kinase CK2 α ko (casein kinase II) (transgenic)	*Csnk2a1* *Csnk2a2* *Csnk2b*	ND 8 17	— 50.0 19.02	MRL-*lpr*	Accelerated lymphoproliferation, auto-Ab, GN	375

[a] Genes are listed by their approximate chromosomal locations. Genes are deficiencies by homologous recombinant knockout unless otherwise stated.
[b] MRL-*lpr*F_2, mixed background derived from (MRL-*Fas*^{lpr} × (B6 × 129)F1)F2; *lpr*, *Fas*^{lpr}, *gld*, *Fasl*^{gld}.
[c] Antibody, Ab, glom. dep., glomerular IgG deposits.
[d] Not determined.

demonstrated that B cells alone could promote local cellular infiltration and inflammation, but not GN [257].

T-Cell-Related Genes

A wide diversity of intercross or congenic mice rendered defective for MHC, CD4, CD8, or T-cell receptor genes have been used to assess the role of helper and cytotoxic T cells and αβ- and γδT-cell subsets in lupus. In MRL-*Fas*^{lpr} mice, deletion of MHC class II [258] or CD4 [259] reduced autoantibodies and GN, but had no effect on lymphadenopathy, indicating that the class II-selected CD4 T cells are important for autoimmunity, whereas DN B220$^+$ T cells are not selected on class II molecules and are not necessary for autoimmunity to occur. In contrast, β2m- (class I) [260–263] or CD8-deficient [259] MRL-*Fas*^{lpr} or C3H-*Fas*^{lpr} mice showed reduced lymphoproliferation and expansion of DN B220$^+$ cells, but only partial diminution in autoantibody levels and GN. Combined with previous studies on TCR Vβ repertoires and anti-CD8 antibody treatment (reviewed in Theofilopoulos and Kono [2]), these findings indicate that DN B220$^+$ T cells in *Fas*-deficient mice are of CD8 origin.

Of interest, MRL-*Fas*^{lpr} mice deficient in β2m were shown to be discordant in skin and kidney disease, with the former accelerated and the latter ameliorated [264]. The skin disease was not accelerated in CD1-deficient MRL-*Fas*^{lpr} mice, thereby suggesting that the effect of β2m deletion (affecting both classical MHC class I as well as CD1) in this manifestation was not mediated by NK T cells that depend on CD1 or by CD1 expression itself on skin Langerhans or B cells. CD1 deficiency in itself also appears not to affect disease severity, including nephritis and vasculitis. The overall findings suggest that end organ disease is not an inevitable consequence of initial autoreactive cell activation in secondary lymphoid organs, but local conditions in target organs can control disease manifestations. In addition, a delayed onset and reduced incidence of anti-RBC antibodies were found in NZB mice deficient for CD4 [265] and even for β2m, despite the fact that in the littermate mice, the characteristic NZB thymic abnormalities and IgM or IgG autoantibody levels to ss or dsDNA remained unchanged [266].

Although the combined results clearly demonstrate the importance of TCRαβ cells in spontaneous lupus, TCRα gene deletion in MRL-*Fas*^{lpr} mice only partially

inhibited disease [267, 268]. Based on the fact that TCRαβ/TCRγδ double-knockout MRL-*Fas*^{lpr} mice fail to generate class-switched autoantibodies and immune complex GN [269, 270], this suggests that TCRγδ cells can also help drive the autoimmune B cells. Paradoxically, however, MRL-*Fas*^{lpr} TCRγδ-deficient mice showed disease exacerbation. Taken together, these results suggest that TCRαβ cells are important for cognate MHC-restricted autoantibody production and are the major provider of B-cell help in intact MRL-*Fas*^{lpr} mice, whereas TCRγδ cells suppress this process, but can concomitantly provide lesser degrees of non-MHC-restricted polyclonal B-cell help. In addition, knockout of the perforin gene in MRL-*Fas*^{lpr} mice resulted in disease exacerbation, suggesting that cytolytic cells may be involved in suppressing autoreactivity [271].

Costimulatory Molecules

Studies in MRL-*Fas*^{lpr} mice deficient for CD40L [272, 273] and CD28 [274] have clearly demonstrated the role of costimulation in lupus development, and the reduced disease progression was similar to that observed in BXSB and BWF1 mice treated with anti-CD40L or CTLA-4Ig (reviewed in Theofilopoulos and Kono [2]). MRL-*Fas*^{lpr} mice deficient for either B7.1 or B7.2 were used to further dissect the CD28-B7 axis [275]. Although neither of these deletions affected autoantibody levels compared to wild-type MRL-*Fas*^{lpr} mice, GN was substantially worse in B7.1-deleted mice, but less severe in B7.2 knockouts, consistent with findings in BWF1 mice showing that antibodies to B7.2, but not B7.1, suppressed disease [276]. Thus, B7.2 clearly plays a crucial role in lupus pathogenesis.

Cytokine Genes

Congenic cytokine knockout or transgenic lupus mice were used to investigate the effects of Th1 and Th2 cytokines, including IFN-γ, IL-12, IL-10, and IL-4. The results obtained in IFN-γ knockout mice clearly contradict the earlier notion that Th1-type responses primarily promote cellular immunity, whereas Th2-type responses promote mainly humoral immunity. There was a uniformly marked reduction of lupus in IFN-γ- or IFN-γR-deficient MRL-*Fas*^{lpr} or BWF1 mice [277–280], and this reduction even applied in the lupus-like mercury-induced autoimmunity model [281], which was previously considered to be a prototypic Th2-mediated disease. In fact, these results correlated well with previous observations of elevated IFN-γ levels in all lupus-prone strains, accelerated disease with IFN-γ treatment, and reduced severity following anti-IFN-γ treatment [2]. Thus far, data indicate that IFN-γ is important in at least two steps of lupus pathogenesis: (1) promotion of the response to self-antigens, perhaps because they are of low antigenicity [279, 281], and (2) promotion of the local inflammatory process, i.e., disease manifestations prevented, even in IFN-γ$^{+/-}$ MRL-*Fas*^{lpr} mice, which have autoantibody levels and glomerular immunoglobulin deposits similar to wild-type animals. The implied requirement for IFN-γ for the progression of local inflammatory responses [279] is supported by findings that IFN-γ signaling is critical for local production of the nephritis-promoting cytokines, CSF-1 and TNF-α [282]. Furthermore, IFN-γ may be responsible directly or indirectly for the apoptosis of kidney tubular epithelial and mesangial cells. This uncoupling of immune and inflammatory processes is reminiscent of FcRγ chain and MCP-1 deletions, as noted later. Clearly, intervention based on a blockade of IFN-γ action is warranted based on the central role of IFN-γ in the induction and effector phases of systemic autoimmunity. The finding that intramuscular injections of a plasmid encoding IFN-γ/Fc could significantly reduce and retard lupus in MRL-*Fas*^{lpr} mice even at advanced stage of disease strongly supports this possibility [283].

A variety of protooncogenes, kinases, and transcription factors have been implicated in the Th1 versus Th2 polarization process. These include the interferon regulatory factor 1 (IRF-1) and T box expressed in T-cell (T-bet, T-box 21) proteins for Th1 cells and, for Th2 cells, the c-Maf protooncogene and the GATA3 zinc finger protein [284]. T-bet regulates Th1 lineage commitment by CD4$^+$ T cells and selectively induces IFN-γ-mediated IgG2a class switching in B cells [285]. A deficiency of T-bet in lupus-prone mixed background MRL-*Fas*^{lpr} mice resulted in reductions in mortality, GN, autoantibody production, and hypergammaglobulinemia [285]. Interestingly, this was not associated with reduced Th1-related T-cell function, apoptosis, or disease manifestations (lymphocytic infiltrates). Instead, an *in vitro* intrinsic impairment of B cells in the production of IgG2a, IgG2b, and IgG3, along with an inability of B cells to generate germline or postswitch IgG2a transcripts in response to IFN-γ, was demonstrated.

IL-12 is an important regulator of Th1 cell differentiation and IFN-γ production [286]. In MRL-*Fas*^{lpr} mice, high levels of IL-12, hyperproduction of IL-12 by peritoneal macrophages after *in vitro* stimulation, acceleration of disease with recombinant IL-12 or intramuscular injection of plasmids encoding IL-12p40 or IL-12p70, and reduction of lymphoproliferation by neutralizing anti-IL-12 antibodies have suggested a major role for IL-12 in lupus [287–289]. However, although transgenic expression of the potent IL-12 antagonist, IL-12p40 in

lupus-susceptible MRL-*Fas*^{lprcg} mice, suppressed the production of Th1 cells, IFN-γ, and anti-dsDNA antibodies, it did not significantly improve clinical manifestations of disease, including lymphoproliferation, GN, and mortality [290]. Similarly, in a previous study, treatment of (NZB × NZW)F1 mice with antibodies to IL-12 inhibited the production of anti-dsDNA antibodies, but did not improve GN [291]. These studies suggest that IL-12 is important for autoantibody production, but that it may play a lesser role in end organ disease. In sharp contrast, intramuscular injection of MRL-*Fas*^{lpr} mice with a plasmid encoding the heterodimeric IL-12 was reported to inhibit disease manifestations, including GN, despite increased IFN-γ and unchanged IL-12 levels [292]. The reason for this discrepancy may be related to dose, timing, and type of treatment; however, if confirmed, it suggests a complicated relationship between IL-12 and the development of autoimmunity.

In contrast to the dramatic effects seen with |IFN-γ, deletion of IL-4 results in only partial or no reduction in disease, depending on the lupus-prone strain. IL-4$^{-/-}$ MRL-*Fas*^{lpr} mice produced less serum IgG1 and IgE (Th2-dependent subclasses), but maintained comparable levels of IgG2a, IgG2b, and autoantibodies [277], although reduced lymphoadenopathy and end organ disease were also observed. In contrast, IL-4-deficient male BXSB mice had similar autoantibody levels, GN severity, and mortality as their wild-type counterparts [293], suggesting that IL-4 plays little role in the immunopathogenesis of disease in this strain.

Previous studies have shown that administration of IL-10 accelerated disease onset and severity in (NZB × NZW)F1 mice, while repeated injections of anti-10 inhibited disease, presumably by the upregulation of endogenous TNF-α and preferential depletion of B1 cells [294, 295]. IL-10 has also been reported to promote autoantibody production by human SLE peripheral blood cells [296]. In contrast, studies in IL-10 gene-deleted MRL-*Fas*^{lpr} mice have shown that disease parameters (skin lesions, lymphadenopathy, increased IFN-γ levels, IgG2a anti-DNA autoantibodies, GN, and mortality) were increased, whereas administration of IL-10 was protective [297]. The protective effect of IL-10 in MRL-*Fas*^{lpr} mice was attributed to inhibition of the T_H1 responses that predominate in this mouse. In contrast, disease-promoting effects of IL-10 in the (NZB × NZW)F1 disease may be due to the importance of this cytokine for B1 cell generation and maintenance [298–300], thought to be a dominant autoantibody-producing cell type in New Zealand mice [1]. Furthermore, Fas-deficient mice expressing a transgenic antierythrocyte antibody develop autoreactive B1 cells that produce anti-RBC antibodies, leading to severe hemolytic anemia [300]. Treatment of these mice with anti-IL-10 antibody prevented autoantibody production and anemia. Because B-1 cells are the main source of B-cell-derived IL-10, it appears that IL-10 may act as an autocrine differentiation factor for these autoantibody-producing cells [301].

Eta-1 (osteopontin)-deficient normal background mice were shown to have reduced type 1 immunity for viral and bacterial infection attributed to diminished IL-12 and IFN-γ and increased IL-10 production [302]. C57BL/6-*Fas*^{lpr} mice rendered Eta-1 deficient were reported to display a delayed onset of polyclonal B-cell activation and somewhat delayed lymphoaccumulation and kidney disease [303]. Earlier studies have shown an increased expression of Eta-1 in MRL-*Fas*^{lpr} mice [304].

Cell-Signaling Molecules

A major pathognomonic finding in Fas deficiency is the expansion of DN B220$^+$ T cells derived predominantly from CD8$^+$ precursors. Although early *in vitro* functional studies suggested that these DN cells were inert, other characteristics, such as constitutive phosphorylation of the CD3ζ chain and increases in p59^{fyn}, certain oncogenes, and cell surface activation markers (TCR^{low}, CD44^{hi}), indicated otherwise. *In vivo* BrdU labeling confirmed the latter notion when DN B220$^+$ T cells were shown to be highly cycling early in life with an age-related decline in the frequency of cycling cells [305]. Furthermore, MRL-*Fas*^{lpr} fyn-deficient mice showed a reduced frequency of DN B220$^+$ T cells along with decreased autoantibody levels and immunopathology, indicating an essential role for fyn in signal transduction and expansion of DN B220$^+$ T cells [305]. Thus, DN B220$^+$ T cells are functional, but lose the capacity to proliferate as they accumulate, presumably due to replicative senescence (a loss of entry into cell cycle after a finite number of divisions). Replicative senescence has also been suggested to explain the rapid accumulation of apoptosis-resistant memory/effector (CD44^{hi}) CD4 cells in older BXSB male mice, perhaps a consequence of increased levels of cyclin kinase inhibitors [235]. Such senescent cells may secrete proinflammatory cytokines that contribute to autoimmunity.

Of interest, *Fasl*^{gld} mice with only one functional CD45 allelle (CD45$^{+/-}$) were reported to have a 10-fold reduction in DN T-cell population and decreased Ig and anti-DNA autoantibodies [306]. The finding suggests that CD45 plays an essential role in the activation of DN cells, which then accumulate due to the FasL defect.

Complement Components

MRL-*Fas*^{lpr} mice deficient for complement factor B (Bf) were utilized to investigate the role of the alternative pathway of complement activation in lupus pathogenesis [307]. Bf, produced by a variety of cell types, such as hepatocytes, phagocytes, fibroblasts, and endothelial cells, is an acute-phase reactant required to activate the alternative pathway. Local production of Bf can be detected in the kidneys of lupus-prone mice [308], where the homozygous deletion of Bf resulted in a significant reduction in GN severity and incidence of vasculitis, along with lower levels of anti-dsDNA, IgG3 anti-IgG2a RF, and IgG3 [307]. Serum C3 also remained at normal levels in Bf$^{-/-}$ mice. These findings support a significant role for Bf and the alternative pathway in the immune complex autoimmune pathology of these mice.

In contrast, MRL-*Fas*^{lpr} deficient for complement component C3 had the opposite effect, with slightly accelerated proteinuria and greater glomerular IgG deposition [309]. Thus, C3, instead of promoting the development of GN, may have a mild beneficial role possibly in the clearance of immune complexes. Similarly, disease severity was the same in mixed 129 × MRL-*Fas*^{lpr} C4 knockout mice with or without the C3 null mutation, suggesting little to no role for C3 [310].

MRL-*Fas*^{lpr} mice were made transgenic for overexpression of a soluble complement regulator, soluble CR1-related gene Y (*Crry*), both systemically and in the kidney [311]. Significant inhibition of complement activation led to reduced mortality, kidney disease, and C3 deposition. No effect was observed on lymphadenopathy, autoantibody production, or IgG kidney deposits. Thus, long-term complement inhibition reduces renal disease and increases the survival of MRL-*Fas*^{lpr} mice. Of potential relevance to these findings is a report [312] wherein normal mice deficient in factor H (Cfh$^{-/-}$; the main inhibitor of spontaneous C3 activation) were shown to develop membranoproliferative GN and to be hypersensitive to developing renal injury caused by immune complexes. In contrast, introduction of a second mutation in the gene encoding complement factor B, which prevents C3 turnover *in vivo*, obviated the phenotype of the Cfh$^{-/-}$ mice.

Local Immune and Inflammatory Response Regulation

Local upregulation of a variety of molecules, including MHC, ICAM-1, nitric oxide, and MCP-1, which may contribute to the immune and inflammatory responses essential for end organ damage, has been reported for lupus strains [2]. The role of such molecules in disease pathogenesis has been addressed by examining lupus-prone mice with homozygous deletions of these genes. MRL-*Fas*^{lpr} mice deficient for ICAM-1 showed improved survival, more as a result of reduced vasculitis than changes in autoantibody levels and GN [313, 314]. Similarly, deletion of the NOS2 gene in MRL-*Fas*^{lpr} mice resulted in partial disease abrogation, with a significant reduction in vasculitis and IgG rheumatoid factor, but anti-DNA antibody levels and GN were equivalent [315]. In contrast, deletion of the FcRγ chain in (NZB × NZW)F1 mice resulted in decoupling of the immune and inflammatory processes, resulting in similar autoantibody levels and glomerular deposits of immunoglobulin or complement, but siginificantly less glomerular destruction and mortality [316]. A similar result was demonstrated in the anti-GBM antibody model [317], wherein administration of the anti-GBM antibody bypassed the requirement for generating pathogenic autoantibodies. Thus, FcγRIII appears to play an important, nonredundant, role in the induction of local inflammatory glomerular injury. Similar decoupling was observed in MRL-*Fas*^{lpr} mice with deletion of macrophage chemoattractant protein-1 (MCP-1), a chemokine that recruits macrophages and T cells to tissues [318]. Thus, MCP-1 appears to be another factor critical for the progression from immune deposits to inflammatory tissue damage.

Miscellaneous Genes

Terminal deoxynucleotidyl transferase (TdT) is essential for adding nucleotides to the N regions at the V(D)J junctions during B- and T-cell antigen receptor rearrangement, which enhances B- and T-cell repertoire diversity both by the random addition of nucleotides and by disrupting the formation of repetitive homology-directed junctions [319–321]. Knockout of the *Tdt* gene in normal background mice resulted in reduced CDR3 region diversity with essentially fetal antigen receptor repertoires. Still, these mice were viable and responded effectively to a broad variety of immune challenges. Nevertheless, frequencies of anti-DNA-expressing B cells following LPS stimulation (natural antibody repertoire) were lower in TdT$^{-/-}$ mice, mainly due to a lower incidence of polyreactivity. In addition, the lack of N-region diversity appeared to reduce the affinity of anti-DNA antibodies [322]. Accordingly, TdT-deficient BWF_1 background mice had significant reductions in GN and mortality, but, curiously, no substantial differences in immunoglobulin levels or antinuclear antibody profiles. In contrast, TdT$^{-/-}$ C57BL/6-*Fas*^{lpr} mice had substantially lower anti-DNA and rheumatoid factor (RF) levels than their TdT$^{+/+}$ littermates [323]. Although

the mechanism(s) by which TdT enhances lupus remains to be determined, apparently it promotes autoreactivity by directly altering the fine specificies and affinities of autoantibodies and/or the T-cell receptor repertoire.

Protein kinase CK2 (casein kinase II) is a ubiquitous heterotetrameric serine-theonine kinase composed of two α or α′ catalytic and two β regulatory subunits [324]. It can phosphorylate a large range of protein substrates, including those involved in nucleic acid synthesis, nuclear transcription, signal transduction, protein synthesis, and the cytoskeleton. CK2 is active in proliferating cells, and high levels are found in certain human cancers. Mice transgenic for the α catalytic subunit of CK2 (CKα) show enhanced susceptibility to T-cell lymphomas in a stochastic manner [325], and overexpression of CK2 accelerates lymphoma development in myc or tal-1-transgenic or p53-knockout mice [326]. Crossing the CK2 transgene onto the MRL-*Fas*^{lpr} background did not change lymphoma incidence, but markedly accelerated lymphoproliferation and autoimmunity, with higher levels of serum IgG2a and earlier development of ANA positivity and proliferative GN [326]. CK2-mediated enhancement of T-cell proliferative responses was postulated to cause this disease acceleration.

Leukotrienes are potent proinflammatory lipid mediators produced through the 5-lipoxygenase pathway of arachidonic acid metabolism. Arachidonic acid metabolites, including leukotrienes, have been suggested to promote autoimmune pathogenesis in MRL-*Fas*^{lpr} mice [327], but 5-lipoxygenase deficiency in these mice resulted in a modest acceleration of mortality and a slight increase in the prevalence of arthritis in males only.

CONCLUSIONS

Advances in transgenic, gene knockout, and quantitative trait mapping have greatly aided identification of the genes and genetically related changes operative in murine models of spontaneous lupus. Genome-wide QTL scans of several lupus strains have resulted not only in the identification of multiple predisposing loci, but have advanced our understanding of the nature and complexity of genetic susceptibility. The relationship of these loci to component phenotypes and precise mapping of specific trait locations are being carried out in interval-specific congenic lines, which will eventually result in definitive characterization of the genetic alterations that predispose to autoimmune disease development. Studies in genetically manipulated normal background mice with an altered expression of specific immune-related genes have revealed a diversity of perturbations that can lead to tolerance defects and manifestations of systemic autoimmunity. Importantly, these findings suggest that spontaneous systemic autoimmunity can be induced by independent genetic abnormalities affecting the various checkpoints that normally control immune responses. Gene alterations affecting B- and T-cell activation, apoptosis, complement, clearance of self-antigens, certain cytokines, and the cell cycle have thus far been implicated. Similar studies assessing germline alterations in lupus-predisposing mice have also identified a variety of genes that are primarily inhibitory, although a few can enhance disease severity. The former are probably involved in downstream effector mechanisms, whereas the latter might play minor roles in lupus predisposition. Combined mapping and genetic manipulation studies have made it apparent that murine lupus susceptibility involves considerable genetic heterogeneity, which will complicate identification of the predisposing genes. Once accomplished, however, specific therapeutic interventions can be based on the individual genotypes and their associated traits.

Since submission of the manuscript additional genetic alterations were reported. Those promoting lupus include deletion of platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) (376), E2F2 knockout (377), STAT6 deficiency (378), deletion of decay-accelerating factor (CD55) in MRL-*Fas*^{lpr} mice (379), lack of TACI (380), transgenic expression of LIGHT (*Tnfsf14*) in T cells (381), and a targeted gain-of-function mutation of lyn (382). In contrast, deletions of the IFN-α/β receptor (383) or STAT-4 (378) in lupus-prone mice reduced disease susceptibility, A NZB polymorphism of C1q on chromosome 4 within the *Nba1/Lbw2/lmh1/Mott* interval was also reported (384).

Acknowledgments

This is Publication Number 15799-IMM from the Department of Immunology, Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. The work of the authors reported herein was supported by National Institutes of Health Grants AR31203, AR39555, AG15061, AR42242, and ES08666. The authors thank M. Kat Occhipinti for editorial assistance.

References

1. Theofilopoulos, A. N., and Dixon, F. J. (1985). Murine models of systemic lupus erythematosus. *Adv. Immunol.* **37**, 269.
2. Theofilopoulos, A. N., and Kono, D. H. (1999). Murine lupus models: Gene-specific and genome-wide studies. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), p. 145. Academic Press, San Diego.
3. Schoenecker, J. G., Johnson, R. K., Lesher, A. P., Day, J. D., Love, S. D., Hoffman, M. R., Ortel, T. L., Parker, W., and Lawson, J. H. (2001). Exposure of mice to

topical bovine thrombin induces systemic autoimmunity. *Am. J. Pathol.* **159**, 1957.
4. Silveira, P. A., and Baxter, A. G. (2001). The NOD mouse as a model of SLE. *Autoimmunity* **34**, 53.
5. Jordan, M. A., Silveira, P. A., Shepherd, D. P., Chu, C., Kinder, S. J., Chen, J., Palmisano, L. J., Poulton, L. D., and Baxter, A. G. (2000). Linkage analysis of systemic lupus erythematosus induced in diabetes-prone nonobese diabetic mice by *Mycobacterium bovis*. *J. Immunol.* **165**, 1673.
6. Chiang, B., Bearer, E., Ansari, A., Dorshkind, K., and Gershwin, M. E. (1990). The BM12 mutation and autoantibodies to dsDNA in NZB.H-2bm12 mice. *J. Immunol.* **145**, 94.
7. Merino, R., Fossati, L., Lacour, M., Lemoine, R., Higaki, M., and Izui, S. (1992). H-2-linked control of the Yaa gene-induced acceleration of lupus-like autoimmune disease in BXSB mice. *Eur. J. Immunol.* **22**, 295.
8. Iwamoto, M., Ibnou-Zekri, N., Araki, K., and Izui, S. (1996). Prevention of murine lupus by an I-E alpha chain transgene: Protective role of I-E alpha chain-derived peptides with a high affinity to $I\text{-}A^b$ molecules. *Eur. J. Immunol.* **26**, 307.
9. Hirose, S., Nagasawa, R., Sekikawa, I., Hamaoka, M., Ishida, Y., Sata, H., and Shirai, T. (1983). Enhancing effect of H-2 linked NZW genes on the autoimmune traits of (NZB × NZW)F1 mice. *J. Exp. Med.* **158**, 228.
10. Kotzin, B. L., and Palmer, E. (1987). The contribution of NZW genes to lupus-like disease in (NZB × NZW)F1 mice. *J. Exp. Med.* **165**, 1237.
11. Kawano, H., Abe, M., Zhang, D., Saikawa, T., Fujimori, M., Hirose, S., and Shirai, T. (1992). Heterozygosity of the major histocompatibility complex controls the autoimmune disease in (NZW × BXSB)F1 mice. *Clin. Immunol. Immunopathol.* **65**, 308.
12. Morel, L., Rudofsky, U. H., Longmate, J. A., Schiffenbauer, J., and Wakeland, E. K. (1994). Polygenic control of susceptibility to murine systemic lupus erythematosus. *Immunity* **1**, 219.
13. Drake, C. G., Rozzo, S. J., Hirschfeld, H. F., Smarnworawong, N. P., Palmer, E., and Kotzin, B. L. (1995). Analysis of the New Zealand Black contribution to lupus-like renal disease: Multiple genes that operate in a threshold manner. *J. Immunol.* **154**, 2441.
14. Vyse, T. J., Rozzo, S. J., Drake, C. G., Appel, V. B., Lemeur, M., Izui, S., Palmer, E., and Kotzin, B. L. (1998). Contributions of Ea(z) and Eb(z) MHC genes to lupus susceptibility in New Zealand mice. *J. Immunol.* **160**, 2757.
15. Rozzo, S. J., Vyse, T. J., David, C. S., Palmer, E., Izui, S., and Kotzin, B. L. (1999). Analysis of MHC class II genes in the susceptibility to lupus in New Zealand mice. *J. Immunol.* **162**, 2623.
16. Ibnou-Zekri, N., Vyse, T. J., Rozzo, S. J., Iwamoto, M., Kobayakawa, T., Kotzin, B. L., and Izui, S. (1999). MHC-linked control of murine SLE. *Curr. Top. Microbiol. Immunol.* **246**, 275.
17. Merino, R., Iwamoto, M., Fossati, L., Muniesa, P., Araki, K., Takahashi, S., Huarte, J., Yamamura, K., Vassalli, J. D., and Izui, S. (1993). Prevention of systemic lupus erythematosus in autoimmune BXSB mice by a transgene encoding I-E alpha chain. *J. Exp. Med.* **178**, 1189.
18. Ibnou-Zekri, N., Iwamoto, M., Fossati, L., McConahey, P., and Izui, S. (1997). Role of the major histocompatibility complex class II Ea gene in lupus susceptibility in mice. *Proc. Natl. Acad. Sci. USA* **94**, 14654.
19. Fujimura, T., Hirose, S., Jiang, Y., Kodera, S., Ohmuro, H., Zhang, D., Hamano, Y., Ishida, H., Furukawa, S., and Shirai, T. (1998). Dissection of the effects of tumor necrosis factor-alpha and class II gene polymorphisms within the MHC on murine systemic lupus erythematosus (SLE). *Int. Immunol.* **10**, 1467.
20. Jacob, C. O., Lee, S. K., and Strassmann, G. (1996). Mutational analysis of TNF-alpha gene reveals a regulatory role for the 3′-untranslated region in the genetic predisposition to lupus-like autoimmune disease. *J. Immunol.* **156**, 3043.
21. Carroll, M. C. (2000). The role of complement in B cell activation and tolerance. *Adv. Immunol.* **74**, 61.
22. Zhang, Y., Schlossman, S. F., Edwards, R. A., Ou, C. N., Gu, J., and Wu, M. X. (2002). Impaired apoptosis, extended duration of immune responses, and a lupus-like autoimmune disease in IEX-1-transgenic mice. *Proc. Natl. Acad. Sci. USA* **99**, 878.
23. Morel, L., Tian, X. H., Croker, B. P., and Wakeland, E. K. (1999). Epistatic modifiers of autoimmunity in a murine model of lupus nephritis. *Immunity* **11**, 131.
24. Watanabe-Fukunaga, R., Brannan, C. I., Itoh, N., Yonehara, S., Copeland, N. G., Jenkins, N. A., and Nagata, S. (1992). The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. *J. Immunol.* **148**, 1274.
25. Drappa, J., Brot, N., and Elkon, K. B. (1993). The Fas protein is expressed at high levels on CD4+CD8+ thymocytes and activated mature lymphocytes in normal mice but not in the lupus-prone strain, MRL lpr/lpr. *Proc. Natl. Acad. Sci. USA* **90**, 10340.
26. Alderson, M. R., Armitage, R. J., Maraskovsky, E., Tough, T. W., Roux, E., Schooley, Ramsdell, F., and Lynch, D. H. (1993). Fas transduces activation signals in normal human T lymphocytes. *J. Exp. Med.* **178**, 2231.
27. Lynch, D. H., Ramsdell, F., and Alderson, M. R. (1995). Fas and FasL in the homeostatic regulation of immune responses. *Immunol. Today* **16**, 569.
28. Suda, T., Okazaki, T., Naito, Y., Yokota, T., Arai, N., Ozaki, S., Nakao, K., and Nagata, S. (1995). Expression of the Fas ligand in cells of T cell lineage. *J. Immunol.* **154**, 3806.
29. Bellgrau, D., Gold, D., Selawry, H., Moore, J., Franzusoff, A., and Duke, R. C. (1995). A role for CD95 ligand in preventing graft rejection. *Nature* **377**, 630.
30. Griffin, T. S., Brunner, T., Fletcher, S. M., Green, D. R., and Ferguson, T. A. (1995). Fas ligand-induced apoptosis as a mechanism of immune privilege. *Science* **270**, 1189.
31. Hunt, J. S., Vassmer, D., Ferguson, T. A., and Miller, L. (1997). Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus. *J. Immunol.* **158**, 4122.
32. Gao, Y., Herndon, J. M., Zhang, H., Griffith, T. S., and Ferguson, T. A. (1998). Anti-inflammatory effects of CD95 ligand (FasL)-induced apoptosis. *J. Exp. Med.* **188**, 887.

33. Kaplan, H. J., Leibole, M. A., Tezel, T., and Ferguson, T. A. (1999). Fas ligand (CD95 ligand) controls angiogenesis beneath the retina. *Natural Med.* **5**, 292.
34. Oehm, A., Behrmann, I., Falk, W., Pawlita, M., Maier, G., Klas, C., Li-Weber, M., Richards, S., Dhein, J., Trauth, B. C., Ponstingl, H., and Krammer, P. H. (1992). Purification and molecular cloning of the APO-1 cell surface antigen, a member of the tumor necrosis factor/nerve growth factor receptor superfamily: Sequence identity with the Fas antigen. *J. Biol. Chem.* **267**, 10709.
35. Watanabe-Fukunaga, R., Brannan, C. I., Copeland, N. G., Jenkins, N. A., and Nagata, S. (1992). Lymphoproliferative disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* **356**, 314.
36. Adachi, M., Watanabe-Fukunaga, R., and Nagata, S. (1993). Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. *Proc. Natl. Acad. Sci. USA* **90**, 1756.
37. Chu, J. L., Drappa, J., Parnassa, A., and Elkon, K. B. (1993). The defect in Fas mRNA expression in MRL/lpr mice is associated with insertion of the retrotransposon, ETn. *J. Exp. Med.* **178**, 723.
38. Wu, J., Zhou, T., He, J., and Mountz, J. D. (1993). Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene. *J. Exp. Med.* **178**, 461.
39. Mariani, S. M., Matiba, B., Armandola, E. A., and Krammer, P. H. (1994). The APO-1/Fas (CD95) receptor is expressed in homozygous MRL/lpr mice. *Eur. J. Immunol*. **24**, 3119.
40. Booker, J. K., Reap, E. A., and Cohen, P. L. (1998). Expression and function of Fas on cells damaged by gamma-irradiation in B6 and B6/lpr mice. *J. Immunol.* **161**, 4536.
41. Adachi, M., Suematsu, S., Kondo, T., Ogasawara, J., Tanaka, T., Yoshida, N., and Nagata, S. (1995). Targeted mutation in the *Fas* gene causes hyperplasia of peripheral lymphoid organs and liver. *Nature Genet*. **11**, 294.
42. Roths, J. B., Murphy, E. D., and Eicher, E. M. (1984). A new mutation, *gld*, that produces lymphoproliferation and autoimmunity in C3H/HeJ mice. *J. Exp. Med.* **159**, 1.
43. Lynch, D. H., Watson, M. L., Alderson, M. R., Baum, P. R., Miller, R. E., Tough, T., Gibson, M., Davis-Smith, T., Smith, C. A., Hunter, K., Bhat, D., Din, W., Goodwin, R. G., and Seldin, M. F. (1994). The mouse Fas-ligand gene is mutated in gld mice and is part of a TNF family gene cluster. *Immunity* **1**, 131.
44. Takahashi, T., Tanaka, M., Brannan, C. I., Jenkins, N. A., Copeland, N. G., Suda, T., and Nagata, S. (1994). Generalized lymphoproliferative disease in mice, caused by a point mutation in the Fas ligand. *Cell* **76**, 969.
45. Hahne, M., Peitsch, M. C., Irmier, M., Schroter, M., Lowin, B., Rousseau, M., Bron, C., Renno, T., French, L., and Tschopp, J. (1995). Characterization of the nonfunctional Fas ligand of gld mice. *Int. Immunol.* **7**, 1381.
46. Murphy, E. D., and Roths, J. B. (1976). A single gene for massive lymphoproliferation with immune complex disease in a new mouse strain MRL. **69**, 1976.
47. Ogata, Y., Kimura, M., Shimada, K., Wakabayashi, T., Onoda, H., Katagiri, T., and Matsuzawa, A. (1993). Distinctive expression of *lpr*^{cg} in the heterozygous state on different genetic backgrounds. *Cell. Immunol.* **148**, 91.
48. Rieux-Laucat, F., Le Deist, F., Hivroz, C., Roberts, I. A., Debatin, K. M., Fischer, A., and de Villartay, J. P. (1995). Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* **268**, 1347.
49. Fisher, G. H., Rosenberg, F. J., Straus, S. E., Dale, J. K., Middleton, L. A., Lin, A. Y., Strober, W., Lenardo, M. J., and Puck, J. M. (1995). Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell* **81**, 935.
50. Drappa, J., Vaishnaw, A. K., Sullivan, K. E., Chu, J. L., and Elkon, K. B. (1996). Fas gene mutations in the Canale-Smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. *N. Engl. J. Med.* **335**,1643.
51. Wang, J., Zheng, L., Lobito, A., Chan, F. K., Dale, J., Sneller, M., Yao, X., Puck, J. M., Straus, S. E., and Lenardo, M. J. (1999). Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. *Cell* **98**, 47.
52. Mysler, E., Bini, P., Drappa, J., Ramos, P., Friedman, S. M., Krammer, P. H., and Elkon, K. B. (1994). The apoptosis-1/Fas protein in human systemic lupus erythematosus. *J. Clin. Invest.* **93**, 1029.
53. Wu, J., Wilson, J., He, J., Xiang, L., Schur, P. H., and Mountz, J. D. (1996). Fas ligand mutation in a patient with systemic lupus erythematosus and lymphoproliferative disease. *J. Clin. Invest.* **98**, 1077.
54. Kojima, T., Horiuchi, T., Nishizaka, H., Sawabe, T., Higuchi, M., Harashima, S. I., Yoshizawa, S., Tsukamoto, H., Nagasawa, K., and Niho, Y. (2000). Analysis of fas ligand gene mutation in patients with systemic lupus erythematosus. *Arthritis Rheum.* **43**, 135.
55. McNally, J., Yoo, D. H., Drappa, J., Chu, J. L., Yagita, H., Friedman, S. M., and Elkon, K. B. (1997). Fas ligand expression and function in systemic lupus erythematosus. *J. Immunol.* **159**, 4628.
56. Shultz, L. D., Schweitzer, P. A., Rajan, T. V., Yi, T., Ihle, J. N., Matthews, R. J., Thomas, M. L., and Beier, D. R. (1993). Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. *Cell* **73**, 1445.
57. Tsui, H. W., Siminovitch, K. A., deSouza, L., and Tsui, F. W. L. (1993). *Motheaten* and *viable motheaten* mice have mutations in the haematopoietic cell phosphatase gene. *Nature Genet.* **4**, 124.
58. Van Zant, G., and Shultz, L. D. (1989). Hematologic abnormalities of the immunodeficient mouse mutant, viable motheaten (*me*^{v}). *Exp. Hematol.* **17**, 81.
59. Scribner, C. L., Hansen, C. T., Klinman, D. M., and Steinberg, A. D. (1987). The interaction of the xid and me genes. *J. Immunol.* **138**, 3611.
60. Yu, C. C., Tsui, H. W., Ngan, B. Y., Shulman, M. J., Wu, G. E., and Tsui, F. W. (1996). B and T cells are not required for the viable motheaten phenotype. *J. Exp. Med.* **183**, 371.
61. Thomas, M. L. (1995). Of ITAMs and ITIMs: Turning on and off the B cell antigen receptor. *J. Exp. Med.* **181**, 1953.

62. Doody, G. M., Justement, L. B., Delibrias, C. C., Matthews, R. J., Lin, J., Thomas, M. L., and Fearon, D. T. (1995). A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. *Science* **269**, 242.
63. Murphy, E. D., and Roths, J. B. (1979). A Y chromosome associated factor in strain BXSB producing accelerated autoimmunity and lymphoproliferation. *Arthritis Rheum*. **22**, 1188.
64. Eisenberg, R. A., Izui, S., McConahey, P. J., Hang, L. M., Peters, C. J., Theofilopoulos, A. N., and Dixon, F. J. (1980). Male determined accelerated autoimmune disease in BXSB mice: Transfer by bone marrow and spleen cells. *J. Immunol.* **125**, 1032.
65. Izui, S., Iwamoto, M., Fossati, L., Merino, R., Takahashi, S., and Ibnou-Zekri, N. (1995). The Yaa gene model of systemic lupus erythematosus. *Immunol. Rev.* **144**, 137.
66. Hudgins, C. C., Steinberg, R. T., Klinman, D. M., Reeves, M. J. P., Steinberg, A. D., and Reeves, M. J. (1985). Studies of consomic mice bearing the Y chromosome of the BXSB mouse. *J. Immunol.* **134**, 3849.
67. Izui, S., Higaki, M., Morrow, D., and Merino, R. (1988). The Y chromosome from autoimmune BXSB/MpJ mice induces a lupus-like syndrome in (NZW x C57BL/6)F1 male mice, but not in C57BL/6 male mice. *Eur. J. Immunol.* **18**, 911.
68. Merino, R., Shibata, T., de Kossodo, S., and Izui, S. (1989). Differential effect of the autoimmune Yaa and lpr genes on the acceleration of lupus-like syndrome in MRL/MpJ mice. *Eur. J. Immunol.* **19**, 2131.
69. Suzuka, H., Yoshifusa, H., Nakamura, Y., Miyawaki, S., and Shibata, Y. (1993). Morphological analysis of autoimmune disease in MRL-lpr,Yaa male mice with rapidly progressive systemic lupus erythematosus. *Autoimmunity* **14**, 275.
70. Jansson, L., and Holmdahl, R. (1994). The Y chromosome-linked "autoimmune accelerating" yaa gene suppresses collagen-induced arthritis. *Eur. J. Immunol.* **24**, 1213.
71. Merino, R., Fossati, L., Lacour, M., and Izui, S. (1991). Selective autoantibody production by Yaa^+ B cells in autoimmune Yaa^+-Yaa^- bone marrow chimeric mice. *J. Exp. Med.* **174**, 1023.
72. Fossati, L., Iwamoto, M., Merino, R., and Izui, S. (1995). Selective enhancing effect of the Yaa gene on immune responses against self and foreign antigens. *Eur. J. Immunol.* **25**, 166.
73. Hugin, A. W., Fossati-Jimack, L., and Izui, S. (2000). The autoimmune accelerating yaa mutation does not accelerate murine AIDS. *Cell. Immunol.* **200**, 76.
74. Fossati, L., Sobel, E. S., Iwamoto, M., Cohen, P. L., Eisenberg, R. A., and Izui, S. (1995). The *Yaa* gene-mediated acceleration of murine lupus: Yaa^- T cells from non-autoimmune mice collaborate with Yaa^+ B cells to produce lupus autoantibodies in vivo. *Eur. J. Immunol.* **25**, 3412.
75. Lawson, B. R., Koundouris, S., Barnhouse, M., Dummer, W., Baccala, R., Kono, D. H., and Theofilopoulos, A. N. (2001). The role of ab^+ T cells and homeostatic T cell proliferation in Yaa^+-associated murine lupus. *J. Immunol.* **167**, 2354.
76. DesJardin, L. E., Butfiloski, E. J., Sobel, E. S., and Schiffenbauer, J. (1996). Hyperproliferation of BXSB B cells is linked to the Yaa allele. *Clin. Immunol. Immunopathol.* **81**, 145.
77. Darvasi, A. (1998). Experimental strategies for the genetic dissection of complex traits in animal models. *Nature Genet.* **18**, 19.
78. Kono, D. H., Burlingame, R. W., Owens, D. G., Kuramochi, A., Balderas, R. S., Balomenos, D., and Theofilopoulos, A. N. (1994). Lupus susceptibility loci in New Zealand mice. *Proc. Natl. Acad. Sci. USA* **91**, 10168.
79. Vyse, T. J., and Kotzin, B. L. (1998). Genetic susceptibility to systemic lupus erythematosus. *Annu. Rev. Immunol.* **16**, 261.
80. Morel, L., and Wakeland, E. K. (1998). Susceptibility to lupus nephritis in the NZB/W model system. *Curr. Opin. Immunol.* **10**, 718.
81. Morel, L., Mohan, C., Yu, Y., Croker, B. P., Tian, N., Deng, A., and Wakeland, E. K. (1997). Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J. Immunol.* **158**, 6019.
82. Morel, L., Yu, Y., Blenman, K. R., Caldwell, R. A., and Wakeland, E. K. (1996). Production of congenic mouse strains carrying genomic intervals containing SLE-susceptibility genes derived from the SLE-prone NZM2410 strain. *Mamm. Genome* **7**, 335.
83. Mohan, C., Alas, E., Morel, L., Yang, P., and Wakeland, E. K. (1998). Genetic dissection of SLE pathogenesis: Sle1 on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J. Clin. Invest.* **101**, 1362.
84. Sobel, E. S., Mohan, C., Morel, L., Schiffenbauer, J., and Wakeland, E. K. (1999). Genetic dissection of SLE pathogenesis: Adoptive transfer of Sle1 mediates the loss of tolerance by bone marrow-derived B cells. *J. Immunol.* **162**, 2415.
85. Mohan, C., Morel, L., Yang, P., and Wakeland, E. K. (1997). Genetic dissection of systemic lupus erythematosus pathogenesis: Sle2 on murine chromosome 4 leads to B cell hyperactivity. *J. Immunol.* **159**, 454.
86. Mohan, C., Morel, L., Yang, P., and Wakeland, E. K. (1998). Accumulation of splenic B1a cells with potent antigen-presenting capability in NZM2410 lupus-prone mice. *Arthritis Rheum.* **41**, 1652.
87. Mohan, C., Yu, Y., Morel, L., Yang, P., and Wakeland, E. K. (1999). Genetic dissection of Sle pathogenesis: Sle3 on murine chromosome 7 impacts T cell activation, differentiation, and cell death. *J. Immunol.* **162**, 6492.
88. Morel, L., Mohan, C., Yu, Y., Schiffenbauer, J., Rudofsky, U. H., Tian, N., Longmate, J. A., and Wakeland, E. K. (1999). Multiplex inheritance of component phenotypes in a murine model of lupus. *Mamm. Genome* **10**, 176.
89. Mohan, C., Morel, L., Yang, P., Watanabe, H., Croker, B., Gilkeson, G., and Wakeland, E. K. (1999). Genetic dissection of lupus pathogenesis: A recipe for nephrophilic autoantibodies. *J. Clin. Invest.* **103**, 1685.
90. Morel, L., Croker, B. P., Blenman, K. R., Mohan, C., Huang, G., Gilkeson, G., and Wakeland, E. K. (2000). Genetic reconstitution of systemic lupus erythematosus

immunopathology with polycongenic murine strains. *Proc. Natl. Acad. Sci. USA* **97**, 6670.
91. Morel, L., Blenman, K. R., Croker, B. P., and Wakeland, E. K. (2001). The major murine systemic lupus erythematosus susceptibility locus, Sle1, is a cluster of functionally related genes. *Proc. Natl. Acad. Sci. USA* **98**, 1787.
92. Boackle, S. A., Holers, V. M., Chen, X., Szakonyi, G., Karp, D. R., Wakeland, E. K., and Morel, L. (2001). Cr2, a candidate gene in the murine Sle1c lupus susceptibility locus, encodes a dysfunctional protein. *Immunity* **15**, 775.
93. Fearon, D. T., and Carroll, M. C. (2000). Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. *Annu. Rev. Immunol.* **18**, 393.
94. Prodeus, A. P., Goerg, S., Shen, L. M., Pozdnyakova, O. O., Chu, L., Alicot, E. M., Goodnow, C. C., and Carroll, M. C. (1998). A critical role for complement in maintenance of self-tolerance. *Immunity* **9**, 721.
95. Wu, X., Jiang, N., Deppong, C., Singh, J., Dolecki, G., Mao, D., Morel, L., and Molina, H. D. (2002). A role for the Cr2 gene in modifying autoantibody production in systemic lupus erythematosus. *J. Immunol.* **169**, 1587.
96. Marquart, H. V., Svendsen, A., Rasmussen, J. M., Nielsen, C. H., Junker, P., Svehag, S. E., and Leslie, R. G. (1995). Complement receptor expression and activation of the complement cascade on B lymphocytes from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **101**, 60.
97. Rozzo, S. J., Allard, J. D., Choubey, D., Vyse, T. J., Izui, S., Peltz, G., and Kotzin, B. L. (2001). Evidence for an interferon-inducible gene, Ifi202, in the susceptibility to systemic lupus. *Immunity* **15**, 435.
98. Choubey, D., and Kotzin, B. L. (2002). Interferon-inducible p202 in the susceptibility to systemic lupus. *Front. Biosci.* **7**, e252.
99. Jiang, Y., Hirose, S., Sanokawa-Akakura, R., Abe, M., Mi, X., Li, N., Miura, Y., Shirai, J., Zhang, D., Hamano, Y., and Shirai, T. (1999). Genetically determined aberrant down-regulation of FcgammaRIIB1 in germinal center B cells associated with hyper-IgG and IgG autoantibodies in murine systemic lupus erythematosus. *Int. Immunol.* **11**, 1685.
100. Mary, C., Laporte, C., Parzy, D., Santiago, M. L., Stefani, F., Lajaunias, F., Parkhouse, R. M., O'Keefe, T. L., Neuberger, M. S., Izui, S., and Reininger, L. (2000). Dysregulated expression of the Cd22 gene as a result of a short interspersed nucleotide element insertion in Cd22a lupus-prone mice. *J. Immunol.* **165**, 2987.
101. Vidal, S., Kono, D. H., and Theofilopoulos, A. N. (1998). Loci predisposing to autoimmunity in MRL-*Fas*^{lpr} and C57BL/6-*Fas*^{lpr} mice. *J. Clin. Invest.* **101**, 696.
102. Wang, Y., Nose, M., Kamoto, T., Nishimura, M., and Hiai, H. (1997). Host modifier genes affect mouse autoimmunity induced by the lpr gene. *Am. J. Pathol.* **151**, 1791.
103. Watson, M. L., Rao, J. K., Gilkeson, G. S., Ruiz, P., Eicher, E. M., Pisetsky, D. S., Matsuzawa, A., Rochelle, J. M., and Seldin, M. F. (1992). Genetic analysis of MRL-lpr mice: Relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. *J. Exp. Med.* **176**, 1645.
104. Nishihara, M., Terada, M., Kamogawa, J., Ohashi, Y., Mori, S., Nakatsuru, S., Nakamura, Y., and Nose, M. (1999). Genetic basis of autoimmune sialadenitis in MRL/lpr lupus-prone mice: Additive and hierarchical properties of polygenic inheritance. *Arthritis Rheum.* **42**, 2616.
105. Parnes, J. R., and Pan, C. (2000). CD72, a negative regulator of B-cell responsiveness. *Immunol. Rev.* **176**, 75.
106. Qu, W., Miyazaki, T., Terada, M., Lu, L., Nishihara, M., Yamada, A., Mori, S., Nakamura, Y., Ogasawara, H., Yazawa, C., Nakatsuru, S., and Nose, M. (2000). Genetic dissection of vasculitis in MRL/lpr lupus mice: A novel susceptibility locus involving the CD72^{c} allele. *Eur. J. Immunol.* **30**, 2027.
107. Hogarth, M. B., Slingsby, J. H., Allen, P. J., Thompson, E. M., Chandler, P., Davies, K. A., Simpson, E., Morley, B. J., and Walport, M. J. (1998). Multiple lupus susceptibility loci map to chromosome 1 in BXSB mice. *J. Immunol.* **161**, 2753.
108. Haywood, M. E., Hogarth, M. B., Slingsby, J. H., Rose, S. J., Allen, P. J., Thompson, E. M., Maibaum, M. A., Chandler, P., Davies, K. A., Simpson, E., Walport, M. J., and Morley, B. J. (2000). Identification of intervals on chromosomes 1, 3, and 13 linked to the development of lupus in BXSB mice. *Arthritis Rheum.* **43**, 349.
109. Ida, A., Hirose, S., Hamano, Y., Kodera, S., Jiang, Y., Abe, M., Zhang, D., Nishimura, H., and Shirai, T. (1998). Multigenic control of lupus-associated antiphospholipid syndrome in a model of (NZW x BXSB) F1 mice. *Eur. J. Immunol.* **28**, 2694.
110. Nishizumi, H., Taniuchi, I., Yamanashi, Y., Kitamura, D., Ilic, D., Mori, S., Watanabe, T., and Yamamoto, T. (1995). Impaired proliferation of peripheral B cells and indication of autoimmune disease in *lyn*-deficient mice. *Immunity* **3**, 549.
111. Hibbs, M. L., Tarlinton, D. M., Armes, J., Grail, D., Hodgson, G., Maglitto, R., Stacker, S. A., and Dunn, A. R. (1995). Multiple defects in the immune system of *Lyn*-deficient mice, culminating in autoimmune disease. *Cell* **83**, 301.
112. O'Keefe, T. L., Williams, G. T., Davies, S. L., and Neuberger, M. S. (1996). Hyperresponsive B cells in CD22-deficient mice. *Science* **274**, 798.
113. Otipoby, K. L., Andersson, K. B., Draves, K. E., Klaus, S. J., Carr, A. G., Kerner, J. D., Perlmutter, R. M., Law, C. L., and Clark, E. A. (1996). CD22 regulates thymus-independent responses and the lifespan of B cells. *Nature* **384**, 634.
114. Sato, S., Miller, A. S., Inaoki, M., Bock, C. B., Jansen, P. J., Tang, M. L. K., and Tedder, T. F. (1996). CD22 is both a positive and negative regulator of B lymphocyte antigen receptor signal transduction: Altered signaling in CD22 deficient mice. *Immunity* **5**, 551.
115. Cornall, R. J., Cyster, J. G., Hibbs, M. L., Dunn, A. R., Otipoby, K. L., Clark, E. A., and Goodnow, C. C. (1998).

Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity* **8**, 497.
116. Tedder, T. F., Inaoki, M., and Sato, S. (1997). The CD19-CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity. *Immunity* **6**, 107.
117. Inaoki, M., Sato, S., Weintraub, B. C., Goodnow, C. C., and Tedder, T. F. (1997). CD19-regulated signaling thresholds control peripheral tolerance and autoantibody production in B lymphocytes. *J. Exp. Med.* **186**, 1923.
118. Coggeshall, K. M. (1998). Inhibitory signaling by B cell Fc gamma RIIb. *Curr. Opin. Immunol.* **10**, 306.
119. Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J. V. (1997). Deletion of SHIP or SHP-1 reveals two distinct pathways for inhibitory signaling. *Cell* **90**, 293.
120. Nadler, M. J. S., Chen, B., Anderson, J. S., Wortis, H. H., and Neel, B. G. (1997). Protein-tyrosine phosphatase SHP-1 is dispensable for FcgammaRIIB-mediated inhibition of B cell antigen receptor activation. *J. Biol. Chem.* **272**, 20038.
121. Takai, T., Ono, M., Hikida, M., Ohmori, H., and Ravetch, J. V. (1996). Augmented humoral and anaphylactic responses in FcgRII-deficient mice. *Nature* **379**, 346.
122. Bolland, S., and Ravetch, J. V. (2000). Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. *Immunity* **13**, 277.
123. Yuasa, T., Kubo, S., Yoshino, T., Ujike, A., Matsumura, K., Ono, M., Ravetch, J. V., and Takai, T. (1999). Deletion of fcgamma receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. *J. Exp. Med.* **189**, 187.
124. Nakamura, A., Yuasa, T., Ujike, A., Ono, M., Nukiwa, T., Ravetch, J. V., and Takai, T. (2000). Fcg Receptor IIB-deficient mice develop Goodpasture's syndrome upon immunization with type IV collagen: A novel murine model for autoimmune glomerular basement membrane disease. *J. Exp. Med.* **191**, 899.
125. Bolland, S., Yim, Y. S., Tus, K., Wakeland, E. K., and Ravetch, J. V. (2002). Genetic modifiers of systemic lupus erythematosus in FcgammaRIIB(-/-) mice. *J. Exp. Med.* **195**, 1167.
126. Morgan, B., Sun, L., Avitahl, N., Andrikopoulos, K., Ikeda, T., Gonzales, E., Wu, P., Neben, S., and Georgopoulos, K. (1997). Aiolos, a lymphoid restricted transcription factor that interacts with Ikaros to regulate lymphocyte differentiation. *EMBO J.* **16**, 2004.
127. Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J., and Wang, W. (1998). NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol. Cell.* **2**, 851.
128. Zhang, Y., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Bird, A., and Reinberg, D. (1999). Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. *Genes Dev.* **13**, 1924.
129. Knoepfler, P. S., and Eisenman, R. N. (1999). Sin meets NuRD and other tails of repression. *Cell* **99**, 447.
130. Koipally, J., Renold, A., Kim, J., and Georgopoulos, K. (1999). Repression by Ikaros and Aiolos is mediated through histone deacetylase complexes. *EMBO J.* **18**, 3090.
131. Wang, J. H., Avitahl, N., Cariappa, A., Friedrich, C., Ikeda, T., Renold, A., Andrikopoulos, K., Liang, L., Pillai, S., Morgan, B. A., and Georgopoulos, K. (1998). Aiolos regulates B cell activation and maturation to effector state. *Immunity* **9**, 543.
132. Moore, P. A., Belvedere, O., Orr, A., Pieri, K., LaFleur, D. W., Feng, P., Soppet, D., Charters, M., Gentz, R., Parmelee, D., Li, Y., Galperina, O., Giri, J., Roschke, V., Nardelli, B., Carrell, J., Sosnovtseva, S., Greenfield, W., Ruben, S. M., Olsen, H. S., Fikes, J., and Hilbert, D. M. (1999). BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* **285**, 260.
133. Schneider, P., MacKay, F., Steiner, V., Hofmann, K., Bodmer, J. L., Holler, N., Ambrose, C., Lawton, P., Bixler, S., Acha-Orbea, H., Valmori, D., Romero, P., Werner-Favre, C., Zubler, R. H., Browning, J. L., and Tschopp, J. (1999). BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J. Exp. Med.* **189**, 1747.
134. Shu, H. B., Hu, W. H., and Johnson, H. (1999). TALL-1 is a novel member of the TNF family that is down-regulated by mitogens. *J. Leukocyte Biol.* **65**, 680.
135. Mukhopadhyay, A., Ni, J., Zhai, Y., Yu, G. L., and Aggarwal, B. B. (1999). Identification and characterization of a novel cytokine, THANK, a TNF homologue that activates apoptosis, nuclear factor-kappaB, and c-Jun NH2-terminal kinase. *J. Biol. Chem.* **274**, 15978.
136. Gross, J. A., Johnston, J., Mudri, S., Enselman, R., Dillon, S. R., Madden, K., Wenfeng, X., Parrish-Novak, J., Foster, D., Lofton-Day, C., Moore, M., Littau, A., Grossman, A., Haugen, H., Foley, K., Blumberg, H., Harrison, K., kindsvogel, W., and Clegg, C. H. (2000). TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* **404**, 995.
137. Mackay, F., Woodcock, S. A., Lawton, P., Ambrose, C., Baetscher, M., Schneider, P., Tschopp, J., and Browning, J. L. (1999). Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J. Exp. Med.* **190**, 1697.
138. Khare, S. D., Sarosi, I., Xia, X. Z., McCabe, S., Miner, K., Solovyev, I., Hawkins, N., Kelley, M., Chang, D., Van, G., Ross, L., Delaney, J., Wang, L., Lacey, D., Boyle, W. J., and Hsu, H. (2000). Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. *Proc. Natl. Acad. Sci. USA* **97**, 3370.
139. Mecklenbrauker, I., Saijo, K., Zheng, N. Y., Leitges, M., and Tarakhovsky, A. (2002). Protein kinase Cdelta controls self-antigen-induced B-cell tolerance. *Nature* **416**, 860.
140. Miyamoto, A., Nakayama, K., Imaki, H., Hirose, S., Jiang, Y., Abe, M., Tsukiyama, T., Nagahama, H., Ohno, S., Hatakeyama, S., and Nakayama, K. I. (2002). Increased proliferation of B cells and auto-immunity in mice lacking protein kinase Cdelta. *Nature* **416**, 865.
141. Zhang, L., Eddy, A., Teng, Y. T., Fritzler, M., Kluppel, M., Melet, F., and Bernstein, A. (1995). An immunological renal disease in transgenic mice that overexpress Fli-1, a

member of the ets family of transcription factor genes. *Mol. Cell. Biol.* **15**, 6961.
142. Higuchi, T., Aiba, Y., Nomura, T., Matsuda, J., Mochida, K., Suzuki, M., Kikutani, H., Honjo, T., Nishioka, K., and Tsubata, T. (2002). Cutting edge: Ectopic expression of CD40 ligand on B cells induces lupus-like autoimmune disease. *J. Immunol.* **168**, 9.
143. van Kooten, C., and Banchereau, J. (2000). CD40-CD40 ligand. *J. Leukocyte Biol.* **67**, 2.
144. Desai-Mehta, A., Lu, L., Ramsey-Goldman, R., and Datta, S. K. (1996). Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J. Clin. Invest.* **97**, 2063.
145. Koshy, M., Berger, D., and Crow, M. K. (1996). Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J. Clin. Invest.* **98**, 826.
146. Blossom, S., Chu, E. B., Weigle, W. O., and Gilbert, K. M. (1997). CD40 ligand expressed on B cells in the BXSB mouse model of systemic lupus erythematosus. *J. Immunol.* **159**, 4580.
147. Waterhouse, P., Penninger, J. M., Timms, E., Wakeham, A., Shahinian, A., Lee, K. P., Thompson, C. B., Griesser, H., and Mak, T. W. (1995). Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* **270**, 985.
148. Tivol, E. A., Borriello, F., Schweitzer, A. N., Lynch, W. P., Bluestone, J. A., and Sharpe, A. H. (1995). Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* **3**, 541.
149. Tivol, E. A., Boyd, S. D., McKeon, S., Borriello, F., Nickerson, P., Strom, T. B., and Sharpe, A. H. (1997). CTLA4Ig prevents lymphoproliferation and fatal multiorgan tissue destruction in CTLA-4-deficient mice. *J. Immunol.* **158**, 5091.
150. Chambers, C. A., Cado, D., Truong, T., and Allison, J. P. (1997). Thymocyte development is normal in CTLA-4-deficient mice. *Proc. Natl. Acad. Sci. USA* **94**, 9296.
151. Chambers, C. A., Sullivan, T. J., and Allison, J. P. (1997). Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. *Immunity* **7**, 885.
152. Chambers, C. A., Kuhns, M. S., and Allison, J. P. (1999). Cytotoxic T lymphocyte antigen-4 (CTLA-4) regulates primary and secondary peptide-specific CD4(+) T cell responses. *Proc. Natl. Acad. Sci. USA* **96**, 8603.
153. Bachmann, M. F., Kohler, G., Ecabert, B., Mak, T. W., and Kopf, M. (1999). Cutting edge: Lymphoproliferative disease in the absence of CTLA-4 is not T cell autonomous. *J. Immunol.* **163**, 1128.
154. Schorle, H., Holtschke, T., Hunig, T., Schimpl, A., and Horak, I. (1991). Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature* **352**, 621.
155. Kundig, T. M., Schorle, H., Bachmann, M. F., Hengartner, H., Zinkernagel, R. M., and Horak, I. (1993). Immune responses in interleukin-2-deficient mice. *Science* **262**, 1059.
156. Sadlack, B., Lohler, J., Schorle, H., Klebb, G., Haber, H., Sickel, E., Noelle, R.J., and Horak, I. (1995). Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells. *Eur. J. Immunol.* **25**, 3053.
157. Willerford, D. M., Chen, J., Ferry, J. A., Davidson, L., Ma, A., and Alt, F. W. (1995). Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* **3**, 521.
158. Suzuki, H., Kundig, T. M., Furlonger, C., Wakeham, A., Timms, E., Matsuyama, T., Schmits, R., Simard, J. J. L., Ohashi, P. S., Griesser, H., Taniguchi, T., Paige, C. J., and Mak, T. W. (1995). Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor. *Science* **268**, 1472.
159. Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A. C., and Horak, I. (1993). Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* **75**, 253.
160. Van Parijs, L., Biuckians, A., Ibragimov, A., Alt, F. W., Willerford, D. M., and Abbas, A. K. (1997). Functional responses and apoptosis of CD25 (IL-2R alpha)-deficient T cells expressing a transgenic antigen receptor. *J. Immunol.* **158**, 3738.
161. Contractor, N. V., Bassiri, H., Reya, T., Park, A. Y., Baumgart, D. C., Wasik, M. A., Emerson, S. G., and Carding, S. R. (1998). Lymphoid hyperplasia, autoimmunity, and compromised intestinal intraepithelial lymphocyte development in colitis-free gnotobiotic IL-2-deficient mice. *J. Immunol.* **160**, 385.
162. Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., Annunziata, N., and Doetschman, T. (1992). Trageted disruption of the mouse transforming growth factor-1 gene results in multifocal inflammatory disease. *Nature* **359**, 693.
163. Kulkarni, A. B., and Karlsson, S. (1993). Transforming growth factor-beta 1 knockout mice: A mutation in one cytokine gene causes a dramatic inflammatory disease. *Am. J. Pathol.* **143**, 3.
164. Dang, H., Geiser, A. G., Letterio, J. J., Nakabayashi, T., Kong, L., Fernandes, G., and Talal, N. (1995). SLE-like autoantibodies and Sjögren's syndrome-like lymphoproliferation in TGF-knockout mice. *J. Immunol.* **155**, 3205.
165. Letterio, J. J., Geiser, A. G., Kulkarni, A. B., Dang, H., Kong, L., Nakabayashi, T., Mackall, C. L., Gress, R. E., and Roberts, A. B. (1996). Autoimmunity associated with TGF-beta1-deficiency in mice is dependent on MHC class II antigen expression. *J. Clin. Invest.* **98**, 2109.
166. Kobayashi, S., Yoshida, K., Ward, J. M., Letterio, J. J., Longenecker, G., Yaswen, L., Mittleman, B., Mozes, E., Roberts, A.B., Karlsson, S., and Kulkarni, A. B. (1999). Beta 2-microglobulin-deficient background ameliorates lethal phenotype of the TGF-beta 1 null mouse. *J. Immunol.* **163**, 4013.
167. Gorelik, L., and Flavell, R. A. (2000). Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* **12**, 171.
168. Lupher, M. L., Jr., Rao, N., Eck, M. J., and Band, H. (1999). The Cbl protooncoprotein: A negative regulator

of immune receptor signal transduction. *Immunol. Today* **20**, 375.
169. Keane, M. M., Rivero-Lezcano, O. M., Mitchell, J. A., Robbins, K. C., and Lipkowitz, S. (1995). Cloning and characterization of cbl-b: A SH3 binding protein with homology to the c-cbl protooncogene. *Oncogene* **10**, 2367.
170. Keane, M. M., Ettenberg, S. A., Nau, M. M., Banerjee, P., Cuello, M., Penninger, J., and Lipkowitz, S. (1999). cbl-3: A new mammalian cbl family protein. *Oncogene* **18**, 3365.
171. Kim, M., Tezuka, T., Suziki, Y., Sugano, S., Hirai, M., and Yamamoto, T. (1999). Molecular cloning and characterization of a novel cbl-family gene, cbl-c. *Gene* **239**, 145.
172. Clements, J. L., Boerth, N. J., Lee, J. R., and Koretzky, G. A. (1999). Integration of T cell receptor-dependent signaling pathways by adapter proteins. *Annu. Rev. Immunol.* **17**, 89.
173. Rudd, C. E. (1999). Adaptors and molecular scaffolds in immune cell signaling. *Cell* **96**, 5.
174. Chiang, Y. J., Kole, H. K., Brown, K., Naramura, M., Fukuhara, S., Hu, R. J., Jang, I. K., Gutkind, J. S., Shevach, E., and Gu, H. (2000). Cbl-b regulates the CD28 dependence of T-cell activation. *Nature* **403**, 216.
175. Bachmaier, K., Krawczyk, C., Kozieradzki, I., Kong, Y. Y., Sasaki, T., Oliveira-dos-Santos, A., Mariathasan, S., Bouchard, D., Wakeham, A., Itie, A., Le, J., Ohashi, P. S., Sarosi, I., Nishina, H., Lipkowitz, S., and Penninger, J. M. (2000). Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-b. *Nature* **403**, 211.
176. Bustelo, X. R., Crespo, P., Lopez-Barahona, M., Gutkind, J. S., and Barbacid, M. (1997). Cbl-b, a member of the Sli-1/c-Cbl protein family, inhibits Vav- mediated c-Jun N-terminal kinase activation. *Oncogene* **15**, 2511.
177. Kishihara, K., Penninger, J., Wallace, V. A., Kundig, T. M., Kawai, K., Wakeham, A., Timms, E., Pfeffer, K., Ohashi, P. S., Thomas, M. L., *et al.* (1993). Normal B lymphocyte development but impaired T cell maturation in CD45-exon6 protein tyrosine phosphatase-deficient mice. *Cell* **74**, 143.
178. Byth, K. F., Conroy, L. A., Howlett, S., Smith, A. J., May, J., Alexander, D. R., and Holmes, N. (1996). CD45-null transgenic mice reveal a positive regulatory role for CD45 in early thymocyte development, in the selection of CD4+CD8+ thymocytes, and B cell maturation. *J. Exp. Med.* **183**, 1707.
179. Cale, C. M., Klein, N. J., Novelli, V., Veys, P., Jones, A. M., and Morgan, G. (1997). Severe combined immunodeficiency with abnormalities in expression of the common leucocyte antigen, CD45. *Arch. Dis. Child.* **76**, 163.
180. Bilwes, A. M., den Hertog, J., Hunter, T., and Noel, J. P. (1996). Structural basis for inhibition of receptor protein-tyrosine phosphatase-alpha by dimerization. *Nature* **382**, 555.
181. Majeti, R., Xu, Z., Parslow, T. G., Olson, J. L., Daikh, D. I., Killeen, N., and Weiss, A. (2000). An inactivating point mutation in the inhibitory wedge of CD45 causes lymphoproliferation and autoimmunity. *Cell* **103**, 1059.
182. Ishida, Y., Agata, Y., Shibahara, K., and Honjo, T. (1992). Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO. J.* **11**, 3887.
183. Nishimura, H., Minato, N., Nakano, T., and Honjo, T. (1998). Immunological studies on PD-1 deficient mice: Implication of PD-1 as a negative regulator for B cell responses. *Int. Immunol.* **10**, 1563.
184. Nishimura, H., Nose, M., Hiai, H., Minato, N., and Honjo, T. (1999). Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* **11**, 141.
185. Fan, W., Richter, G., Cereseto, A., Beadling, C., and Smith, K. A. (1999). Cytokine response gene 6 induces p21 and regulates both cell growth and arrest. *Oncogene* **18**, 6573.
186. Vairapandi, M., Balliet, A. G., Fornace, A. J., Jr., Hoffman, B., and Liebermann, D. A. (1996). The differentiation primary response gene MyD118, related to GADD45, encodes for a nuclear protein which interacts with PCNA and p21WAF1/CIP1. *Oncogene* **12**, 2579.
187. Salvador, J. M., Hollander, M. C., Nguyen, A. T., Kopp, J. B., Barisoni, L., Moore, J. K., Ashwell, J. D., and Fornace, A. J., Jr. (2002). Mice lacking the p53-effector gene Gadd45a develop a lupus-like syndrome. *Immunity* **16**, 499.
188. Yablonski, D., and Weiss, A. (2001). Mechanisms of signaling by the hematopoietic-specific adaptor proteins, SLP-76 and LAT and their B cell counterpart, BLNK/SLP-65. *Adv. Immunol.* **79**, 93.
189. Sommers, C. L., Park, C. S., Lee, J., Feng, C., Fuller, C. L., Grinberg, A., Hildebrand, J. A., Lacana, E., Menon, R. K., Shores, E. W., Samelson, L. E., and Love, P. E. (2002). A LAT mutation that inhibits T cell development yet induces lymphoproliferation. *Science* **296**, 2040.
190. Aguado, M. T., Balderas, R. S., Rubin, R. L., Duchosal, M. A., Kofler, R., Birshtein, B. K., Secher, D. S., Dixon, F. J., and Theofilopoulos, A. N. (1987). Specificity and molecular characteristics of monoclonal IgM rheumatoid factors from arthritic and non-arthritic mice. *J. Immunol.* **139**, 1080.
191. Le, L. Q., Kabarowski, J. H., Weng, Z., Satterthwaite, A. B., Harvill, E. T., Jensen, E. R., Miller, J. F., and Witte, O. N. (2001). Mice lacking the orphan G protein-coupled receptor G2A develop a late-onset autoimmune syndrome. *Immunity* **14**, 561.
192. Vratsanos, G. S., Jung, S., Park, Y. M., and Craft, J. (2001). CD4(+) T cells from lupus-prone mice are hyperresponsive to T cell receptor engagement with low and high affinity peptide antigens: A model to explain spontaneous T cell activation in lupus. *J. Exp. Med.* **193**, 329.
193. O'Connor, L., Strasser, A., O'Reilly, L. A., Hausmann, G., Adams, J. M., Cory, S., and Huang, D. C. (1998). Bim: A novel member of the Bcl-2 family that promotes apoptosis. *EMBO J.* **17**, 384.
194. Puthalakath, H., Huang, D. C., O'Reilly, L. A., King, S. M., and Strasser, A. (1999). The proapoptotic activity of the Bcl-2 family member Bim is regulated by interaction with the dynein motor complex. *Mol. Cell.* **3**, 287.

195. Bouillet, P., Metcalf, D., Huang, D. C., Tarlinton, D. M., Kay, T. W., Kontgen, F., Adams, J. M., and Strasser, A. (1999). Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* **286**, 1735.
196. Strasser, A., Whittingham, S., Vaux, D. L., Bath, M. L., Adams, J. M., Cory, S., and Harris, A. W. (1991). Enforced bcl-2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. USA* **88**, 8661.
197. Ray, S. K., Putterman, C., and Diamond, B. (1996). Pathogenic autoantibodies are routinely generated during the response to foreign antigen: A paradigm for autoimmune disease. *Proc. Natl. Acad. Sci. USA* **93**, 2019.
198. Kuo, P., Bynoe, M., and Diamond, B. (1999). Crossreactive B cells are present during a primary but not secondary response in BALB/c mice expressing a bcl-2 transgene. *Mol. Immunol.* **36**, 471.
199. Mandik-Nayak, L., Nayak, S., Sokol, C., Eaton-Bassiri, A., Madaio, M. P., Caton, A. J., and Erikson, J. (2000). The origin of anti-nuclear antibodies in bcl-2 transgenic mice. *Int. Immunol.* **12**, 353.
200. Cantley, L. C., and Neel, B. G. (1999). New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc. Natl. Acad. Sci. USA* **96**, 4240.
201. Di Cristofano, A., and Pandolfi, P. P. (2000). The multiple roles of PTEN in tumor suppression. *Cell* **100**, 387.
202. Di Cristofano, A., Kotsi, P., Peng, Y. F., Cordon-Cardo, C., Elkon, K. B., and Pandolfi, P. P. (1999). Impaired Fas response and autoimmunity in Pten+/– mice. *Science* **285**, 2122.
203. Maehama, T., and Dixon, J. E.(1998). The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* **273**, 13375.
204. Irmler, M., Thome, M., Hahne, M., Schneider, P., Hofmann, K., Steiner, V., Bodmer, J. L., Schroter, M., Burns, K., Mattmann, C., Rimoldi, D., French, L. E., and Tschopp, J. (1997). Inhibition of death receptor signals by cellular FLIP. *Nature* **388**, 190.
205. Tschopp, J., Irmler, M., and Thome, M. (1998). Inhibition of fas death signals by FLIPs. *Curr. Opin. Immunol.* **10**, 552.
206. Van Parijs, L., Refaeli, Y., Abbas, A. K., and Baltimore, D. (1999). Autoimmunity as a consequence of retrovirus-mediated expression of C-FLIP in lymphocytes. *Immunity* **11**, 763.
207. Im, H. J., Pittelkow, M. R., and Kumar, R. (2002). Divergent regulation of the growth-promoting gene IEX-1 by the p53 tumor suppressor and Sp1. *J. Biol. Chem.* **277**, 14612.
208. Lu, Q., and Lemke, G. (2001). Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science* **293**, 306.
209. Schwartzberg, P. L. (2001). Immunology: Tampering with the immune system. *Science* **293**, 228.
210. Lu, Q., Gore, M., Zhang, Q., Camenisch, T., Boast, S., Casagranda, F., Lai, C., Skinner, M. K., Klein, R., Matsushima, G. K., Earp, H. S., Goff, S. P., and Lemke, G. (1999). Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature* **398**, 723.
211. Scott, R. S., McMahon, E. J., Pop, S. M., Reap, E. A., Caricchio, R., Cohen, P. L., Earp, H. S., and Matsushima, G. K. (2001). Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* **411**, 207.
212. Cohen, P. L., Caricchio, R., Abraham, V., Camenisch, T. D., Jennette, J. C., Roubey, R. A., Earp, H. S., Matsushima, G., and Reap, E. A. (2002). Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the c-mer membrane tyrosine kinase. *J. Exp. Med.* **196**, 135.
213. Mehling, A., Loser, K., Varga, G., Metze, D., Luger, T. A., Schwarz, T., Grabbe, S., and Beissert, S. (2001). Overexpression of CD40 ligand in murine epidermis results in chronic skin inflammation and systemic autoimmunity. *J. Exp. Med.* **194**, 615.
214. Seery, J. P., Carroll, J. M., Cattell, V., and Watt, F. M. (1997). Antinuclear autoantibodies and lupus nephritis in transgenic mice expressing interferon gamma in the epidermis. *J. Exp. Med.* **186**, 1451.
215. Botto, M., Dell'Agnola, C., Bygrave, A. E., Thompson, E. M., Cook, H. T., Petry, F., Loos, M., Pandolfi, P. P., and Walport, M. J. (1998). Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature Genet.* **19**, 56.
216. Bickerstaff, M. C., Botto, M., Hutchinson, W. L., Herbert, J., Tennent, G. A., Bybee, A., Mitchell, D. A., Cook, H. T., Butler, P. J., Walport, M. J., and Pepys, M. B. (1999). Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. *Nature Med.* **5**, 694.
217. Paul, E., and Carroll, M. C. (1999). SAP-less chromatin triggers systemic lupus erythematosus. *Nature Med.* **5**, 607.
218. Erb, K. J., Ruger, B., von Brevern, M., Ryffel, B., Schimpl, A., and Rivett, K. (1997). Constitutive expression of interleukin (IL)-4 in vivo causes autoimmune-type disorders in mice. *J. Exp. Med.* **185**, 329.
219. Muller, W., Kuhn, R., and Rajewsky, K. (1991). Major histocompatibility complex class II hyperexpression on B cells in interleukin 4-transgenic mice does not lead to B cell proliferation and hypergammaglobulinemia. *Eur. J. Immunol.* **21**, 921.
220. Burstein, H. J., Tepper, R. I., Leder, P., and Abbas, A. K. (1991). Humoral immune functions in IL-4 transgenic mice. *J. Immunol.* **147**, 2950.
221. Tepper, R. I., Levinson, D. A., Stanger, B. Z., Campos-Torres, J., Abbas, A. K., and Leder, P. (1990). IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. *Cell* **62**, 457.
222. Lewis, D. B., Yu, C. C., Forbush, K. A., Carpenter, J., Sato, T. A., Grossman, A., Liggitt, D. H., and Perlmutter, R. M. (1991). Interleukin 4 expressed in situ selectively alters thymocyte development. *J. Exp. Med.* **173**, 89.
223. Santiago, M., Fossati, L., Jacquiet, C., Muller, W., Izui, S., and Reininger, L. (1997). Interleukin-4 protects against a genetically linked lupus-like autoimmune syndrome. *J. Exp. Med.* **185**, 65.

224. Worthington, M. T., Amann, B. T., Nathans, D., and Berg, J. M. (1996). Metal binding properties and secondary structure of the zinc-binding domain of Nup475. *Proc. Natl. Acad. Sci. USA* **93**, 13754.
225. Taylor, G. A., Carballo, E., Lee, D. M., Lai, W. S., Thompson, M. J., Patel, D. D., Schenkman, D., Gilkeson, G. S., Broxmeyer, H. E., Haynes, B. F., and. Blackshear, P. J. (1996). A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. *Immunity* **4**, 445.
226. Carballo, E., Gilkeson, G. S., and Blackshear, P. J. (1997). Bone marrow transplantation reproduces the tristetraprolin-deficiency syndrome in recombination activating gene-2 (-/-) mice: Evidence that monocyte/macrophage progenitors may be responsible for TNFalpha overproduction. *J. Clin. Invest.* **100**, 986.
227. Carballo, E., Lai, W. S., and Blackshear, P. J. (1998). Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. *Science* **281**, 1001.
228. Rothe, J., Lesslauer, W., Lotscher, H., Lang, Y., Koebel, P., Kontgen, F., Althage, A., Zinkernagel, R., Steinmetz, M., and Bluethmann, H. (1993). Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature* **364**, 798.
229. Pfeffer, K., Matsuyama, T., Kundig, T. M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P. S., Kronke, M., and Mak, T. W. (1993). Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. *Cell* **73**, 457.
230. Zhou, T., Edwards, C. K., Yang, P., Wang, Z., Bluethmann, H., and Mountz, J. D. (1996). Greatly accelerated lymphadenopathy and autoimmune disease in lpr mice lacking tumor necrosis factor receptor I. *J. Immunol.* **156**, 2661.
231. Sytwu, H. K., Liblau, R. S., and McDevitt, H. O. (1996). The roles of Fas/APO-1 (CD95) and TNF in antigen-induced programmed cell death in T cell receptor transgenic mice. *Immunity* **5**, 17.
232. Balomenos, D., Martin-Caballero, J., Garcia, M. I., Prieto, I., Flores, J. M., Serrano, M., and Martinez, A. C. (2000). The cell cycle inhibitor p21 controls T-cell proliferation and sex-linked lupus development. *Nature Med.* **6**, 171.
233. Santiago-Raber, M. L., Lawson, B. R., Dummer, W., Barnhouse, M., Koundouris, S., Wilson, C. B., Kono, D. H., and Theofilopoulos, A. N. (2001). The role of cyclin kinase inhibitor p21 in systemic autoimmunity. *J. Immunol.* **167**, 4067.
234. Lawson, B. R., Kono, D. H., and Theofilopoulos, A. N. (2002). Deletion of p21 (WAF-1/Cip1) does not induce systemic autoimmunity in female BXSB mice. *J. Immunol.* **168**, 5928.
235. Sabzevari, H., Propp, S., Kono, D. H., and Theofilopoulos, A. N. (1997). G1 arrest and high expression of cyclin kinase and apoptosis inhibitors in accumulated activated/memory phenotype CD4+ cells of older lupus mice. *Eur. J. Immunol.* **27**, 1901.
236. Chui, D., Oh-Eda, M., Liao, Y. F., Panneerselvam, K., Lal, A., Marek, K. W., Freeze, H. H., Moremen, K. W., Fukuda, M. N., and Marth, J. D. (1997). Alpha-mannosidase-II deficiency results in dyserythropoiesis and unveils an alternate pathway in oligosaccharide biosynthesis. *Cell* **90**, 157.
237. Moremen, K. W., Trimble, R. B., and Herscovics, A. (1994). Glycosidases of the asparagine-linked oligosaccharide processing pathway. *Glycobiology* **4**, 113.
238. Chui, D., Sellakumar, G., Green, R., Sutton-Smith, M., McQuistan, T., Marek, K., Morris, H., Dell, A., and Marth, J. (2001). Genetic remodeling of protein glycosylation in vivo induces autoimmune disease. *Proc. Natl. Acad. Sci. USA* **98**, 1142.
239. Demetriou, M., Granovsky, M., Quaggin, S., and Dennis, J. W. (2001). Negative regulation of T-cell activation and autoimmunity by Mgat5 N- glycosylation. *Nature* **409**, 733.
240. Perillo, N. L., Pace, K. E., Seilhamer, J. J., and Baum, L. G. (1995). Apoptosis of T cells mediated by galectin-1. *Nature* **378**, 736.
241. Vespa, G. N., Lewis, L. A., Kozak, K. R., Moran, M., Nguyen, J. T., Baum, L. G., and Miceli, M. C. (1999). Galectin-1 specifically modulates TCR signals to enhance TCR apoptosis but inhibit IL-2 production and proliferation. *J. Immunol.* **162**, 799.
242. Napirei, M., Karsunky, H., Zevnik, B., Stephan, H., Mannherz, H. G., and Moroy, T. (2000). Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nature Genet.* **25**, 177.
243. Yasutomo, K., Horiuchi, T., Kagami, S., Tsukamoto, H., Hashimura, C., Urushihara, M., and Kuroda, Y. (2001). Mutation of DNASE1 in people with systemic lupus erythematosus. *Nature Genet.* **28**, 313.
244. Inglis, J. D., Lee, M., and Hill, R. E. (1993). Emk, a protein kinase with homologs in yeast maps to mouse chromosome 19. *Mamm Genome* **4**, 401.
245. Guo, S., and Kemphues, K. J. (1995). par-1, a gene required for establishing polarity in *C. elegans* embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. *Cell* **81**, 611.
246. Hurov, J. B., Stappenbeck, T. S., Zmasek, C. M., White, L. S., Ranganath, S. H., Russell, J. H., Chan, A. C., Murphy, K. M., and Piwnica-Worms, H. (2001). Immune system dysfunction and autoimmune disease in mice lacking Emk (Par-1) protein kinase. *Mol. Cell. Biol.* **21**, 3206.
247. Bessone, S., Vidal, F., Le Bouc, Y., Epelbaum, J., Bluet-Pajot, M. T., and Darmon, M. (1999). EMK protein kinase-null mice: Dwarfism and hypofertility associated with alterations in the somatotrope and prolactin pathways. *Dev. Biol.* **214**, 87.
248. Zipper, L. M., and Mulcahy, R. T. (2002). The Keap1 BTB/POZ dimerization function is required to sequester Nrf2 in cytoplasm. *J. Biol. Chem.*
249. Ishii, T., Itoh, K., and Yamamoto, M. (2002). Roles of Nrf2 in activation of antioxidant enzyme genes via antioxidant responsive elements. *Methods Enzymol.* **348**, 182.
250. Yoh, K., Itoh, K., Enomoto, A., Hirayama, A., Yamaguchi, N., Kobayashi, M., Morito, N., Koyama, A., Yamamoto,

M., and Takahashi, S. (2001). Nrf2-deficient female mice develop lupus-like autoimmune nephritis. *Kidney. Int.* **60**, 1343.
251. Ollier, W., Davies, E., Snowden, N., Alldersea, J., Fryer, A., Jones, P., and Strange, R. (1996). Association of homozygosity for glutathione-S-transferase GSTM1 null alleles with the Ro+/La- autoantibody profile in patients with systemic lupus erythematosus. *Arthritis Rheum.* **39**, 1763.
252. Poss, K. D. and Tonegawa, S. (1997). Heme oxygenase 1 is required for mammalian iron reutilization. *Proc. Natl. Acad. Sci. USA* **94**, 10919.
253. Shlomchik, M. J., Madaio, M. P., Ni, D., Trounstein, M., and Huszar, D. (1994). The role of B cells in lpr/lpr-induced autoimmunity. *J. Exp. Med.* **180**, 1295.
254. Chan, O. T., Madaio, M. P., and Shlomchik, M. J. (1999). B cells are required for lupus nephritis in the polygenic, Fas-intact MRL model of systemic autoimmunity. *J. Immunol.* **163**, 3592.
255. Chan, O., and Shlomchik, M. J. (1998). A new role for B cells in systemic autoimmunity: B cells promote spontaneous T cell activation in MRL-lpr/lpr mice. *J. Immunol.* **160**, 51.
256. Chan, O. T., and Shlomchik, M. J. (2000). B cells promote CD8+ T cell activation in MRL-Fas(lpr) mice independently of MHC class I antigen presentation. *J. Immunol.* **164**, 1658.
257. Chan, O. T., Hannum, L. G., Haberman, A. M., Madaio, M. P., and Shlomchik, M. J. (1999). A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. *J. Exp. Med.* **189**, 1639.
258. Jevnikar, A. M., Grusby, M. J., and Glimcher, L. H. (1994). Prevention of nephritis in major histocompatibility complex class II-deficient MRL-lpr mice. *J. Exp. Med.* **179**, 1137.
259. Koh, D. R., Ho, A., Rahemtulla, A., Fung-Leung, W. P., Griesser, H., and Mak, T. W. (1995). Murine lupus in MRL/lpr mice lacking CD4 or CD8 T cells. *Eur. J. Immunol.* **25**, 2558.
260. Giese, T., and Davidson, W. F. (1995). In CD8+ T cell-deficient lpr/lpr mice, CD4+B220+ and CD4+B220– T cells replace B220+ double-negative T cells as the predominant populations in enlarged lymph nodes. *J. Immunol.* **154**, 4986.
261. Ohteki, T., Iwamoto, M., Izui, S., and MacDonald, H. R. (1995). Reduced development of CD4-8-B220+ T cells but normal autoantibody production in lpr/lpr mice lacking major histocompatibility complex class I molecules. *Eur. J. Immunol.* **25**, 37.
262. Maldonado, M. A., Eisenberg, R. A., Roper, E., Cohen, P. L., and Kotzin, B. L. (1995). Greatly reduced lymphoproliferation in lpr mice lacking major histocompatibility complex class I. *J. Exp. Med.* **181**, 641.
263. Christianson, G. J., Blankenburg, R. L., Duffy, T. M., Panka, D., Roths, J. B., Marshak-Rothstein, A., and Roopenian, D. C. (1996). beta2-microglobulin dependence of the lupus-like autoimmune syndrome of MRL-lpr mice. *J. Immunol.* **156**, 4932.
264. Chan, O. T., Paliwal, V., McNiff, J. M., Park, S. H., Bendelac, A., and Shlomchik, M. J. (2001). Deficiency in beta(2)-microglobulin, but not CD1, accelerates spontaneous lupus skin disease while inhibiting nephritis in MRL-Fas(lpr) nice: An example of disease regulation at the organ level. *J. Immunol.* **167**, 2985.
265. Chen, S. Y., Takeoka, Y., Ansari, A. A., Boyd, R., Klinman, D. M., and Gershwin, M. E. (1994). The natural history of disease expression in CD4 and CD8 gene-deleted New Zealand black (NZB) mice. *J. Immunol.* **157**, 2676.
266. Chen, S. Y., Takeoka, Y., Pike-Nobile, L., Ansari, A. A., Boyd, R., and Gershwin, M. E. (1997). Autoantibody production and cytokine profiles of MHC class I (beta2-microglobulin) gene deleted New Zealand black (NZB) mice. *Clin. Immunol. Immunopathol.* **84**, 318.
267. Peng, S. L., Cappadona, J., McNiff, J. M., Madaio, M. P., Owen, M. J., Hayday, A. C., and Craft, J. (1998). Pathogenesis of autoimmunity in alphabeta T cell-deficient lupus-prone mice. *Clin. Exp. Immunol.* **111**, 107.
268. Peng, S. L., Madaio, M. P., Hughes, D. P., Crispe, I. N., Owen, M. J., Wen, Hayday, A. C., and Craft, J. (1996). Murine lupus in the absence of alpha beta T cells. *J. Immunol.* **156**, 4041.
269. Peng, S. L., and Craft, J. (1997). The regulation of murine lupus. *Ann. N. Y. Acad. Sci.* **815**, 128.
270. Craft, J., Peng, S., Fujii, T., Okada, M., and Fatenejad, S. (1999). Autoreactive T cells in murine lupus: Origins and roles in autoantibody production. *Immunol. Res.* **19**, 245.
271. Peng, S. L., Moslehi, J., Robert, M. E., and Craft, J. (1998). Perforin protects against autoimmunity in lupus-prone mice. *J. Immunol.* **160**, 652.
272. Ma, J., Xu, J., Madaio, M. P., Peng, Q., Zhang, J., Grewal, I. S., Flavell, R. A., and Craft, J. (1996). Autoimmune lpr/lpr mice deficient in CD40 ligand: Spontaneous Ig class switching with dichotomy of autoantibody responses. *J. Immunol.* **157**, 417.
273. Peng, S. L., McNiff, J. M., Madaio, M. P., Ma, J., Owen, M. J., Flavell, R. A., Hayday, A. C., and Craft, J. (1997). alpha-beta T cell regulation and CD40 ligand dependence in murine systemic autoimmunity. *J. Immunol.* **158**, 2464.
274. Tada, Y., Nagasawa, K., Ho, A., Morito, F., Koarada, S., Ushiyama, O., Suzuki, N., Ohta, A., and Mak, T. W. (1999). Role of the costimulatory molecule CD28 in the development of lupus in MRL/lpr mice. *J. Immunol.* **163**, 3153.
275. Liang, B., Gee, R. J., Kashgarian, M. J., Sharpe, A. H., and Mamula, M. J. (1999). B7 costimulation in the development of lupus: Autoimmunity arises either in the absence of B7.1/B7.2 or in the presence of anti-B7.1/B7.2 blocking antibodies. *J. Immunol.* **163**, 2322.
276. Nakajima, A., Azuma, M., Kodera, S., Nuriya, S., Terashi, A., Abe, M., Hirose, S., Shirai, T., Yagita, H., and Okumura, K. (1995). Preferential dependence of autoantibody production in murine lupus on CD86 costimulatory molecule. *Eur. J. Immunol.* **25**, 3060.
277. Peng, S. L., Moslehi, J., and Craft, J. (1997). Roles of interferon-gamma and interleukin-4 in murine lupus. *J. Clin. Invest.* **99**, 1936.

278. Haas, C., Ryffel, B., and Le, H. M. (1997). IFN-gamma is essential for the development of autoimmune glomerulonephritis in MRL/Ipr mice. *J. Immunol.* **158**, 5484.
279. Balomenos, D., Rumold, R., and Theofilopoulos, A. N. (1998). Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J. Clin. Invest.* **101**, 364.
280. Haas, C., Ryffel, B., and Le Hir, M. (1998). IFN-gamma receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB × NZW)F1 mice. *J. Immunol.* **160**, 3713.
281. Kono, D. H., Balomenos, D., Pearson, D. L., Park, M. S., Hildebrandt, B., Hultman, P., and Pollard, K. M. (1998). The prototypic Th2 autoimmunity induced by mercury is dependent on IFN-gamma and not Th1/Th2 imbalance. *J. Immunol.* **161**, 234.
282. Schwarting, A., Wada, T., Kinoshita, K., Tesch, G., and Kelley, V. R. (1998). IFN-gamma receptor signaling is essential for the initiation, acceleration, and destruction of autoimmune kidney disease in MRL-Fas(lpr) mice. *J. Immunol.* **161**, 494.
283. Lawson, B. R., Prud'homme, G. J., Chang, Y., Gardner, H. A., Kuan, J., Kono, D. H., and Theofilopoulos, A. N. (2000). Treatment of murine lupus with cDNA encoding IFN-gammaR/Fc. *J. Clin. Invest.* **106**, 207.
284. Ho, I. C., and Glimcher, L. H. (2002). Transcription: Tantalizing times for T cells. *Cell* **109**(Suppl.), S109.
285. Peng, S. L., Szabo, S. J., and Glimcher, L. H. (2002). T-bet regulates IgG class switching and pathogenic autoantibody production. *Proc. Natl. Acad. Sci. USA* **99**, 5545.
286. Robinson, D. S., and O'Garra, A. (2002). Further checkpoints in Th1 development. *Immunity* **16**, 755.
287. Huang, F. P., Feng, G. J., Lindop, G., Stott, D. I., and Liew, F. Y. (1996). The role of interleukin 12 and nitric oxide in the development of spontaneous autoimmune disease in MRL/MP-lpr/lpr mice. *J. Exp. Med.* **183**, 1447.
288. Fan, X., Oertli, B., and Wuthrich, R. P. (1997). Up-regulation of tubular epithelial interleukin-12 in autoimmune MRL-Fas(lpr) mice with renal injury. *Kidney Int.* **51**, 79.
289. Xu, H., Kurihara, H., Ito, T., Nakajima, S., Hagiwara, E., Yamanokuchi, H., and Asari, A. (2001). IL-12 enhances lymphoaccumulation by suppressing cell death of T cells in MRL-lpr/lpr mice. *J. Autoimmun.* **16**, 87.
290. Yasuda, T., Yoshimoto, T., Tsubura, A., and Matsuzawa, A. (2001). Clear suppression of Th1 responses but marginal amelioration of autoimmune manifestations by IL-12p40 transgene in MRL-FAS(lprcg)/FAS(lprcg) mice. *Cell Immunol.* **210**, 77.
291. Nakajima, A., Hirose, S., Yagita, H., and Okumura, K. (1997). Role of IL-4 and IL-12 in the development of lupus in NZB/W F1 mice. *J. Immunol.* **158**, 1466.
292. Hagiwara, E., Okubo, T., Aoki, I., Ohno, S., Tsuji, T., Ihata, A., Ueda, A., Shirai, A., Okuda, K., Miyazaki, J., and Ishigatsubo, Y. (2000). IL-12-encoding plasmid has a beneficial effect on spontaneous autoimmune disease in MRL/MP-lpr/lpr mice. *Cytokine* **12**, 1035.
293. Kono, D. H., Balomenos, D., Park, M. S., and Theofilopoulos, A. N. (2000). Development of lupus in BXSB mice is independent of IL-4. *J. Immunol.* **164**, 38.
294. Ishida, H., Hastings, R., Kearney, J., and Howard, M. (1992). Continuous anti-interleukin 10 antibody administration depletes mice of Ly-1 B cells but not conventional B cells. *J. Exp. Med.* **175**, 1213.
295. Ishida, H., Muchamuel, T., Sakaguchi, S., Andrade, S., Menon, S., and Howard, M. (1994). Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* **179**, 305.
296. Llorente, L., Zou, W., Levy, Y., Richaud-Patin, Y., Wijdenes, J., Alcocer-Varela, J., Morel-Fourrier, B., Brouet, J. C., Alarcon-Segovia, D., Galanaud, P., *et al.* (1995). Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med.* **181**, 839.
297. Yin, Z., Bahtiyar, G., Zhang, N., Liu, L., Zhu, P., Robert, M. E., McNiff, J., Madaio, M. P., and Craft, J. (2002). IL-10 regulates murine lupus. *J. Immunol.* **169**, 2148.
298. Ramachandra, S., Metcalf, R. A., Fredrickson, T., Marti, G. E., and Raveche, E. (1996). Requirement for increased IL-10 in the development of B-1 lymphoproliferative disease in a murine model of CLL. *J. Clin. Invest.* **98**, 1788.
299. Balabanian, K., Foussat, A., Bouchet-Delbos, L., Couderc, J., Krzysiek, R., Amara, A., Baleux, F., Portier, A., Galanaud, P., and Emilie, D. (2002). Interleukin-10 modulates the sensitivity of peritoneal B lymphocytes to chemokines with opposite effects on stromal cell-derived factor-1 and B-lymphocyte chemoattractant. *Blood* **99**, 427.
300. Watanabe, N., Ikuta, K., Nisitani, S., Chiba, T., and Honjo, T. (2002). Activation and differentiation of autoreactive B-1 cells by interleukin 10 induce autoimmune hemolytic anemia in Fas-deficient antierythrocyte immunoglobulin transgenic mice. *J. Exp. Med.* **196**, 141.
301. O'Garra, A., Chang, R., Go, N., Hastings, R., Haughton, G., and Howard, M. (1992). Ly-1 B (B-1) cells are the main source of B cell-derived interleukin 10. *Eur. J. Immunol.* **22**, 711.
302. Ashkar, S., Weber, G. F., Panoutsakopoulou, V., Sanchirico, M. E., Jansson, M., Zawaideh, S., Rittling, S. R., Denhardt, D. T., Glimcher, M. J., and Cantor, H. (2000). Eta-1 (osteopontin): An early component of type-1 (cell-mediated) immunity. *Science* **287**, 860.
303. Weber, G. F., and Cantor, H. (2001). Differential roles of osteopontin/Eta-1 in early and late lpr disease. *Clin. Exp. Immunol.* **126**, 578.
304. Patarca, R., Wei, F. Y., Singh, P., Morasso, M. I., and Cantor, H. (1990). Dysregulated expression of the T cell cytokine Eta-1 in CD4-8-lymphocytes during the development of murine autoimmune disease. *J. Exp. Med.* **172**, 1177.
305. Balomenos, D., Rumold, R., and Theofilopoulos, A. N. (1997). The proliferative *in vivo* activities of lpr double-negative T cells and the primary role of p59fyn in their activation and expansion. *J. Immunol.* **159**, 2265.
306. Brooks, W. P., and Lynes, M. A. (2001). Effects of hemizygous CD45 expression in the autoimmune Fasl(gld/gld) syndrome. *Cell. Immunol.* **212**, 24.

307. Watanabe, H., Garnier, G., Circolo, A., Wetsel, R. A., Ruiz, P., Holers, V. M., Boackle, S. A., Colten, H. R., and Gilkeson, G. S. (2000). Modulation of renal disease in MRL/lpr mice genetically deficient in the alternative complement pathway factor B. *J. Immunol.* **164**, 786.
308. Passwell, J., Schreiner, G. F., Nonaka, M., Beuscher, H. U., and Colten, H. R. (1988). Local extrahepatic expression of complement genes C3, factor B, C2, and C4 is increased in murine lupus nephritis. *J. Clin. Invest.* **82**, 1676.
309. Sekine, H., Reilly, C. M., Molano, I. D., Garnier, G., Circolo, A., Ruiz, P., Holers, V. M., Boackle, S. A., and Gilkeson, G. S. (2001). Complement component C3 is not required for full expression of immune complex glomerulonephritis in MRL/lpr mice. *J. Immunol.* **166**, 6444.
310. Einav, S., Pozdnyakova, O. O., Ma, M., and Carroll, M. C. (2002). Complement C4 is protective for lupus disease independent of C3. *J. Immunol.* **168**, 1036.
311. Bao, L., Haas, M., Boackle, S. A., Kraus, D. M., Cunningham, P. N., Park, P., Alexander, J. J., Anderson, R. K., Culhane, K., Holers, V. M., and Quigg, R. J. (2002). Transgenic expression of a soluble complement inhibitor protects against renal disease and promotes survival in MRL/lpr mice. *J. Immunol.* **168**, 3601.
312. Pickering, M. C., Cook, H. T., Warren, J., Bygrave, A. E., Moss, J., Walport, M. J., and Botto, M. (2002). Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nature Genet.* **31**, 424.
313. Lloyd, C. M., Gonzalo, J. A., Salant, D. J., Just, J., and Gutierrez-Ramos, J. C. (1997). Intercellular adhesion molecule-1 deficiency prolongs survival and protects against the development of pulmonary inflammation during murine lupus. *J. Clin. Invest.* **100**, 963.
314. Bullard, D. C., King, P. D., Hicks, M. J., Dupont, B., Beaudet, A. L., and Elkon, K. B. (1997). Intercellular adhesion molecule-1 deficiency protects MRL/MpJ-Fas(lpr) mice from early lethality. *J. Immunol.* **159**, 2058.
315. Gilkeson, G. S., Mudgett, J. S., Seldin, M. F., Ruiz, P., Alexander, A. A., Misukonis, M. A., Pisetsky, D. S., and Weinberg, J. B. (1997). Clinical and serologic manifestations of autoimmune disease in MRL-lpr/lpr mice lacking nitric oxide synthase type 2. *J. Exp. Med.* **186**, 365.
316. Clynes, R., Dumitru, C., and Ravetch, J. V. (1998). Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* **279**, 1052.
317. Park, S. Y., Ueda, S., Ohno, H., Hamano, Y., Tanaka, M., Shiratori, T., Yamazaki, T., Arase, H., Arase, N., Karasawa, A., Sato, S., Ledermann, B., Kondo, Y., Okumura, K., Ra, C., and Saito, T. (1998). Resistance of Fc receptor-deficient mice to fatal glomerulonephritis. *J. Clin. Invest.* **102**, 1229.
318. Tesch, G. H., Maifert, S., Schwarting, A., Rollins, B. J., and Kelley, V. R. (1999). Monocyte chemoattractant protein 1-dependent leukocytic infiltrates are responsible for autoimmune disease in MRL-Fas(lpr) mice. *J. Exp. Med.* **190**, 1813.
319. Gilfillan, S., Benoist, C., and Mathis, D. (1995). Mice lacking terminal deoxynucleotidyl transferase: Adult mice with a fetal antigen receptor repertoire. *Immunol. Rev.* **148**, 201.
320. Komori, T., Okada, A., Stewart, V., and Alt, F. W. (1993). Lack of N regions in antigen receptor variable region genes of TdT-deficient lymphocytes. *Science* **261**, 1171.
321. Komori, T., Pricop, L., Hatakeyama, A., Bona, C. A., and Alt, F. W. (1996). Repertoires of antigen receptors in Tdt congenitally deficient mice. *Int. Rev. Immunol.* **13**, 317.
322. Weller, S., Conde, C., Knapp, A. M., Levallois, H., Gilfillan, S., Pasquali, J. L., and Martin, T. (1997). Autoantibodies in mice lacking terminal deoxynucleotidyl transferase: Evidence for a role of N region addition in the polyreactivity and in the affinities of anti-DNA antibodies. *J. Immunol.* **159**, 3890.
323. Molano, I. D., Wloch, M. K., Alexander, A. A., Watanabe, H., and Gilkeson, G. S. (2000). Effect of a genetic deficiency of terminal deoxynucleotidyl transferase on autoantibody production by C57BL6 fas(lpr) mice. *Clin. Immunol.* **94**, 24.
324. Allende, C. C., and Allende, J. E. (1998). Promiscuous subunit interactions: A possible mechanism for the regulation of protein kinase CK2. *J. Cell. Biochem. Suppl.* **31**, 129.
325. Seldin, D. C., and Leder, P. (1995). Casein kinase II alpha transgene-induced murine lymphoma: Relation to theileriosis in cattle. *Science* **267**, 894.
326. Xu, X., Landesman-Bollag, E., Channavajhala, P. L., and Seldin, D. C. (1999). Murine protein kinase CK2: gene and oncogene. *Mol. Cell. Biochem.* **191**, 65.
327. Spurney, R. F., Ruiz, P., Pisetsky, D. S., and Coffman, T. M. (1991). Enhanced renal leukotriene production in murine lupus: Role of lipoxygenase metabolites. *Kidney Int.* **39**, 95.
328. Rudofsky, U. H., Evans, B. D., Balaban, S. L., Mottironi, V. D., and Gabrielsen, A. E. (1993). Differences in expression of lupus nephritis in New Zealand mixed H-2z homozygous inbred strains of mice derived from New Zealand black and New Zealand white mice: Origins and initial characterization. *Lab. Invest.* **68**, 419.
329. Fossati, L., Takahashi, S., Merino, R., Iwamoto, M., Aubry, J. P., Nose, M., Spach, Motta, R., and Izui, S. (1993). An MRL/MpJ-lpr/lpr substrain with a limited expansion of lpr double-negative T cells and a reduced autoimmune syndrome. *Int. Immunol.* **5**, 525.
330. Kinjoh, K., Kyogoku, M., and Good, R. A. (1993). Genetic selection for crescent formation yields mouse strain with rapidly progressive glomerulonephritis and small vessel vasculitis. *Proc. Natl. Acad. Sci. USA* **90**, 3413.
331. Kofler, R., McConahey, P. J., Duchosal, M. A., Balderas, R. S., Theofilopoulos, A. N., and Dixon, F. J. (1991). An autosomal recessive gene that delays expression of lupus in BXSB mice. *J. Immunol.* **146**, 1375.
332. Vidal, S., Gelpi, C., and Rodriguez-Sanchez, J. L. (1994). (SWR × SJL)F1 mice: A new model of lupus-like disease. *J. Exp. Med.* **179**, 1429.

333. Walker, S. E., Gray, R. H., Fulton, M., Wigley, R. D., and Schnitzer, B. (1978). Palmerston North mice, a new animal model of systemic lupus erythematosus. *J. Lab. Clin. Med.* **92**, 932.
334. Bignon, J. S., and Siminovitch, K. A. (1994). Identification of PTP1C mutation as the genetic defect in motheaten and viable motheaten mice: A step toward defining the roles of protein tyrosine phosphatases in the regulation of hemopoietic cell differentiation and function. *Clin. Immunol. Immunopathol.* **73**, 168.
335. Pollard, K. M., and Hultman, P. (1997). Effects of mercury on the immune system. *Met. Ions. Biol. Syst.* **34**, 421.
336. Rubin, R. L. (1997). Drug-induced lupus. **47**, 871.
337. Satoh, M., Kumar, A., Kanwar, Y. S., and Reeves, W. H. (1995). Anti-nuclear antibody production and immune-complex glomerulonephritis in BALB/c mice treated with pristane. *Proc. Natl. Acad. Sci. USA* **92**, 10934.
338. Mendlovic, S., Fricke, B. H., Shoenfeld, Y., Bakimer, R., and Mozes, E. (1990). The genetic regulation of the induction of experimental SLE. *Immunology* **69**, 228.
339. Vyse, T. J., Morel, L., Tanner, F. J., Wakeland, E. K., and Kotzin, B. L. (1996). Backcross analysis of genes linked to autoantibody production in New Zealand White mice. *J. Immunol.* **157**, 2719.
340. Xie, S., Chang, S., Yang, P., Jacob, C., Kaliyaperumal, A., Datta, S. K., and Mohan, C. (2001). Genetic contributions of nonautoimmune SWR mice toward lupus nephritis. *J. Immunol.* **167**, 7141.
341. Kono, D. H., Park, M. S., Szydlik, A., Haraldsson, K. M., Kuan, J. D., Pearson, D. L., Hultman, P., and Pollard, K. M. (2001). Resistance to xenobiotic-induced autoimmunity maps to chromosome 1. *J. Immunol.* **167**, 2396.
342. Waters, S. T., Fu, S. M., Gaskin, F., Deshmukh, U. S., Sung, S. S., Kannapell, C. C., Tung, K. S., McEwen, S. B., and McDuffie, M. (2001). NZM2328: A new mouse model of systemic lupus erythematosus with unique genetic susceptibility loci. *Clin. Immunol.* **100**, 372.
343. Vyse, T. J., Rozzo, S. J., Drake, C. G., Izui, S., and Kotzin, B. L. (1997). Control of multiple autoantibodies linked with a lupus nephritis susceptibility locus in New Zealand black mice. *J. Immunol.* **158**, 5566.
344. Rozzo, S. J., Vyse, T. J., Drake, C. G., and Kotzin, B. L. (1996). Effect of genetic background on the contribution of New Zealand black loci to autoimmune lupus nephritis. *Proc. Natl. Acad. Sci. USA* **93**, 15164.
345. Gu, L., Weinreb, A., Wang, X. P., Zack, D. J., Qiao, J. H., Weisbart, R., and Lusis, A. J. (1998). Genetic determinants of autoimmune disease and coronary vasculitis in the MRL-lpr/lpr mouse model of systemic lupus erythematosus. *J. Immunol.* **161**, 6999.
346. Rahman, Z. S., Tin, S. K., Buenaventura, P. N., Ho, C. H., Yap, E. P., Yong, R. Y., and Koh, D. R. (2002). A novel susceptibility locus on chromosome 2 in the (New Zealand Black × New Zealand White)F1 hybrid mouse model of systemic lupus erythematosus. *J. Immunol.* **168**, 3042.
347. Ochiai, K., Ozaki, S., Tanino, A., Watanabe, S., Ueno, T., Mitsui, K., Toei, J., Inada, Y., Hirose, S., Shirai, T., and Nishimura, H. (2000). Genetic regulation of anti-erythrocyte autoantibodies and splenomegaly in autoimmune hemolytic anemia-prone new zealand black mice. *Int. Immunol.* **12**, 1.
348. Drake, C. G., Babcock, S. K., Palmer, E., and Kotzin, B. L. (1994). Genetic analysis of the NZB contribution to lupus-like autoimmune disease in (NZB × NZW)F1 mice. *Proc. Natl. Acad. Sci. USA* **91**, 4062.
349. Hirose, S., Tsurui, H., Nishimura, H., Jiang, Y., and Shirai, T. (1994). Mapping of a gene for hypergammaglobulinemia to the distal region on chromosome 4 in NZB mice and its contribution to systemic lupus erythematosus in (NZB × NZW)F1 mice. *Int. Immunol.* **6**, 1857.
350. Jiang, Y., Hirose, S., Hamano, Y., Kodera, S., Tsurui, H., Abe, M., Terashima, K., Ishikawa, S., and Shirai, T. (1997). Mapping of a gene for the increased susceptibility of B1 cells to Mott cell formation in murine autoimmune disease. *J. Immunol.* **158**, 992.
351. Nakatsuru, S., Terada, M., Nishihara, M., Kamogawa, J., Miyazaki, T., Qu, W. M., Morimoto, K., Yazawa, C., Ogasawara, H., Abe, Y., Fukui, K., Ichien, G., Ito, M. R., Mori, S., Nakamura, Y., and Nose, M. (1999). Genetic dissection of the complex pathological manifestations of collagen disease in MRL/lpr mice. *Pathol. Int.* **49**, 974.
352. Vyse, T. J., Drake, C. G., Rozzo, S. J., Roper, E., Izui, S., and Kotzin, B. L. (1996). Genetic linkage of IgG autoantibody production in relation to lupus nephritis in New Zealand hybrid mice. *J. Clin. Invest.* **98**, 1762.
353. Haywood, M. E., Vyse, T. J., McDermott, A., Thompson, E. M., Ida, A., Walport, M. J., Izui, S., and Morley, B. J. (2001). Autoantigen glycoprotein 70 expression is regulated by a single locus, which acts as a checkpoint for pathogenic anti-glycoprotein 70 autoantibody production and hence for the corresponding development of severe nephritis, in lupus-prone PXSB mice. *J. Immunol.* **167**, 1728.
354. Rozzo, S. J., Vyse, T. J., Menze, K., Izui, S., and Kotzin, B. L. (2000). Enhanced susceptibility to lupus contributed from the nonautoimmune C57BL/10, but not C57BL/6, genome. *J. Immunol.* **164**, 5515.
355. Santiago, M. L., Mary, C., Parzy, D., Jacquet, C., Montagutelli, X., Parkhouse, R. M., Lemoine, R., Izui, S., and Reininger, L. (1998). Linkage of a major quantitative trait locus to Yaa gene-induced lupus- like nephritis in (NZW × C57BL/6)F1 mice. *Eur. J. Immunol.* **28**, 4257.
356. Kamogawa, J., Terada, M., Mizuki, S., Nishihara, M., Yamamoto, H., Mori, S., Abe, Y., Morimoto, K., Nakatsuru, S., Nakamura, Y., and Nose, M. (2002). Arthritis in MRL/lpr mice is under the control of multiple gene loci with an allelic combination derived from the original inbred strains. *Arthritis Rheum.* **46**, 1067.
357. Tucker, R. M., Vyse, T. J., Rozzo, S., Roark, C. L., Izui. S., and Kotzin, B. L. (2000). Genetic control of glycoprotein 70 autoantigen production and its influence on immune complex levels and nephritis in murine lupus. *J. Immunol.* **165**, 1665.
358. Walport, M. J., Davies, K. A., and Botto, M. (1998). C1q and systemic lupus erythematosus. *Immunobiology* **199**, 265.

359. Mitchell, D. A., Pickering, M. C., Warren, J., Fossati-Jimack, L., Cortes-Hernandez, J., Cook, H. T., Botto, M., and Walport, M. J. (2002). C1q deficiency and autoimmunity: The effects of genetic background on disease expression. *J. Immunol.* **168**, 2538.
360. O'Keefe, T. L., Williams, G. T., Batista, F. D., and Neuberger, M. S. (1999). Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. *J. Exp. Med.* **189**, 1307.
361. Lai, W. S., Carballo, E., Strum, J. R., Kennington, E. A., Phillips, R. S., and Blackshear, P. J. (1999). Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. *Mol. Cell. Biol.* **19**, 4311.
362. Yu, C. C., Yen, T. S., Lowell, C. A., and DeFranco, A. L. (2001). Lupus-like kidney disease in mice deficient in the Src family tyrosine kinases Lyn and Fyn. *Curr. Biol.* **11**, 34.
363. Chen, Z., Koralov, S. B., and Kelsoe, G. (2000). Complement C4 inhibits systemic autoimmunity through a mechanism independent of complement receptors CR1 and CR2. *J. Exp. Med.* **192**, 1339.
364. Goulet, J. L., Griffiths, R. C., Ruiz, P., Spurney, R. F., Pisetsky, D. S., Koller, B. H., and Coffman, T. M. (1999). Deficiency of 5-lipoxygenase abolishes sex-related survival differences in MRL-lpr/lpr mice. *J. Immunol.* **163**, 359.
365. Chesnutt, M. S., Finck, B. K., Killeen, N., Connolly, M. K., Goodman, H., and Wofsy, D. (1998). Enhanced lymphoproliferation and diminished autoimmunity in CD4-deficient MRL/lpr mice. *Clin. Immunol. Immunopathol.* **87**, 23.
366. Cattell, V. (1999). Nitric oxide and glomerulonephritis. *Semin. Nephrol.* **19**, 277.
367. Peng, S. L., and Craft, J. (1996). T cells in murine lupus: Propagation and regulation of disease. *Mol. Biol. Rep.* **23**, 247.
368. Peng, S. L., Madaio, M. P., Hayday, A. C., and Craft, J. (1996). Propagation and regulation of systemic autoimmunity by gammadelta T cells. *J. Immunol.* **157**, 5689.
369. Wen, L., Pao, W., Wong, F. S., Peng, Q., Craft, J., Zheng, B., Kelsoe, G., Dianda, L., Owen, M. J., and Hayday, A. C. (1996). Germinal center formation, immunoglobulin class switching, and autoantibody production driven by "non alpha/beta" T cells. *J. Exp. Med.* **183**, 2271.
370. Kinoshita, K., Tesch, G., Schwarting, A., Maron, R., Sharpe, A. H., and Kelley, V. R. (2000). Costimulation by B7-1 and B7-2 is required for autoimmune disease in MRL-*fas*^{lpr} mice. *J. Immunol.* **164**, 6046.
371. Paisansinsup, T., Vallejo, A. N., Luthra, H., and David, C. S. (2001). HLA-DR modulates autoantibody repertoire, but not mortality, in a humanized mouse model of systemic lupus erythematosus. *J. Immunol.* **167**, 4083.
372. Kontoyiannis, D., and Kollias, G. (2000). Accelerated autoimmunity and lupus nephritis in NZB mice with an engineered heterozygous deficiency in tumor necrosis factor. *Eur. J. Immunol.* **30**, 2038.
373. Conde, C., Weller, S., Gilfillan, S., Marcellin, L., Martin, T., and Pasquali, J. L. (1998). Terminal deoxynucleotidyl transferase deficiency reduces the incidence of autoimmune nephritis in (New Zealand Black × New Zealand White)F1 mice. *J. Immunol.* **161**, 7023.
374. Feeney, A. J., Lawson, B. R., Kono, D. H., and Theofilopoulos, A. N. (2001). Terminal deoxynucleotidyl transferase deficiency decreases autoimmune disease in MRL-*Fas*^{lpr} mice. *J. Immunol.* **167**, 3486.
375. Rifkin, I. R., Channavajhala, P. L., Kiefer, H. L., Carmack, A. J., Landesman-Bollag, E., Beaudette, B. C., Jersky, B., Salant, D. J., Ju, S. T., Marshak-Rothstein, A., and Seldin, D. C. (1998). Acceleration of lpr lymphoproliferative and autoimmune disease by transgenic protein kinase CK2 alpha. *J. Immunol.* **161**, 5164.
376. Wilkinson, R., Lyons, A. B., Roberts, D., Wong, M. X., Bartley, P. A., and Jackson, D. E. (2002). Platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) acts as a regulator of B-cell development, B-cell antigen receptor (BCR)-mediated activation, and autoimmune disease. *Blood* **100**, 184.
377. Murga, M., Fernandez-Capetillo, O., Field, S. J., Moreno, B., L., R. B., Fujiwara, Y., Balomenos, D., Vicario, A., Carrera, A. C., Orkin, S. H., Greenberg, M. E., and Zubiaga, A. M. (2001). Mutation of E2F2 in mice causes enhanced T lymphocyte proliferation, leading to the development of autoimmunity. *Immunity*. **15**, 959.
378. Singh, R. R., Saxena, V., Zang, S., Li, L., Finkelman, F. D., Witte, D. P., and Jacob, C. O. (2003). Differential contribution of IL-4 and STAT6 vs STAT4 to the development of lupus nephritis. *J. Immunol.* **170**, 4818.
379. Miwa, T., Maldonado, M. A., Zhou, L., Sun, X., Luo H. Y., Cai, D., Werth, V. P., Madaio, M. P., Eisenberg, R. A., and Song, W. C. (2002). Deletion of decay-accelerating factor (CD55) exacerbates autoimmune disease development in MRL/*lpr* mice, *Am. J. Pathol.* **161**, 1077.
380. Seshasayee, D., Valdez, P., Yan, M., Dixit, V. M., Tumas, D., and Grewal, I. S. (2003). Loss of TACI causes fatal lymphoproliferation and autoimmunity, establishing TACI as an inhibitory BLyS receptor. *Immunity* **18**, 279.
381. Wang, J., Lo, J. C., Foster, A., Yu, P., Chen, H. M., Wang, Y., Tamada, K., Chen, L., and Fu, Y. X. (2001). The regulation of T cell homeostasis and autoimmunity by T cell-derived LIGHT. *J. Clin. Invest.* **108**, 1771.
382. Hibbs, M. L., Harder, K. W., Armes, J., Kountouri, N., Quilici, C., Casagranda, F., Dunn, A. R., and Tarlinton, D. M. (2002). Sustained activation of Lyn tyrosine kinase in vivo leads to autoimmunity. *J. Exp. Med.* **196**, 1593.
383. Santiago-Raber, M. L., Baccala, R., Haraldsson, K. M., Choubey, D., Stewart, T. A., Kono, D. H., and Theofilopoulos, A. N. (2003). Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. *J. Exp. Med.* **197**, 777.
384. Miura-Shimura, Y., Nakamura, K., Ohtsuji, M., Tomita, H., Jiang, Y., Abe, M., Zhang, D., Hamano, Y., Tsuda, H., Hashimoto, H., Nishimura, H., Taki, S., Shirai, T., and Hirose, S. (2002), C1q regulatory region polymorphism down-regulating murine C1q protein levels with linkage to lupus nephritis. *J. Immunol.* **169**, 1334.

9

SYSTEMIC LUPUS ERYTHEMATOSUS IN DOMESTIC ANIMALS

Richard E. W. Halliwell

INTRODUCTION

The study of spontaneous diseases of domestic animals is of great value in research into the pathogenesis and treatment of many spontaneous diseases of humans. Although generally more expensive than the use of traditional animal models, and despite mounting sensitivities regarding the use of dogs and cats in medical research, their close environmental proximity to humans and their outbred nature make them particularly appropriate for such investigations.

This is most certainly true in case of spontaneous autoimmune diseases. Investigations of canine immune-mediated thrombocytopenia, autoimmune hemolytic anemia, lymphocytic thyroiditis, rheumatoid arthritis, and systemic lupus erythematosus (SLE), to name but a few, have all contributed to better understanding of the counterpart disease in humans [1]. The most significant work, however, has probably involved investigations of canine SLE. Although contributions enhancing our knowledge of the clinical aspects of the disease continue on both sides of the Atlantic, in-depth investigations of the pathogenesis commenced in the United States in the late 1960s and in recent years has taken place largely in France and Scandinavia.

CANINE SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction

The first suggestion that SLE occurred occur in dogs appeared in the 1960s with the work of Lewis and colleagues [2]. Seven cases were described, all of which suffered from autoimmune hemolytic anemia, and six of which in addition had immune-mediated thrombocytopenia and evidence of renal disease. A further report [3] detailed a case of erosive polyarthritis, which in addition suffered from anemia, leucopenia, amyloidosis, pleuritis, and whose serum had antinuclear antibodies (ANA). These workers utilized dogs suffering from SLE to create the first of three breeding colonies, details of which have appeared over the years [4] and also to conduct classical transmission experiments with cell-free filtrates [5].

Clinical Signs

As in humans, canine SLE is a multisystem disease, and the systems can be affected in random association and in random sequence. However, the appearance of

three or more signs simultaneously generally implies a poor prognosis [6]. The different studies, however, report varying incidence of the clinical signs, which is probably a reflection of the clinical bias of the clinician to whom the patients are presented. For example, the dermatologist is likely to receive a higher proportion of cases with skin disease, the orthopedic specialist will see mostly patients with joint disease, and the hematologist will see predominantly animals with anemia or thrombocytopenia. As in humans, canine SLE generally takes a chronic course, with periods of acute or subacute disease interspersed with periods of mild disease or clinical remission.

Joint Disease

Overall, this appears to be the most common presenting sign and is present in 40 to >90% of patients, and is multiarticular and generally symmetrical and nondeforming [1, 7–12]. The arthritis is almost always nonerosive, with <2% of cases being erosive arthritis. Affected animals have a sudden or gradual onset of generalized stiffness, and involved joints may be swollen and painful. In some advanced cases the temporomandibular joints become involved, leading to pain on eating and pronounced muscular wasting of the temporal muscles. Radiographic evidence of a bony change is usually minor to absent, with soft tissue swelling alone often being evident. Synovial fluid aspirates contain elevated neutrophil counts, and occasional LE cells are seen (Fig. 1). Histopathology of the synovial membrane reveals hypertrophy and hyperplasia of the lining cells, and a mixed cellular infiltrate with mononuclear cells predominating. Vasculitis is also commonly seen.

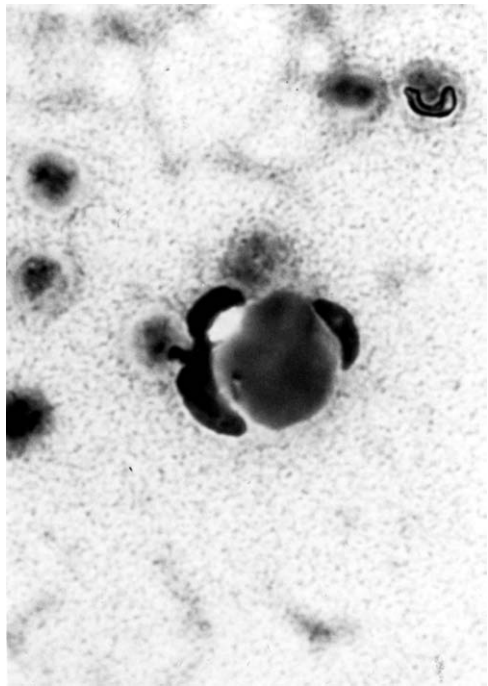


FIGURE 1 LE cell from the joint fluid of a dog with SLE-associated arthritis. Courtesy of Dr. D. Meyer, University of Florida. Reproduced from Halliwell, R.E.W., and Gorman, N.T. (1989). "Veterinary Clinical Immunology," with permission.

Polymyositis

This may accompany arthritis and, unless biopsies are taken routinely, may escape diagnosis. Occasionally it may be severe and lead to pronounced muscular atrophy, often involving the temporal muscles. It is reported in some 5% of cases.

When associated with arthritis of the temporomandibular joints, the resultant muscular wasting can be profound.

Skin Disease

The appearance of skin disease is highly variable and ranges from trivial, mild, alopecic, scarring lesion to severe and widespread ulceration [13]. Symmetry is a striking feature. Some cases present with a pruritic, generalized seborrhoeic dermatitis. In others, ulceration of the mucocutaneous junctions is apparent, which may spread to involve other parts of the body. Ulcerative and/or hyperkeratotic footpads may be seen. Focal areas of alopecia are often noted, which may extend to involve large areas. Apparent photosensitization may occur, with a history of lesions involving the hairless areas of the body flaring up following sunlight exposure. Loss of pigment from the nose and the eyelids may be apparent, and facial edema is sometimes noted. Some cases proceed to severe ulceration of the nose (Fig. 2).

Although vesiculation with subsequent ulceration is not reported as a feature of the cutaneous signs of canine SLE, a recent report features a bichon frisé with severe erosive lesions on the right elbow, axilla, and lateral thorax [14] (Fig 3). Histopathology revealed subepidermal clefting with an absence of dermal inflammation. Immunological studies revealed a circulating antibasement membrane antibody in a titer of >20,000, and the serum bound to the NC1 domain of recombinant human type VII collagen. In addition, the patient suffered from persistant proteinuria, a Coombs'-positive anemia, thrombocytopenia, pleuritis, and hepatitis. The authors proposed that this represented the canine equivalent of type 1 bullous SLE of humans [15]. Another report details a case of SLE showing a

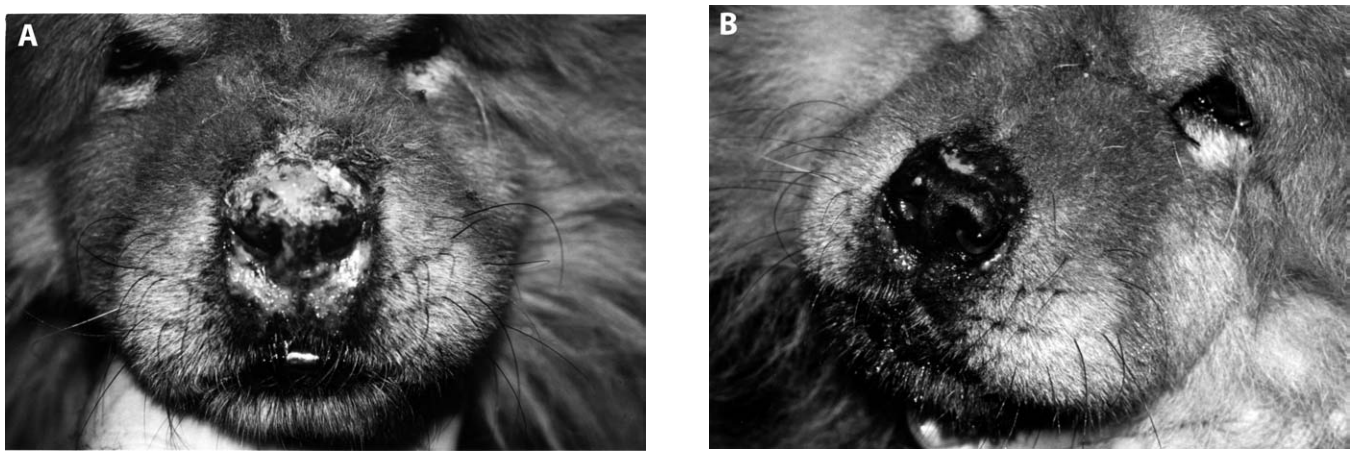


FIGURE 2 Depigmentation and ulceration of the nose of a chow (a). The dog also had oral ulcers and arthritis. There was a good response to prednisolone and azathioprine within 8 weeks (b).

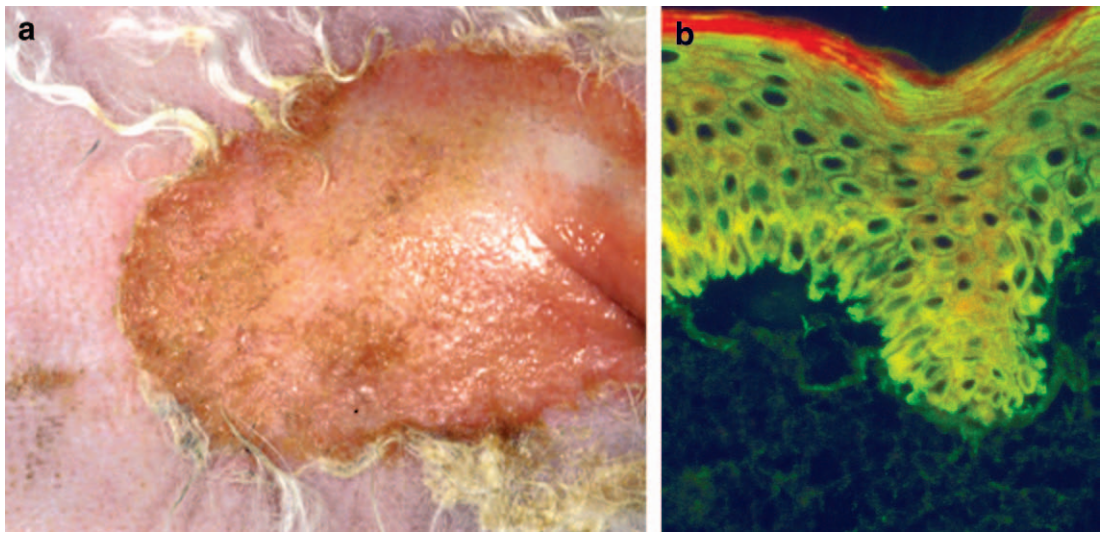


FIGURE 3 (a) Ulcerative lesions in a bichon frisé suffering from type 1 bullous SLE. (b) Indirect immunofluorescence in a case of type 1 bullous SLE using canine salt-spit lip. From Olivry *et al.* [4], with permission.

Coombs'-positive anemia, thrombocytopenia, and polyarthritis with an ANA titer of 10,240 [16]. However, instead of cutaneous signs of SLE, the patient had both clinical and histopathological signs suggestive of pemphigus foliaceus. Immunohistochemistry revealed desmosomal deposition of IgG. The dog subsequently developed a lymphoma. This appears to represent concomitant SLE and pemphigus—a phenomenon also reported occasionally in humans [15].

Histopathology of the cutaneous changes in SLE is ordinarily characterized by a lichenoid, largely lymphoid infiltrate that hugs the dermoepidermal junction (Fig. 4) [13, 17, 18]. Vacuolar alteration and apoptosis of basal cells are major diagnostic criteria, leading to the formation of colloid or Civatte bodies. Occasionally, this may lead to intraepidermal pustules. A leucocytoclastic vasculitis may be seen in the dermis, and thickening of the basement membrane with pigmentary incontinence is often a feature of chronic cases. Immunofluorescent studies reveal immunoglobulin deposits at the dermoepidermal junction (Fig. 5).

Dogs also suffer from discoid LE, or cutaneous LE, in which skin signs alone are noted and in which ANA is absent [19]. The predominant part of the body affected is the nose, with initial depigmentation being followed by erythema and ulceration (Fig. 6). Lesions are exacerbated by sunlight. Histopathology is similar to that of skin lesions in SLE, and immunofluorescent

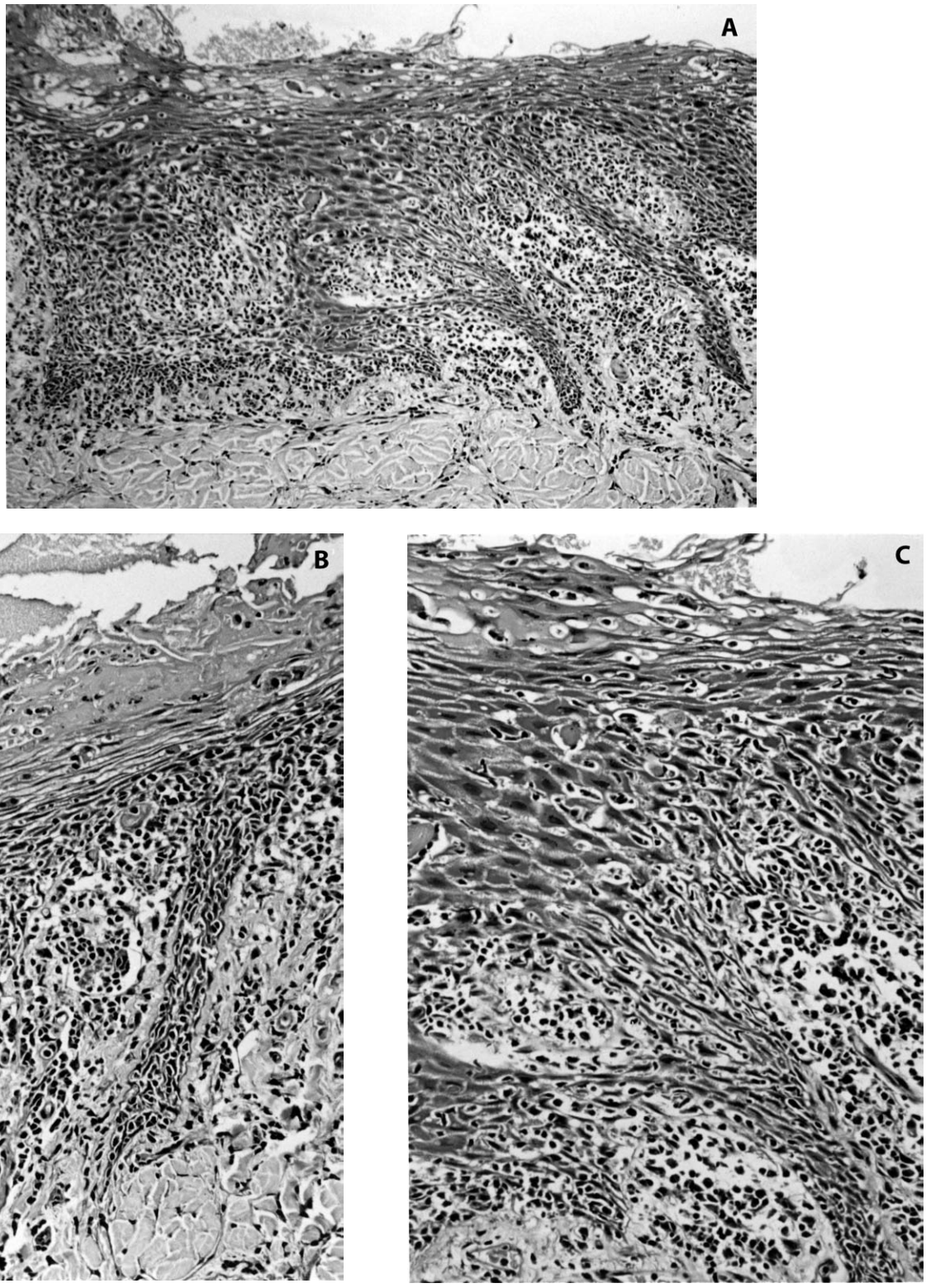


FIGURE 4 Histopathology in a case of SLE (a, ×100; b, ×200; c, ×450). There is a striking mononuclear cell infiltrate below the demoepidermal functions, and hydropic charge with disruption in the epidermis. Courtesy of Dr. R. Else, University of Edinburgh.

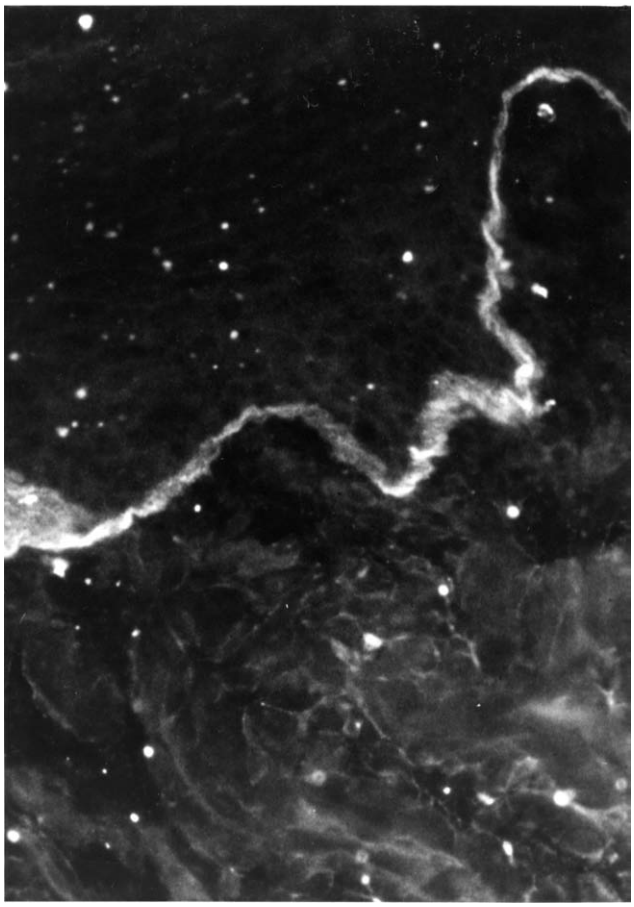


FIGURE 5 Immunoglobulin deposits at the dermoepidermal junction in a case of SLE. Courtesy of Dr. L. Werner, University of California. Reproduced from Halliwell, R.E.W., and Gorman, N.T. (1989). "Veterinary Clinical Immunology," with permission.

studies reveal immunoglobulin deposits at the dermoepidermal junction.

An ulcerative dermatitis of Shetland sheepdogs and rough collies appears to represent a vesicular variant of cutaneous LE [20]. Affected animals showed ulceration of the hairless portion of the ventral abdomen and chest that was exacerbated in the summer (Fig. 7). Some also showed buccal ulceration. There were no systemic signs, except for evidence of granular casts in 4/8 cases that could represent early renal disease. Antinuclear antibody tests were negative, and the presence or absence of dermoepidermal junction immunoglobulin deposits was not examined.

Ulcerative Stomatitis

This is a feature of between 10 and 20% of cases. It may involve buccal mucosal surfaces, the hard or soft palate, and less frequently the tongue. Histopathology

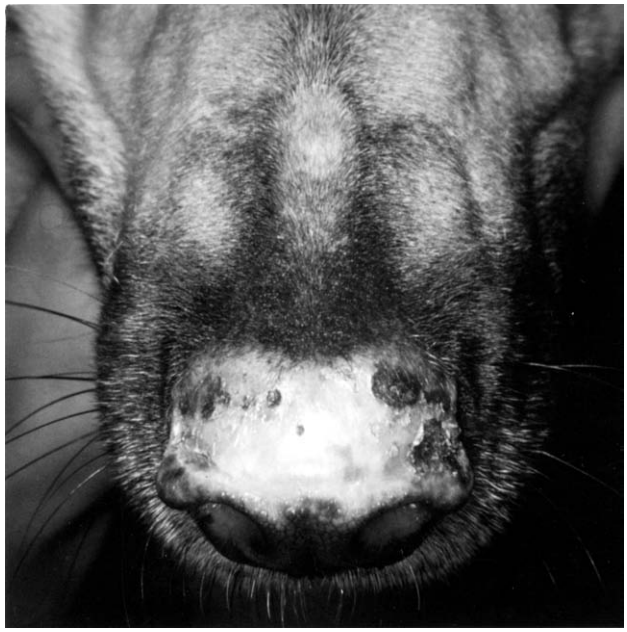


FIGURE 6 Discoid LE in a dog. The depigmentation is followed by erythema and inflammation. The disease was exacerbated by sunlight, but ANA was negative and there were no other systemic signs.

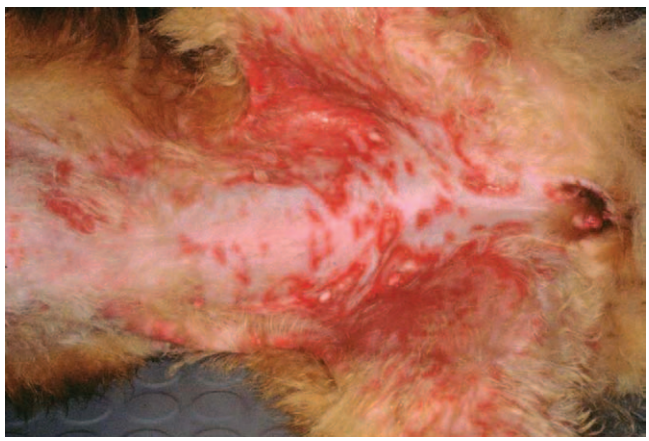


FIGURE 7 Vesicular cutaneous LE in a Shetland sheepdog. From Jackson and Olivry [20], with permission.

of the affected areas reveals a similar picture to that seen in the skin, with an intense mononuclear cell infiltrate submucosally.

Hematological Changes

Some 30–60% of cases are presented with anemia. Of these cases, approximately half are apparently immune mediated, showing evidence of hemolysis with positive direct Coombs' tests or autoagglutination. The remaining patients are apparently suffering from anemia of

chronic disease. Leucopenia and leucocytosis are seen with approximately the same frequency, affecting 20–30% of cases each. In a proportion of the cases with leucopenia, the lymphoid series is preferentially reduced, leading to a marked lymphopenia.

In some cases of SLE, thrombocytopenia may be the initial cause for presentation to the veterinarian [21]. This may be manifested as petechial or ecchymotic hemorrhages, bleeding from any of the natural orifices, or excessive bleeding following minor surgery.

Renal Changes

Profuse proteinuria, as assessed by a four-plus reaction by the sulfosalicylic acid method, or quantitatively at a level >0.5 g/liter, is present in some 50% of cases. Azotemia may be present on presentation and is a frequent and often life-threatening complication of severe cases. Biopsies usually reveal a proliferative, membranous glomerulonephritis, and immune complexes are revealed readily using fluorescein-conjugated anti-IgG and anti-C3 (Fig. 8).

FIGURE 8 Immunoglobulin deposits in the renal glomerulus. Glomerulonephritis is associated with a case of SLE.

Neurological Abnormalities

These are recorded more rarely than in humans; however, psychological and behavioral abnormalities are identified less readily in dogs and may pass unnoticed or be ascribed to other causes. Polyneuropathies characterized by hyperesthesia, convulsions, and/or obvious behavioral changes are noted in some 2–5% of cases.

Lymphadenopathy

Lymphadenopathy is detectable in some 50% of cases and generally affects, symmetrically, the majority of the palpable nodes. It is often accompanied by splenomegaly.

Pleuritis/Pericarditis

This is not an easy diagnosis in dogs. It is reported as affecting some 5–10% of cases, but this is possibly an underestimate [7].

Pulmonary Disease

A chronic pneumonia has occasionally been reported, but is a rare complication [7].

Fever

Fever is present in a high proportion of cases, and indeed some authors contend that it is invariably present [6]. However, it tends to be intermittent and cyclical, and so may be missed unless repeated examinations are made. Characteristically, it is quite briskly responsive to corticosteroids.

Incidence of the Various Changes

Table 1 contains a composite of the various clinical and pathological changes as reported by seven studies over the years. In some studies, details given were less than in others, in which case the lack of information is given as not reported.

Immunological Findings

It is generally held that a diagnosis of SLE is untenable without serological evidence of antinuclear antibody activity. A variety of techniques have been employed to demonstrate this, and some studies have painstakingly addressed the antigenic specificity of the antibody activity.

TABLE 1 Incidence of Clinical and Pathological Sings in Canine SLE Derived from Published Case Studies

Criterion	Reference							Incidence[a] (%)
	17 (*n* = 29)	6 (*n* = 29)	7 (*n* = 30)	8 (*n* = 42)	9 (*n* = 13)	12 (*n* = 26)	11 (*n* = 75)	
Arthritis	17	29	23	25	13	10	69	76.2
Skin disease	13	3	9	19	4	14	45	43.8
Glomerulonephritis	6	15	7	22	2	12	49	46.3
Fever	12	NR[b]	28	NR	NR	8	75[c]	80.4
Central nervous system signs	2	3	0	4	1	0	1	4.5
Pleuritis	0	3	1	NR	1	0	6	5.4
Polymyositis	0	1	2	0	0	2	6	4.5
Pneumonia	0	1	2	0	0	2	2	2.9
Oral ulcers	0	0	5	7	0	10	8	12.2
Hematological signs								
Anemia	8	7	NR	25	5	8	10	29.4
Leucopenia	4	NR	NR	NR	NR	NR	15	14.4
Thrombocytopenia	4	1	NR	7	4	2	3	9.8

[a] Incidence as a percentage of those reports detailing that criterion.
[b] Not recorded.
[c] In this report, although fever was seen in 100% of cases, it was cyclical and not always present.

LE Cell Phenomenon

In earlier studies, the LE preparation, using either clotted or heparinized blood, was generally employed. However, difficulties in identifying LE cells accurately and a perceived lack of sensitivity in some hands have meant that this approach has been gradually abandoned.

Antinuclear Antibody by Immunofluorescence

Substrates employed have included mouse leucocytes, mouse and rat liver, and various tissue culture cell substrates, notably HeLa, Vero, and HEp-2 cells. The reported sensitivity varies between laboratories and with the substrate employed.

It is generally held that positive ANA are encountered in virtually every case of canine SLE—indeed, some hold that a definitive diagnosis of SLE in dogs is not tenable in the absence of supportive serology. However, it is also important to emphasize that, as in humans, positive ANA is by no means restricted to dogs with SLE. For example, positive ANAs are frequently encountered in leishmaniosis [22], a condition also associated with other immunological abnormalities, including circulating immune complexes and lymphadenoapthy. The dermatological signs of leishmaniosis are also strikingly similar to those seen in SLE.

As also is the case in humans, positive ANA may be found in sera from normal dogs. In one study, the incidence of ANA was assessed in sera from 100 normal dogs presented for routine heartworm prophylaxis using HeLa cells [23]. Two of these showed a titer of 320 and two of 80. In 19 animals a titer of 10 was recorded, 3 had a titer of 20, and 1 of 40. Forty-seven were negative when assessed undiluted. Another study examined the incidence in a line-bred colony of English cocker spaniels in which there was a high incidence of cardiomyopathy and in which the existence of at least one case of SLE had been confirmed [24]. Using Vero cells, the incidence in diseased dogs ranged from 47 to 60%. The incidence in 58 normal dogs in the same kennel was 46.5%. Antibody titers ranged from 10 to 2560. In contrast, none of 25 sera from English cockers from a control kennel yielded positive results.

A number of different patterns are seen. Commonest are homogeneous patterns and speckled patterns (which may be either fine or coarse), followed by homogeneous/speckled and homogeneous with large dots [25]. The large dot pattern is observed only when mouse leucocytes are employed as the substrate. However, in reality, a spectrum of patterns is seen, which in some instances fall descriptively in between. Membrane fluorescence is quite rare. Nucleolar fluorescence is occasionally noted, but is more often associated with unclassified immune-mediated diseases than with classical SLE.

Antigen Specificities of Canine ANA

Double-Stranded (ds)DNA

One of the greatest controversies in the field of canine SLE pertains to the frequency of occurrence of anti-dsDNA antibodies. It is now generally accepted that they are rarely encountered. The problem arises largely from the existence of an acidic β-globulin, which is not antibody and which binds to DNA and also to the synthetic double-stranded DNA analogue poly-deoxyadenylate-deoxythymidylate (dAdT) [26]. This protein is absent in primate sera, but has been found in bovine, equine, feline, mink, and canine serum. The highest levels of around 100 μg/ml are found in the latter species. This leads to false-positive reactions when techniques based on the Farr assay are employed [26–28].

Although the exact nature of this protein is still unknown, some features have been elucidated. It is heat labile and destroyed by heating for 30 min at 60°C, but not at 58°C [29]. It does not bind to protein A, would not appear to be a glycoprotein, as evidenced by a failure to bind to lentil lectin, and is partially inhibited by the addition of EDTA and more completely by the addition of dextran sulfate [29]. The interference can also be inhibited by the addition of sodium dodecyl sulfate [27] and by the use of increased ionic strength and pH of the buffers [30]. However, the use of the latter technique did give higher binding of sera from animals with not only SLE, but a variety of inflammatory diseases compared to normals. However, positive binding to dsDNA was not always correlated with binding to dAdT, and *vice versa.*

The detection of anti-dsDNA in canine sera is, therefore, not a simple matter. Other detection systems have included indirect immunofluorescence employing *Crithidia luciliae* or *Trypanosoma brucei* and ELISA techniques employing highly purified dsDNA. In all studies, immunofluorescent techniques show at best a low incidence of weak positive reactions using sera from dogs fulfilling the criteria of the American College of Rheumatology (ACR), although one study indicated weak positive reactions in 4/8 [31]. In two more studies using a combination of indirect immunofluorescence and ELISA, positive reactions were seen in only 2/100 and 0/43, respectively [25, 32], and reactions in ELISA were concordant with those using indirect immunofluorescence. It has been suggested that the DNA-binding protein in normal canine sera could, in addition to interfering with the Farr assay, also interfere with the binding of specific anti-dsDNA antibodies in an ELISA. However, in a study that addressed this question, none of the canine sera assayed were able to inhibit binding of a murine anti-DNA monoclonal antibody [32]. However, one study employing a commercial ELISA system showed relatively high DNA binding in sera from dogs with SLE, and to a lesser extent from dogs with other arthropathies [33]. These differences would appear irreconcilable on the basis of sample differences. The latter authors did, however, note that sera that gave high dsDNA-binding values also bound strongly to single-stranded (ss)DNA, which could suggest a possible contamination of the dsDNA substrate with ssDNA [33].

ssDNA

Demonstration of the presence of anti-ssDNA is of no value in the diagnosis of SLE in dogs. In one study, sera from 21% of 100 dogs with SLE gave positive results, as did 14% of dogs with positive ANA but less than four criteria for SLE, and 26.8% of 56 dogs with miscellaneous infectious diseases, including leishmaniosis [25]. This emphasizes the absolute necessity of utilizing highly purified and stable dsDNA preparations for all assays involving canine serum.

Histones

The presence of antihistone antibodies is highly correlated with a positive diagnosis of SLE [25, 32–35]. In the most extensive study, 71/100 sera from patients fulfilling at least four ACR criteria gave positive results, as compared with 6.7% of 120 normals [25]. In the case of dogs with one or more, but less than four criteria for SLE, 38% of 400 sera gave positive results. A number of these, of course, could later have fulfilled the four criteria. Of the 56 dogs with miscellaneous infectious diseases, 17.9% gave positive results.

The pattern of recognition, however, differs from that in humans. Using immunoblots, 54% of sera from dogs with SLE recognized H4, with the same number recognizing H3. Eight percent recognized H1, 22% H2A, and 20% H2B [25]. In another study, antibodies to H2B were not detected in any of 43 sera from dogs with SLE [32]. In contrast, H1 and H2B are more prominent autoantigens in humans [36, 37]. A further difference is that the histone determinants against which canine antibodies are directed are mostly trypsin resistant [25].

Other Antigens Recognized

Many of the other antigens that are recognized by sera from human patients with SLE are also recognized by canine sera. Some 7% of sera from dogs with SLE precipitate ribonucleoprotein (RNP), and a further 12% detected both RNP and Sm antigen [25]. High-motility group (HMG) proteins were recognized by 20% of sera, with HMG1 detected by 6% and HMG2 by 18%. No sera recognized HMG14 and HMG17. In

the aforementioned study, three sera also contained anti-Sjorgren's syndrome A antibody (anti-SSA), but anti-SSB has yet to be detected.

Of great interest are antibodies with a specificity that appears to be unique for sera from canine patients with SLE, which were initially termed anti-type 1 (or T1) and anti-type 2 (or T2) [25, 34]. These are reactive with soluble nuclear extracts, but the T2 antigen is absent from extracted nuclear antigen preparations. Anti-T1 is directed against a major nuclear antigen of 43 kDa [25]. Studies have confirmed that this is identical with the 43 kDa glycoprotein recognized by some canine SLE sera in the studies of Soulard *et al*. [38]. This has now been identified as hnRNP G [39]. Interestingly, many sera from dogs with SLE also react with a ribosomal antigen of identical molecular weight [40].

Antinucleolar Antibody

Four cases, with clinical signs compatible with SLE, have been reported in which a high-titer antinucleolar antibody was detected. In three of these, polyarthritis was a major presenting sign [41], although one case had concomitant immune-mediated thrombocytopenia, anemia, leucopenia, and skin eruption [42]. In view of the paucity of cases, assumptions regarding the disease associations cannot be made. The antibody specificity has been examined, and sera detect three polypeptides of 110,000, 95,000, and 45,000 kDa, which are antigenically cross-reactive [41].

Antibody Activity and Staining Patterns

A number of studies have attempted to correlate specific antibody activity with staining patterns. Early studies had demonstrated that anti-T1 (hnRNP G) gives a relatively fine speckled pattern, anti-Sm and anti-RNP give a coarser speckled, or reticulonodular pattern, and the antihistone antibody gives a generally homogeneous pattern [25].

More recent studies have employed immunodiffusion [43], ELISA, and immunoblots [44]. It was first shown that sera that exhibit chromosomal reactivity exhibit a homogeneous nuclear fluorescence pattern and fail to precipitate on immunodiffusion analysis using commercially available ENA preparations. Sera that show negative chromosomal reactivity are more likely to exhibit speckled patterns, and a proportion are positive on immunodiffusion. Lines of identity with human reactive sera were shown in the case of anti-RNP and anti-Sm only [43]. Some sera precipitated, showing partial identity with the homologous human protein, and in others there was a complete lack of identity. The autoantigen recognized most frequently in ELISA and immunoblots was hnRNP G—an antigen absent from the commercially available extract made for the examination of human sera. Examination of anti-RNP activity confirmed that canine sera react with the same full major antigenic region of the 70 K protein as human sera [44]. However, attempts to correlate staining patterns and/or antibody specificity with disease expression have thus far proved unsuccessful.

T-Cell Subsets in Canine SLE

A study of 20 dogs with SLE in differing stages showed striking abnormalities in lymphocytes and in T-cell subsets [45]. First, animals in the active phase showed marked lymphopenia, with mean counts of 1050×10^6/liter as compared with 2130×10^6/liter in controls. In the former, $CD4^+$ and $CD8^+$ cells comprised a mean of 56.7 and 10.9%, respectively. Comparative data for the controls were 40.5 and 18%, which implies a striking deficiency of cytotoxic/suppressor cells. Moreover, the percentage of $2B3^+$, or activated T cells, increased in the active phases of the disease from a mean of 46.5 to 64.1%. A combination treatment with prednisone and levamisole was quite effective in inducing long-term remission, and successful therapy was associated with normalization of the $CD4^+$:$CD8^+$ ratio.

Canine MHC Antigens, C4 Allotypes, and SLE

Possible associations between SLE and MHC class I and II antigens, as well as associations with C4 allotypes, have been investigated in two breeding colonies in which there is a high incidence of SLE [46]. There was a significant positive association of SLE with DLA-A7 (relative risk 11.93) and negative associations with both DLA-A1 and DLA-B5 (relative risks 0.06 and 0.05). No associations were found with class II antigens, and there were no associations with the C4 allotype. The latter finding contrasts with a previous study on eight dogs with polyarthritis, all of who had positive ANA, but only two of which satisfied the current ACR criteria for SLE [47]. Five (out of five studied) had the same C4 allotype, and three had markedly lowered C4 levels, which rose following corticosteroid treatment.

Lupus Anticoagulant

Although commonly encountered at some point in cases of SLE in humans, tests for the presence of this abnormality are not undertaken routinely in veterinary medicine. The literature lists just one case in a dog [48], with a further case in a cat [49].

Antithyroglobulin Antibodies

Hypothyroidism as a result of lymphocytic thyroiditis is a relatively common disease in dogs, and the pres-

ence of antithyroglobulin antibodies is a useful diagnostic aid [50]. However, although thyroiditis has been reported in cases of SLE [24], there have been studies that have investigated its incidence systematically.

Epidemiology of Canine SLE: Lessons for Human SLE

Breed and Sex Incidence

In most of the studies, the German shepherd breed appears to be overrepresented, and in a study at Lyon, France, German shepherds comprised 46.7% of 75 cases of SLE fulfilling at least four ACR criteria, as opposed to a 25% incidence of this breed in the base population [12]. In another study of 58 dogs in California, in which the diagnosis was aided by the application of log-linear and logistic regression models in disease prediction, 17.4% of 58 cases were German shepherds, as compared with a base clinic population of 7.8% ($P < 0.01$) [51].

The existence of a sex bias is controversial. A complicating factor in this analysis is that many bitches are spayed, and the proportion of spayed to intact females is not always noted. Also, to a lesser extent, some males may be castrated. At all events, most studies fail to show a female sex bias, which is in sharp contrast to cases of immune-mediated thrombocytopenia and autoimmune hemolytic anemia, occurring as single entities, in which a higher proportion of females than males is reported [1]. The only study showing a female predisposition was that of Drazner [8] in which 23/30 affected animals were female. However, two important points relating to the latter study are that some animal diagnosed as suffering from SLE satisfied only two ACR criteria, and it was also noteworthy that only 3 out of the 23 females were spayed. A male sex bias was shown in both the French study (51 males vs 24 females) and the Californian study in which intact males were significantly overrepresented ($P < 0.025$) [12, 51].

The Development of Breeding Colonies

A clear advantage offered by research using spontaneous animal models for human diseases is the ability to selectively inbreed such animals, and thus explore genetic aspects, as well as enabling the production of more clinical material. It can be argued that such models, occurring in an outbred animal, have more relevance to the human disease than do, for example, disease models employing highly inbred strains of mice.

The first of the canine SLE colonies was established by Lewis and Schwartz [4] in Boston by mating two dogs with SLE. The first significant conclusion was that the incidence of positive ANA could not be accounted for by any conventional mechanism of inheritance, and the authors hypothesized that it was the result of vertical transmission of an infective agent in a genetically susceptible animal. However, for many years, no animal in the colony developed disease, despite having high-titer ANA. Finally, after 10 years, and involving five inbred generations, SLE, autoimmune thyroiditis, and Sjogren's syndrome were all diagnosed [52].

Another colony was established by Monier and colleagues in France, starting with the mating of an affected German shepherd to an affected Belgium shepherd [53]. Both the incidence of positive ANA and clinical signs increased with each inbred generation. In the F1 family, the incidence of positive ANA and of clinical signs was 3/6 and 0/6, respectively. In the F2 it was 2/4 and 1/4, and in the F3 it was 5/6 and 4/6. Lymphocytes from affected animals showed higher sensitivity to concanavalin A (Con A) in lymphocyte blastogenesis assays, reduced Con A-induced suppression, lower levels of thymulin, and resistance to the induction of immunological tolerance using intravenous aggregate-free human IgG. In general, the appearance of ANA preceded that of clinical signs—in one case by as much as 3.8 years.

Hubert and colleagues [54] examined another colony of German shepherds that were being bred for show and sale. In contrast to the earlier study, the incidence of ANA and of clinical signs was reduced in each generation, which could be ascribed to the use of outbreeding rather than inbreeding once the clinical signs had become evident. It was of interest that of the 34 dogs that showed one or more ACR criterion for SLE, 22 were males and 12 were females. It was also noted that the appearance of the different clinical signs could be spread over some time. For example, in one animal, arthritis developed 16 months prior to the onset of proteinuria and cutaneous manifestations [54].

Conclusions of the three studies involving artificial or spontaneous breeding colonies are that the occurrence of SLE cannot be ascribed to genetic influences alone and that environmental influences must also play a role.

Possible Role of Transmissible Agents

The early investigations of Lewis and colleagues provided convincing evidence that serological abnormalities could be transmitted by a filterable agent [5]. Cell-free extracts of spleens from the offspring of dogs with SLE were injected into newborn puppies and into mice and rats. The dogs and the mice, but none of the rats, developed ANA. Some of the mice developed lymphoma, and cell-free extracts from the tumors induced

ANA when injected back into puppies. None of the animals, however, developed SLE. The occasional finding of normal animals with high-titer ANA and anecdotal reports of clusters of both dogs and cats with high-titer ANA are also supportive of the existence of a transmissible agent.

This has naturally led to a search for possible evidence of transmission of serological abnormalities from humans to dogs, and vice versa. The first report concerned two dogs living in a household where a number of family members had SLE [55]. Skin disease and arthritis were noted in the dogs, which were not subjected to detailed veterinary investigations. Both had anti-dsDNA when serum was subjected to the Farr assay.

The implication that a possible transmissible agent might be crossing the species barrier was refuted by three later better controlled studies involving investigation of a broader spectrum of autoantibodies and a greater number of individuals of both species [56–58].

The controversy was reawakened by study in which sera from 15 dogs living with patients with SLE were examined and compared with sera from 9 dogs with various immune-mediated diseases, not all of which were autoimmune in nature. Controls consisted of 10 laboratory-reared and -held Labrador retrievers and beagles [59]. The mean age of 10 of the household dogs living with SLE patients was 9.1 years and that of the control group was approximately 2 years. The control group was thus not matched for breed, age, or environment. Dogs from both the immune-mediated disease group and the SLE patient's group had abnormal serum electrophoretograms using criteria that were not defined. They also had higher levels of dsDNA binding as assessed by ELISA. It was noteworthy, however, that no sera from any group contained anti-dsDNA as assessed by indirect immunofluorescence using *Crithidia luciliae* and that none of the sera from the SLE patient's dogs had positive ANA as detected by indirect immunofluorescence employing both mouse liver and Hep-2 cells. Also, surprisingly, only 1 of the 9 dogs diagnosed as suffering from immune-mediated disease had ANA. Furthermore, no animals from any group had detectable antibodies to any extractable nuclear antigens. In view of the controversy that has surrounded the detection of anti-dsDNA in the dog, it would be important to show that the DNA preparation lacked detectable ssDNA in that there was no evidence of anti-dsDNA antibody using *C. luciliae.* Clearly, one can draw no conclusions from this study.

The issue, however, still fails to go away, and recent review again draws heavily on anecdotal observations [60]. Two dogs are described that lived in the home of a rheumatologist whose mother-in-law had suffered from SLE for >35 years. One of the dogs had clinical signs compatible with SLE, a positive ANA, and elevated anti-RNP as assessed by ELISA. The other dog, also a cocker spaniel, had an ANA titer of 25 and what was described as ocular Sjogren's syndrome. The existence of a true canine analogy of Sjogren's syndrome is quite controversial, and the dog probably was suffering from keratocunjunctivitis sicca, which is quite common in dogs as a stand-alone entity and may not have an immunological basis. It is indeed possible that the dog with SLE "caught" lupus from the mother-in-law. Clearly, the family should obtain a number of further dogs to see if the phenomenon is repeatable!

Diagnosis of SLE in Dogs

Over the years, this has caused just as much controversy as it has in human medicine. Some of the original ARA criteria [61] either did not occur in the dog or were difficult to evaluate. Raynaud's phenomenon and the serologic test for syphilis were among the former, and psychoses would be among the latter. With this in mind, criteria were proposed in which the presence of two major signs (polyarthritis, dermatological signs that cannot be ascribed to any other cause, Coombs' positive anemia, thrombocytopenia, glomerulonephritis, leucopenia, and polymyositis), together with evidence of antinuclear antibody by either immunofluorescence or LE cell preparation, justified a diagnosis of definite SLE [22]. Alternatively, the diagnosis could be made with the presence of one major sign, presence of an antinuclear antibody, and two minor signs (fever of unknown origin, pleuritis or central nervous signs, particularly seizures). A diagnosis of probable SLE was proposed if there was one major, two minor signs and positive serological evidence, or two major signs without serological evidence. The more recent findings that a high proportion of cases of canine SLE apparently suffer from anemia of chronic disease, which is not immune mediated, necessitates dropping the requirement that the anemia should be Coombs' positive. This approach has been found to have a high level of specificity and of sensitivity [49].

A better approach, however, is to adapt the revised ACR criteria [62] to the veterinary context as done by Chabanne and colleagues [63]. The major change is that criteria under immunological disorders are changed to incorporate one or more of

- Antihistone antibody
- Anti-Sm antibody
- Anti-hnRNP G
- A decrease in $CD8^+$ cells to $<200/mm^3$ or a $CD4^+ : CD8^+$ ratio higher than 4.0

The ANA specificities just noted are those associated most reliably with SLE. This approach has been widely commended and appears to represent the preferred way forward.

There remains the problem, as in humans, that the various signs can commence at random times and in random association. It is also clear that a positive ANA is frequently detectable prior to the onset of clinical signs or, rarely, after their onset [54]. However, although the use of such criteria is essential for defining the disease for the publication of data, clinicians continually encounter cases of immune-mediated disease covering a wide spectrum that require aggressive therapy, but whose precise nature will never be defined.

Treatment

Therapy of dogs with SLE generally involves the use of corticosteroids, initially in high doses, perhaps with concomitant use of cytotoxic drugs, such as azathioprine or chlorambucil. In cases where immune-mediated thromobocytopenia is part of the presenting signs, aggressive therapy, which may include the use of vincristine, is employed. Likewise, where the presenting signs include autoimmune hemolytic anemia and there is a life-threatening rapid fall in erythrocyte counts, intravenous cyclophosphamide is often employed. The disease can wax and wane, and there is a tendency to overtreat and maintain animals on continued therapy when it may not be required.

Plasmapheresis was used on four dogs with SLE that was refractory to conventional treatment [64]. Three of the four dogs had good responses lasting from 1 to 6 months. The therapy led to an appreciable fall in the ANA titer and to an increase in the levels of C3 and total hemolytic complement. The authors recommend the treatment only for severe cases that are minimally responsive to anti-inflammatory therapy and cytotoxic drugs. However the, dramatic response seen in some cases appears to emphasize the pathogenic role of the immune complexes.

Excellent results have been reported with the combined use of prednisone with levamisole [12]. In a comparative study, one group of dogs received initial treatment with 1–2 mg/kg of prednisone, which was gradually tapered once control was affected. The other received the same dose of prednisone with levamisole orally at a dose of 3–7 mg/kg/day on alternate days. The first group, continued to receive minimal doses of corticosteroids for maintenance, whereas in the case of the second group, prednisone was discontinued, with levamisole alone used when there was a recrudescence. In the group treated with corticosteroids alone, clinical improvement always occurred, but it was not maintained when prednisone was withdrawn. In the group in which combined therapy was used, prolonged remission was obtained in >50% of cases, which was as long as 9 years. When relapses occurred, levamisole alone was effective in achieving a further sustained remission. Successful treatment with levamisole caused a normalization of the lymphopenia, as well as a normalization in the abnormal $CD4^+$:$CD8^+$ ratio [45], making it tempting to speculate a cause-and-effect relationship between the disease and the underlying abnormalities in T-cell subsets.

SYSTEMIC LUPUS ERYTHEMATOSUS IN CATS

Introduction

As discussed, the dog suffers from a disease that is strikingly similar to that of humans. However, the situation with the cat is not so clear. Nonetheless, cats with a broad spectrum of clinical signs are encountered that are compatible with those found in SLE in humans and dogs; in some instances, the association of a number of these in the same patient is highly suggestive of the existence of SLE in this species.

In addition to the spontaneous disease, cats are of particular interest in the area of drug-induced lupus. This species suffers relatively commonly from hyperthyroidism, the etiology of which is obscure. Of the therapeutic approaches available, the use of propothiouracil leads to a high incidence of side effects in the form of a lupus-like syndrome [65]. In experimental studies, 53% of cats given 150 mg daily became ill within 4–8 weeks, manifesting weight loss and lymphadenopathy. Affected cats also showed a Coombs'-positive anemia and positive ANA [66]. It appears that the sulfur atom is critical in this phenomenon, as administration of propouracil is without effect. Drug-induced serological abnormalities have also been recognized in dogs following treatment with hydralazine, but in the latter case no clinical signs resulted [67].

Clinical Signs

The first report of an SLE-like disease in the cat appeared in 1971 and was described as a case of immune complex glomerulonephritis, which was accompanied by a positive LE cell preparation [68]. This was followed by papers detailing a cat with autoimmune hemolytic anemia [69] and one with immune-mediated thrombocytopenia [70], both accompanied by positive LE cell preparations

Scott and co-authors [71] described two cats whose presenting signs were dermatological and whose sera

showed low-titer positive ANA tests (10 and 40). One case was presented with recurrent fever and lymphadenopathy, paronychia, and a symmetric crusting alopecic rash involving the head and ears. Direct immunofluorescence revealed immunoglobulin deposits at the dermoepidermal junction. The second case had an extensive, ulcerative, crusting skin disease and anemia. Histopathology revealed striking hydropic degeneration of the epidermal basal cells.

A further report discussed results of ANA screening of sera from 107 cats presented to the University of Florida College of Veterinary Medicine over a 4-year period with signs suggestive of immune-mediated disease [72]. Five sera yielded titers >10. Two of the cats had ulcerative plasma cell pododermatitis accompanied by glomerulonephritis and anemia. Two other cats showed skin lesions that were histologically compatible with pemphigus foliaceous—one of which showed linear deposits of immunoglobulin at the dermoepidermal junction. The final case had a history of progressive, erosive polyarthritis and later developed erosive nasal lesions, perioccular erythema, and glomerulonephritis. Three of these cases satisfied the revised ACR criteria for a diagnosis of SLE.

Another report described 11 cats with a wide range of clinical signs, including various neurological and behavioral changes, glomerulonephritis, nonerosive arthritis, anemia, stomatitis, lymphadenopathy, and a range of skin changes [73]. Six of these cats satisfied the revised ACR criteria by showing at least four classical signs. ANA titers ranged from 40 to 1280.

The latest reports feature (i) a cat with symmetrical facial dermatitis (Fig. 9), thrombocytopenia, and an ANA titer of 160 [74] and (ii) a cat with autoimmune hemolytic anemia, thrombocytopenia, probable neurologic disease, and an ANA titer of 4096 [49]. The latter case did not fulfill the criteria for definite SLE. Histopathology of the skin in the former case showed an interface dermatitis and folliculitis with occasional basal cell vacuolation and necrosis and pigmentary incontinence. The cat responded to glucocorticoid therapy, but the skin disease relapsed following cessation of the treatment with, in addition, ulceration involving the hard palate. It was concluded that the cat had now satisfied four of the ACR criteria.

A summary of the incidence of the clinical signs ascribed to feline lupus is detailed in Table 2.

FIGURE 9 Facial lesions in a cat with SLE. From Vitale *et al.* [74], with permission.

TABLE 2 Feline Systemic Lupus Erythematosus: Incidence of Clinical Sings from Case Reports in the Literature ($n = 23$)[a]

Glomerulonephritis	10
Neurological signs	10
Arthritis[b]	9
Anemia	10
Dermatological signs	8
Fever	4
Lymphadenopathy	3
Buccal or mucucutaneous ulcers	4
Thrombocytopenia	3

[a] Data from Refs. 49, 54–59, and 74.
[b] In some cases, no clinical signs were evident, but the diagnosis was made by joint tap cytology.

Diagnosis and Therapy

The approach to the diagnosis in the cat follows broadly the same principles as outlined for the dog. However, care must be taken to exclude two feline viral diseases, namely feline leukemia and feline infectious peritonitis, both of which can induce positive ANA tests [72].

High-dose prednisone or prednisolone represents the cornerstone of the therapeutic approach, with the addition of cyclophosphamide, chlorambucil, or gold salts if the former approach alone proves insufficient or where an alternate day regimen for the corticosteroids cannot be obtained. In comparison to dogs, cats are relatively resistant to the side effects of corticosteroids, which may reflect differences in plasma binding or in

FIGURE 10 Facial lesions of a horse with cutaneous LE. From Stannard [76], with permission.

tissue receptors. This also means that generally higher levels are required than in dogs.

SYSTEMIC LUPUS ERYTHEMATOSUS IN HORSES

SLE is rarely reported in horses [75]. The literature contains full descriptions of only two cases, although there are anecdotal reports of additional cases. The general clinical and dermatological signs are described elsewhere [76] and are depicted in Fig. 10.

The first report described a horse presenting with polyarthritis, proteinuria, thrombocytopenia, and a positive ANA test [77]. The second contained a very full description, with autopsy findings. The patient was presented with a history of weight loss, Coombs'-positive anemia, and bilaterally symmetrical alopecia [78]. Clinical examination in addition showed an ulcerative glossitis and generalized lymphadenopathy. Histopathological examination of skin biopsies showed a superficial and deep lymphocytic infiltrate, with hydropic degeneration and frequent single cell necrosis of the follicular and interfollicular basal cells. Immunoperoxidase staining of paraffin-embedded sections revealed linear deposits of immunoglobulin at the dermoepidermal junction, and the ANA was positive with a titer of 320.

Although the anemia responded to the administration of dexamethasone (0.2 mg/kg iv daily), the animal became recumbent and was euthanized. In addition to the findings noted premortem, an autopsy showed focal lymphoid infiltrates in the liver, a fibrinous synovitis, and a membranous glomerulonephritis accompanied by immunoglobulin deposits on the basement membrane. The diagnosis of SLE was convincingly made.

CONCLUSIONS

The current status of the knowledge of SLE in domestic animals has been presented. Particularly in the case of the dog, striking similarities with the disease in humans are evident.

A major controversy urgently in need of further investigation is the question of the possible transmission of serological abnormalities and/or clinical SLE between the species, especially between the human and the dog. It seems unfortunate that in some of the studies, veterinary involvement has been quite peripheral, which in turn has impacted negatively on the creditability of the data. Close working relationships between medical and veterinary scientists are a prerequisite for the optimal utilization of domestic animal models of human disease.

References

1. Halliwell, R. E. W. (1978). Autoimmune disease in the dog. *Adv. Vet. Sci. Comp. Med.* **22**, 221.
2. Lewis, R. M., Schwartz, R., and Henry, W. B. (1965). Canine systemic lupus erythematosus. *Blood* **25**, 143.
3. Lewis, R. M., and Hathaway, J. E. (1967). Canine systemic lupus erythematosus presenting with symmetrical polyarthritis. *J. Small Anim. Prac.* **8**, 273.
4. Lewis, R. M., and Schwartz, R. S. (1971). Canine systemic lupus erythematosus, genetic analysis of an established breeding colony. *J. Exp. Med.* **134**, 417.
5. Lewis, R. M., Schwartz, A. J., Harris, G., *et al.* (1973). Canine SLE, transmission of serological abnormalities by cell free filtrates. *J. Clin. Invest.* **52**, 1893.
6. Chabanne, L., Fournel, C., Monier, J.-C., and Rigal, D. (1999). Canine systemic lupus erythematosus. 1. Clinical and biological aspects. *Compen Continuing Ed. Prac. Vet.* **21**, 135.
7. Pedersen, N. C., Weisner, K., Castels, J. J., *et al.* (1976). Noninfectious canine arthritis: The inflammatory nonerosive arthritides. *J. Am. Vet. Med. Assoc.* **169**, 304.
8. Drazner, F. H. (1980). Systemic lupus erythematosus in the dog. *Compen Continuing Ed. Prac. Vet.* **3**, 243.
9. Grindem, C. B., and Johnson, K. H. (1983). Systemic lupus erythematosus: Literature review and report of 43 new canine cases. *J. Am. Anim. Hosp. Assoc.* **19**, 489.
10. Bennett, D. (1987). Immune-based non-erosive inflammatory joint disease of the dog. 1. Canine systemic lupus erythematosus. *J. Small Anim. Pract.* **28**, 871.
11. Thoren-Tolling, K., and Ryden, L. (1991). Serum auto antibodies and clinical/pathological features in German shepherd dogs with a lupuslike syndrome. *Acta Vet. Scand.* **32**, 15.
12. Fournel, C., Chabanne, L., and Caux, C., *et al.* (1992). Canine systemic lupus erythematosus. I. A study of 75 cases. *Lupus* **1**, 133.
13. Scott, D. W., Walton, D. K., Manning, *et al.* (1993). Canine lupus erythematosus. I. Systemic lupus erythematosus. *J. Am. Anim. Hosp. Assoc.* **19**, 461.

14. Olivry, T., Savary, K. C. M., Murphy, K. M., *et al.* (1999). Bullous systemic lupus erythematosus (type 1) in a dog. *Vet. Rec.* **145**, 165.
15. Sontheimer, R. D. (1997). The lexicon of cutaneous lupus erythematosus—A review and personal perspective on the nomenclature and classification of the cutaneous manifestations of lupus erythematosus. *Lupus* **6**, 84.
16. Foster, A. P., Sturgess, C. P., Gould, D. J., *et al.* (2000). Pemphigus foliaceus in association with systemic lupus erythematosus, and subsequent lymphoma in a cocker spaniel. *J. Small Anim. Pract.* **41**, 266.
17. Yager, J. A., and Wilcock, B. P. (1994). Systemic lupus erythematosus. *In* "Color Atlas and Text of Surgical Pathology of the Dog and Cat," pp. 95–96. Wolfe, London.
18. Gross, T. L., Ihrke, P. J., and Walder, E. J. (1992). Systemic lupus erythematosus. *In* "Veterinary Dermatopathology," pp. 24–26. Mosby, St. Louis, MO.
19. Griffin, C. E., Stannard, A. A., Ihrke, P. J., *et al.* (1979). Canine discoid lupus erythematosus. *Vet. Immunol. Immunopathol.* **1**, 79.
20. Jackson, H. A., and Olivry, T. (2001). Ulcerative dermatosis of the Shetland sheepdog and rough collie may represent a novel vesicular variant of cutaneous lupus erythematosus. *Vet. Derm.* **12**, 19.
21. Wilkins, R. J., Hurvitz, A. J., and Dodds-Laffin, W. J. (1973). Immunologically mediated thrombocytopenia in the dog. *J. Am. Vet. Med. Assoc.* **163**, 277.
22. Lucena, R., and Ginel, P. J. Immunoglobulin isotype of antinuclear antibodies in dogs with leishmaniasis. *Res. Vet. Sci.* **65**, 205.
23. Halliwell, R. E. W. (1981). Skin diseases associated with autoimmunity. II. The nonbullous autoimmune skin diseases. *Compen. Cont. Ed. Prac. Vet.* **3**, 156.
24. Day, M. J. (1996) Inheritance of serum autoantibody, reduced serum IgA and autoimmune disease in a canine breeding colony. *Vet. Immunol. Immunopathol.* **53**, 207.
25. Monier, J. C., Ritter, J., Caux, C., *et al.* (1992). Canine systemic lupus erythematosus. II. Antinuclear antibodies. *Lupus* **1**, 287.
26. Thorburn, R., Hurvitz, A. I., and Kunkel, H. G. (1972). A DNA-binding protein in the serum of certain mammalian species. *Proc. Natl. Acad. Sci. USA* **69**, 3327.
27. Monier, J. C., Dardenne, M., Rigal, D., *et al*. (1980). Clinical and laboratory features of canine lupus syndromes. *Arthritis Rheum.* **23**, 294.
28. Bennett, D., and Kirkham, D. (1987). The laboratory identification of serum antinuclear antibody in the dog. *J. Comp. Pathol.* **97**, 523.
29. Zeromski, J., Thoren-Tolling, K., Bergqvist, R., and Stejskal, V. (1984). DNA binding proteins in canine sera: A method for removal of nonspecific DNA binding in the Farr assay. *Vet. Immunol. Immunopathol.* **7**, 169.
30. Shull, R. M., Miller, H. A., and Chilina, A. R. (1983). Investigation of the nature and specificity of antinuclear antibody in dogs. *Am. J. Vet. Res.* **44**, 2004.
31. Thoren-Tolling, K., and Ryden, L. (1991). Serum auto antibodies and clinical/pathological features in German shepherd dogs with a lupus like syndrome. *Acta. Vet. Scand.* **32**, 15.
32. Monestier, M., Novick, K. E., Karam, E. T., *et al.* (1995). Autoantibodies to histone, DNA and nucleosome antigens in canine systemic lupus erythematosus. *Clin. Exp. Immunol.* **99**, 37.
33. Bell, S. C., Hughes, D. E., Bennet, D., *et al.* (1997). Analysis and significance of anti-nuclear antibodies in dogs. *Res. Vet. Sci.* **62**, 83.
34. Costa, O., Fournel, C., Lotchouang, E., *et al.* (1984). Specificities of antinuclear antibodies detected in dogs with systemic lupus erythematosus. *Vet. Immunol. Immunopathol.* **7**, 369.
35. Brinet, A., Fournel, C., Faure, J. R., *et al.* (1988). Antihistone antibodies (ELISA and immunoblot) in canine lupus erythematosus. *Clin. Exp. Immunol.* **74**, 105.
36. Costa, O., and Monier, J. C. (1986). Antihistone antibodies detected by ELISA and immunoblotting in systemic lupus erythematosus and rheumatoid arthritis. *J. Rheum.* **13**, 722.
37. Costa, O., Tchouatcha-Tchouassom, J. C., Roux, B., and Monier, J. C. (1986). Anti-H1 histone antibodies in systemic lupus erythematosus: Epitope localization after immunoblotting of chymotrypsin digested H1. *Clin. Exp. Immunol.* **63**, 608.
38. Soulard, M., Barque, J.-P., Della Valle, V., *et al.* (1991). A novel 43-kDa glycoprotein is detected in the nucleus of mammalian cells by autoantibodies from dogs with autoimmune disorders. *Exp. Cell Res.* **193**, 59.
39. Soulard, M., Dellavelle, V., Monod, G., *et al.* (1996). The 1 protein of the heterogeneous nuclear ribonucleoprotien is a novel dog nuclear autoantigen. *J. Autoimmun.* **9**, 599.
40. Absi, M., La Vergne, J. P., Marzouki, A., *et al.* (1989). Heterogeneity of ribosomal autoantibodies from human and canine connective tissue diseases. *Immunol. Lett.* **23**, 35.
41. Soulard, M., Lagaye, S., Della Valle, V., *et al.* (1989). Nucleolar proteins identified in human cells as antigens by sera from dogs with autoimmune disorders. *Exp. Cell Res.* **182**, 482.
42. Werner, L. L., Bloomberg, M. S., Calderwood Mays, M. B., and Ackerman, N. (1985). Progressive polysystemic immune-mediated disease in a dog. *Vet. Immunol. Immunopathol.* **8**, 183.
43. Hansson, H., and Karlsson-Parra, A. (1999). Canine antinuclear antibodies: Comparison of immunofluorescnece staining patterns and precipitin reactivity. *Acta. Vet. Scand.* **40**, 205.
44. Henriksson, E. W., Hansson, H., Karlsson-Parra, A., and Pettersson, I. (1998). Autoantibody profiles in canine ANA-positive sera investigated by immunoblot and ELISA. *Vet. Immunol. Immunopathol.* **61**, 157.
45. Chabanne, L., Fournel, C., Caux, C., *et al.* (1997). Abnormalities of lymphocyte subsets in canine systemic lupus erythematosus. *Autoimmunity* **22**, 1.
46. Teichner, M., Krumbacher, K., Doxiadis, I., *et al.* (1990). Systemic lupus erythematosus in dogs: Association to the major histocompatibility complex antigen DLA-A7. *Clin. Immunol. Immunopathol.* **55**, 255.

47. Day, M. J., Kay, P. H., Clark, W. T., *et al.* (1985). Complement C4 allotype association with and serum C4 concentration in an autoimmune disease in the dog. *Clin. Immunol. Immunopathol.* **35**, 85.
48. Stone, M. S., Johnstone, I. B., Brooks, M., *et al.* (1994). Lupus-type anticoagulant in a dog with hemolysis and thrombosis. *J. Vet. Intern. Med.* **8**, 57.
49. Lusson, D., Billiemaz, B., and Chabanne, J. L. (1999). Circulating lupus anticoagulant and probable systemic lupus erythematosus in a cat. *J. Fel. Med. Surg.* **1**, 193.
50. Beale, K. M., Halliwell, R. E. W., and Chen, C. L. (1990). Prevalence of antithyroglobulin antibodies detected by enzyme-linked immunosorbent assay of canine serum. *Am. J. Vet. Res.* **50**, 475.
51. Kass, P. H., Farver, T. B., Strombeck, D. R., and Ardans, A. A. (1985). Application of the log-linear and logistic regression models in the prediction of systemic lupus erythematosus in the dog. *Am. J. Vet. Res.* **46**, 2340.
52. Schwartz, R. S., Quimby, F. W., and Andre-Schwartz, J. Canine systemic lupus erythematosus: Phenotypic expression of autoimmunity in a closed colony. *In* "Genetic Control of Autoimmune Disease" (N. R. Rose, P. E. Bigazzi, and N. E. Warner, eds.), pp. 287–292. Elsevier Press, Amsterdam.
53. Monier, J. C., Fournel, C., Lapras, M., *et al.* (1988). Systemic lupus erythematosus in a colony of dogs. *Am. J. Vet. Res.* **49**, 46.
54. Hubert, B., Teichner, M., Fournel, C., and Monier, J. C. (1988). Spontaneous familial lupus erythematosus in a canine breeding colony. *J. Comp. Pathol.* **98**, 80.
55. Beaucher, W. N., Garman, R. H., and Condemi, J. J. (1977). Familial LE: Antibodies to DNA in household dogs. *N. Engl. J. Med.* **296**, 982.
56. Clair, D., DeHoratius, R. J., Wolfe. J., and Halliwell, R. E. W. (1980). Autoantibodies in human contacts of SLE dogs. *Arthritis Rheum* **23**, 251.
57. Kristensen, S., Flagstad, A., Jansen, H., *et al.* (1979). The absence of evidence suggesting that systemic lupus erythematosus is a zoonosis of dogs. *Vet. Rec.* **105**, 422.
58. Reinertsen, J. L., Kaslow, R. A., Kippel, J. H., *et al.* (1979). An epidemiologic study of households exposed to canine systemic lupus erythematosus. *Arthritis Rheum.* **23**, 564.
59. Jones, D. R. E., Hopkinson, N. D., and Powell, R. J. (1992). Autoantibodies in pet dogs owned by patients with systemic lupus erythematosus. *Lancet* **339**, 1378.
60. Panush, R. S., Levine, M. L., and Reichlein, M. (2000). Do I need an ANA? Some thoughts about man's best friend and the transmissability of lupus. *J. Rheumatol.* **27**, 287.
61. Cohen, A. S., Reynolds, W. E., Franklin, E. C., *et al.* (1971). Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21**, 643.
62. Tan, E. M., Cohen, A. S., Fries, J. F., *et al.* (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271.
63. Chabanne, L., Fournel, C., Rigal, D., and Monier, J.-C. (1999). Canine systemic lupus erythematosus. II. Diagnosis and treatment. *Compen Cont. Ed. Prac. Vet.* **21**, 402.
64. Matus, R. E., Gordon, B. R., Leifer, C. E., *et al.* (1985). Plasmapheresis in five dogs with systemic immune-mediated disease. *J. Am. Vet. Med. Assoc.* **187**, 595.
65. Petersen, M. E., Hurvitz, A. I., Leib, M. S., *et al.* (1984). Propothiouracil-associated hemolytic anemia, thrombocytopenia, and antinuclear antibodies in cats with hyperthyroidism. *J. Am. Vet. Med. Assoc.* **184**, 806.
66. Aucoin, D. P., Petersen, M. E., Hurvitz, A. I., *et al.* (1985). Propothiouracil-induced immune-mediated disease in the cat. *J. Pharmacol. Exp. Ther.* **234**, 13.
67. Balazs, T., Robinson, C. J. G., and Balter, N. (1981). Hydralazine-induced antinuclear antibodies in dogs. *Toxicol. Appl. Pharmacol.* **57**, 452.
68. Slauson, D. O., Russell, S. W., and Schechter, R. D. (1971). Naturally occurring immune complex glomerulonephritis in a cat. *Am. J. Pathol.* **103**, 131.
69. Heise, S. C., Smith, R. S., and Schalm, O. W. (1973). Lupus erythematosus with hemolytic anemia in an cat. *Feline Pract.* **3**, 14.
70. Gabbert, N. H. (1983). Systemic lupus erythematosus in a cat with thrombocytopenia. *Vet. Med. Small Anim. Clin.* **78**, 77.
71. Scott, D. W., Haupt, K. H., Knowlton, B. F., and Lewis, R. M. (1979). A glucocorticoid-responsive dermatitis in cats, resembling systemic lupus erythematosus in man. *J. Am. Anim. Hosp. Assoc.* **15**, 157.
72. Werner, L. L., and Gorman, N. T. (1984). Immune-mediated disorders of cats. *Vet. Clin. North Am. Small Anim. Pract.* **14**, 1039.
73. Pedersen, N. C., and Barlough, J. E. (1991). Systemic lupus erythematosus in the cat. *Feline Pract*. **19**, 5.
74. Vitale, C. B., Ihrke, P. J., Gross, T. L., and Werner, L. L. (1997). Systemic lupus erythematosus in a cat: Fulfillment of the American Rheumatism Association criteria with supportive skin histopathology. *Vet. Derm.* **8**, 133.
75. Scott, D. W. Immunological diseases. *In* "Large Animal Dermatology," pp. 314–317. W. B. Saunders, Philadelphia.
76. Stannard, A. (2000). Stannards illustrated equine dermatology notes. *Vet. Derm.* **11**, 177.
77. Vrins, A., and Feldman, B. (1983). Lupus-erythematosus-like syndrome in a horse. *Equine Pract.* **5**, 18.
78. Geor, R. J., Clark, E. G., Haines, D. M., and Napier, P. G. (1990). Systemic lupus erythematosus in a filly. *J. Am. Vet. Med. Assoc.* **197**, 1489.

10

ANTI-DNA ANTIBODIES

Structure, Assembly, and Diversity

Elena Peeva
Betty Diamond

A diverse repertoire of antibodies is generated to ensure protection from the world of microorganisms and their toxins. Because mechanisms of diversity proceed randomly, autoantibody production occurs. Antinuclear antibodies are a hallmark of systemic lupus erythematosus (SLE), whereas anti-DNA antibodies are a very specific marker for this disease.

Low-affinity anti-DNA antibodies are nonpathogenic, whereas high-affinity anti-DNA antibodies correlate with disease activity, especially with renal involvement. Pathogenic anti-DNA antibodies are IgG antibodies and somatically mutated and have features suggesting selection in response to a specific antigen. Their pathogenicity may arise from their ability to form immune complexes that deposit in the kidneys, their cross-reactivity with renal antigens leading to direct binding to kidney cells, or their capacity to react with DNA or nucleosomes previously bound to the renal tissues. It has been hypothesized that anti-DNA B cells can be activated by bacterial DNA complexed to protein, environmental pathogens via a process of molecular mimicry, through B-cell somatic mutation, or through activation of novel B-cell costimulatory pathways mediated by toll receptors.

Anti-DNA B cells are normally downregulated by one of three basic mechanisms of tolerance: deletion, anergy, or receptor editing. Survival and activation or tolerance of autoreactive B cells is mediated by several genes, many of which regulate strength of signaling through the B-cell receptor. Dysregulation of any of these may contribute to the development of SLE.

A major goal of understanding of the structure, origin, and pathogenicity of anti-DNA antibodies is to develop novel targeted treatments for SLE.

The protective effects of humoral immunity are mediated by antibodies that cause destruction or inactivation of microbial organisms and their toxins. The immune system generates antibodies to a constantly changing array of foreign antigens, but not to self-antigens. Dysregulation of the immune system, manifested as a production of autoantibodies, leads to autoimmunity. Systemic lupus erythematosus is characterized by the production of high-affinity antibodies directed toward a variety of self-antigens, primarily nuclear antigens [1, 2]. Some of these autoantibodies, such as anti-Sm, anti-RNP, and antinuclear antibody are markers for disease, whereas others, such as anti-DNA, anticardiolipin, and anti-Ro, contribute to tissue damage [3–7].

Antibodies are glycoproteins produced by B lymphocytes in both a membrane-bound and a secreted form. Initially, antibodies serve as antigen receptors on the membrane of resting B lymphocytes. After B cells encounter antigen, they differentiate further to produce a secreted form of antibody. Each immunoglobulin (Ig) is composed of two heavy chains and two light chains. The two heavy chains are linked by disulfide bonds, and each heavy chain is linked to a light chain by a disulfide

bond. Together the heavy and light chains combine to form an antibody molecule with two functional regions: a constant region that determines its effector functions and a variable region that is involved in antigen binding and constitutes the amino-terminal domain. The light chains contribute solely to antigen binding, whereas the heavy chains constant region determines the isotype (IgM, IgD, IgG, IgA, or IgE) of the antibody [8]. A naïve B cell produces IgM antibodies. After encounter with antigen and T-cell help, it can switch to the production of other isotypes; IgG is the predominant isotype of the secondary (also called memory) immune response.

Each antibody molecule contains two identical antigen-binding sites composed of the variable regions of the heavy and light chains. Certain portions of the variable region are highly polymorphic and are known as complementarity-determining regions (CDRs); they serve as contact sites for antigen binding [9]. Other more conserved portions of the variable region are called framework regions (FRs). X-ray crystallographic studies have shown that the amino acids of the CDRs are arranged in flexible loops, whereas the FRs have a more rigid structure that contributes more to maintaining spatial orientation of the antigen-binding pocket than to antigenic specificity [10]. Each variable region of an antibody molecule is composed of four FRs and three CDRs. The most variable portion of the molecule is the CDR3 [11, 12]. The variable region of an antibody contains antigenic determinants defined as idiotype. Anti-idiotypic antibodies are antibodies that bind to specific determinants in the CDRs or FRs of another antibody [13, 14]; they can bind to membrane immunoglobulin and, like antigen, can either tolerize or activate the B cell. Thus, anti-idiotypic antibodies may be considered as a potential therapeutic to tolerize pathogenic autoantibody-producing B cells (see later).

The Ig light and heavy chain variable region genes, which encode the antibody molecules, are formed by a rearrangement of multiple gene segments. This process involves excision of DNA between various gene segments followed by their ligation. V (variable), D (diversity), and J (joining) segments are brought together to form a heavy chain variable region gene [15, 16], and V and J segments form a light chain variable region gene [17, 18]. V(D)J gene segments each come from gene clusters arranged tandemly in the heavy or light chain locus. In humans, the heavy chain gene segments come from the gene locus on chromosome 14, whereas κ and λ light chain gene segments come from gene loci on chromosomes 2 and 22, respectively. There are approximately 50 functional heavy chain V segment genes distributed randomly along the V locus. They share 80% identity at the nucleotide level primarily in FRs [19–22] thirty functional D gene segments and 6 J gene segments for the human immunoglobulin heavy chain have been described [21]. Each V, D, and J gene segment is flanked by a conserved heptamer–spacer–nanomer sequence, known as the recombination signal sequence (RSS) [23], which is esential for Ig rearrangement.

The first rearrangement occurs in the heavy chain locus and starts with the joining of a D segment from the D cluster to a J segment in the J cluster, which is mediated by excision and deletion of the DNA between the segments. Then, a V gene segment is rearranged to the already assembled DJ unit, forming a complete VDJ variable region [24]. The process of variable region recombination requires a complex of enzymes called V(D)J recombinase [25], most of which are present in the majority of cells because of their involvememt in the maintenance of double-stranded DNA. However, recombination activating genes, RAG-1 and RAG-2, are found only in lymphocytes [26]. RAGs start the process of VDJ recombination by generating double-stranded DNA breaks. Joining of the coding segments is mediated by several enzymes (Ku 70, Ku 80, DNA-PKs, XRCC4, ligase, Mre 11) [27–29], whereas the formation and stabilization of the cleavage synaptic complex are regulated by HMG1 and HMG2 from the high mobility group family of proteins [30]. During rearrangement, imprecise VDJ joining may result in junctions of variable length, and nontemplate–derived nucleotides may be inserted at the VD or DJ junctions of the heavy chain, generating CDRs of variable length and sequence. If the single-stranded DNA that is present after the break can form a hairpin loop, the resulting double-stranded (P, palindromic) sequences are added at the junction. Alternatively, N-nucleotides or nontemplate-encoded nucleotides are inserted randomly at the VD and DJ junctions by the enzyme terminal deoxynucleotidyl transferase (TdT) [31].

Light chain gene segment rearrangement can occur from the κ or λ locus and begins after a functional heavy chain is formed. Rearrangement starts in the locus, but if both rearranged κ alleles are not functional, the process will proceed to the λ locus. The ratio between κ and λ light chains varies among species: in humans it is 3:2 and in mice it is 20:1. The light chain variable region is composed of only two gene segments: V and J. The κ light chain gene locus on chromosome 2 contains approximately 40 functional V gene segments that are grouped into seven families and five J segments [32–34]. The λ light chain locus is on chromosome 22 and contains at least seven families with up to 70 members [35–37]. Similar to heavy chain gene rearrangement, V and J elements of the light chain loci also rearrange by recombination at heptamer/nonamer consensus sites. At the time of light chain rearrangement TdT is no longer expressed in the B cells; thus, only rarely are N

sequences inserted at the VJ junction of the light chain [38]. The rearranged heavy and light chain V(D)J segments contribute to antibody structure as follows: the V segments of both heavy and light chains encode FR1, 2, and 3 and CDR1 and 2; CDR3 is encoded by the heavy chain D region and the light chain VJ junction. The J segment from each chain encodes FR4. The rearranged heavy chain VDJ segment is initially associated with an IgM constant region gene, it can undergo a second kind of gene rearrangement during the secondary response to associate with downstream constant region genes in a process called heavy chain class switching [39, 40]. Switching is mediated by switch sequences located upstream of each constant region gene [41]. In general, heavy chain class switching occurs in B cells after they are activated by antigen.

There are two heavy chain loci and four light chain loci from each of which a functional gene can be derived. However, each B cell expresses only one rearranged heavy chain and one rearranged light chain gene through a process called allelic exclusion, in which a productive rearrangement on one heavy and one light chain locus inhibits rearrangements on other loci. Rearrangement on the first chromosome is often unproductive because of deletions, mutations, and DNA reading frame shifts that generate stop codons during recombination or because nonfunctional variable region gene segments called pseudogenes are used [42]. Nonproductive rearrangement on one chromosome permits Ig genes on the other chromosome to undergo rearrangement [8]. The first light chain gene rearrangements occur in the κ locus, but if they are nonproductive on both chromosomes, the V and J segments of the λ locus will rearrange to produce a light chain [42, 43]. If a functional antibody binds to self-antigen, additional rearrangements may take place to change its specificity and prevent autoreactivity. These additional rearrangements are termed receptor editing and occur frequently in developing B cells [44]. There are reports that receptor editing can also occur in mature B cells [45–48], demonstrated by increased RAG protein expression in germinal centers [45, 48], but this issue remains controversial.

The immune system has an ability to assure enormous B-cell diversity. The large antibody repertoire is a result of the process of recombination used to create complete Ig variable regions and is based on the following mechanisms: (1) random combinations of V, D, and J gene segments and V and J segments into heavy and light chain genes, respectively; (2) junctional diversity produced by N or P sequence insertion and/or imprecise joining of gene segments; (3) random pairing of heavy and light chains; and (4) somatic mutation of rearranged genes. The first three mechanisms occur before exposure to antigen and are potentially capable of producing a repertoire of $10^{9–11}$ different antibodies [8], whereas the process of somatic mutation occurs after antigen encounter. Somatic point mutations are single nucleotide substitutions that can occur throughout the heavy and light chain variable region genes, but are most numerous in certain hot-spot motifs [49–51]. They represent site-specific, differentiation stage-specific, and lineage-specific events [50]; they are important in the generation of high-affinity antibodies and their suboptimal frequency leads to immunodeficiency [52]. Mutated antibodies can also acquire novel antigenic specificities. For example, antibodies to foreign antigen can acquire autospecificity through somatic point mutation [53, 54]. Somatic mutation takes place in dividing centroblasts, whose rearranged Ig variable region genes undergo a much higher mutation rate than any other somatic cell (1 bp per 10^3 bp per cell division). The DNA mismatch repair system, which generally functions to correct point mutations in DNA, has been implicated in Ig gene mutation. Deficiency in mismatch repair enzymes MSH2 [55, 56] and MSH6 [57] causes altered nucleotide targeting for mutations. In both MSH2- and MSH6-deficient mice, the mutations are targeted to G and C nucleotides and to RGYW or WRCY hot spots [57, 58]. Controversy exists whether a deficiency in other mismatch repair enzymes PMS 2 and Mlh1 affects the process of somatic hypermutation [59, 60] or not [55, 60]. Activation-induced cytidine deaminase (AID), a putative RNA-editing enzyme discovered in germinal center B cells [61], is also critical for the process of somatic mutation [62]. It has been demonstrated that AID expression can induce hypermutation in hybridoma cells targeting to G/C nucleotides within RGYW or WRCY hot spots [63].

Because the process of somatic mutation occurs concurrently with heavy chain class switching, although by a different mechanism, hypermutation is more common in IgG than in IgM antibodies. Due to degeneracy of the genetic code, not every point mutation causes a change at the amino acid level. Mutations without consequent amino acid changes are termed silent (S). A point mutation that leads to a amino acid change, replacement (R) mutation, may result in an antibody with decreased or increased affinity for the triggering antigen. It is possible to indirectly analyze antigen selection during the course of the germinal center response by calculating the ratio of replacement (R) to silent (S) mutations in rearranged antibody genes. In a process referred to as affinity maturation, only those B cells making antibodies that bind to the eliciting antigen with a high affinity will be selected for proliferation [64]. B cells producing antibodies with low affinity rarely progress to plasma or memory cells, whereas B cells producing anti-

bodies of higher affinity will continue to be amplified. This selection for increased affinity may be reflected in a greater than random R/S ratio in the CDRs and a random or lower than random R/S ratio in the FRs of the selected antibodies [65].

In summary, multiple mechanisms exist for generating a diverse repertoire of antibody molecules and ensure the production of antibodies that are protective against microbial invasion. However, because these mechanisms of diversity proceed randomly in the absence of instruction from external antigens, they permit the production of autoantibodies, which must then be regulated.

DETECTION OF ANTI-DNA ANTIBODIES

Anti-DNA antibodies can be detected by several assays. Some of these assays are specific for high-affinity antibodies, whereas others detect low-affinity antibodies as well (Table 1). The most frequently utilized assays are the Farr assay, the Millipore filter assay, the enzyme-linked immunosorbent assay (ELISA), and the *Crithidia luciliae* assay [66–68].

In the Farr assay, radiolabeled single-stranded (ss) or double-stranded (ds) DNA is added to the sample to be tested for anti-DNA antibodies. The immune complexes can then be precipitated by ammonium sulfate and the results are expressed as a percentage of radioactivity that is present in the precipitate. This test, can detect both IgG and IgM anti-DNA antibodies but cannot distinguish between the two classes. The Farr assay is an excellent assay for the detection of high-affinity antibodies.

The Millipore filter assay also utilizes radiolabeled dsDNA that is added to the test serum and passed through a filter. Because the filters bind protein, but not dsDNA, DNA–antibody complexes are retained on the filter while free dsDNA passes through. The amount of radioactivity retained on the filter is proportional to the anti-dsDNA antibody in the test sample. This assay cannot be used to detect antibodies to ssDNA because ssDNA, unlike dsDNA, will bind directly to the filter. Neither the Farr assay nor the Millipore assay can distinguish between antibody–DNA complexes and complexes of other serum proteins with DNA.

TABLE 1 Assays for the Detection of Anti-DNA Antibodies

Assay	High-affinity anti-DNA antibodies detected	Low-affinity anti-DNA antibodies detected
Farr assay	+	–
Millipore filter method	+	–
ELISA	+	+
Crithidia lucilia	+	+

The ELISA is a rapid, simple, quantitative, and reproducible assay [69] that is now the most commonly used clinical assay. Microtiter plates are coated with ss or dsDNA. The serum is applied to the coated plates. Antibody in the serum is referred to as primary antibody. The presence of bound anti-DNA antibody can then be detected by an enzyme-linked anti-Ig antibody, or secondary antibody. Addition of a substrate that changes color in the presence of the enzyme allows the detection of anti-DNA antibodies. The color change can be read in a spectrophotometer. The advantage of this assay is the ease with which large numbers of samples can be screened. ELISAs are highly sensitive and have the advantage of being able to determine antibody isotype, light chain usage, and idiotypic specificity, depending on the specificity of the secondary antibody. It is currently the most popular assay in clinical use.

The most specific test for anti-dsDNA antibodies utilizes the flagellate *C. luciliae* as a substrate. This organism has a kinetoplast that contains circular dsDNA that is not associated with histone proteins. Test samples are incubated with the organism on a glass slide. The fluoresceinated anti-Ig antibody is then added. Detection of the fluorescent kinetoplast using a fluorescence microscope indicates the presence of anti-DNA antibodies. Although not as sensitive as the ELISA, this assay is highly specific for dsDNA binding and, like the ELISA, can distinguish structurally distinct subsets of anti-DNA antibodies, depending on the specificity of the secondary fluoresceinated antibody.

Accurate detection of high titers of anti-dsDNA antibodies has been shown to be useful in the diagnosis of SLE, and an increasing titer is generally correlated with disease flares [70]. Given the heterogeneity of the anti-DNA antibody detection assays available and their specific limitations, the utility of anti-dsDNA antibody detection in the diagnosis and treatment of SLE is enhanced in clinical decision making by a consideration of the sensitivity and specificity of the assay used.

CELLULAR ORIGIN OF ANTI-DNA ANTIBODIES

B1 cells comprise less than 10% of total B cells and are preferentially localized to the peritoneal and pleural cavity in mice [71], in contrast to conventional B cells, which populate peripheral lymphoid organs [72, 73].

Much controversy exists over the physiologic function of B1 cells. The autoimmune NZB mouse strain displays an increased number of B1 cells and antierythrocyte antibodies, and their elimination by hypotonic shock with intraperitoneal injections of distilled water significantly decreases the occurrence of hemolytic anemia [74]. The same method of elimination of B1 cells from the peritoneal cavity of NZB/W F1 lupus-prone mice leads to diminished anti-DNA serum titers with subsequent amelioration of the kidney disease [74]. Although an increased number of B1 B cells has been reported in some autoimmune diseases, the association with SLE is not firm [75–77]. Based on their membrane expression of the 67-kDa molecule CD5, B1 cells can be divided into two subsets: B1a and B1b. [78]. The functional significance of the CD5 surface marker has not yet been determined. It has been hypothesized that it may be a negative regulator of B-cell receptor signaling in B1 cells [79]. There is a long-standing notion that B1 cells represent a separate, self-replenishing B-cell population [78]. Evidence suggests that the strength of BCR signaling and the presence of particular costimulatory molecules strongly influence the fate of B cells and determine whether they will develop into B1, follicular B2, or marginal zone B cells [80, 81]. Mice with functionally inactive B-cell activating factor (BAFF), a member of the tumor necrosis factor family of proteins [82], or lacking BAFF [83] have a block in B-cell maturation in the periphery with a dramatic decrease in marginal and follicular B2 B cells, but with a normal number of peritoneal B1 B cells.

Although both B1 cells and conventional B2 cells can produce anti-DNA antibodies [77], the anti-DNA antibodies that have been termed natural autoantibodies are produced mainly by B1 cells [84]. They tend to be low-affinity, germline-encoded IgM antibodies [85, 86] that react preferentially with ssDNA, but are often polyreactive [87]. These anti-DNA antibodies are found in the sera of normal mice and humans and are believed to be nonpathogenic, but have been also postulated to help regulate the production of high-affinity pathogenic autoantibodies [88].

The autoantibodies of SLE differ from natural autoantibodies. Anti-DNA antibodies that are correlated with disease manifestations of SLE, specifically renal pathology, are encoded predominantly by somatically mutated Ig genes of the IgG isotype [89, 90] and have specificity for dsDNA or both ssDNA and dsDNA, although they may also exhibit polyreactivity (see cross-reactivity section and Table 2). In the NZB/W mouse, evidence shows that both marginal zone and follicular B cells contribute to anti-DNA antibody production. Follicular B cells are T-cell-dependent, and their involvement in the production of pathogenic autoantibodies is supported by the demonstration that $CD4^+$ T-cell depletion or blockade of B-cell/T-cell costimulatory pathways leads to decreased anti-DNA titers in NZB/W mice [91, 92]. However, NZB/W mice also demonstrate an expansion of marginal zone B cells [93]. Most marginal zone B cells express germline-encoded Ig VH genes [94] and are T cell independent for immunoglobulin production. Newly accumulating data demonstrates that marginal zone B cells may provide help to T-cell-dependent responses by acting as antigen-presenting cells (APCs) [95, 96] or by enhancing antigen presentation by follicular DCs [97]. Several studies have implicated marginal zone B cells in murine SLE. The increased number of autoreactive marginal zone B cells in NZB/W mice is linked to a chromosomal region that overlaps with Nba2, a genetic locus associated with lupus nephritis [98]. In a model of estrogen-induced lupus, the majority of anti-dsDNA antibody-secreting B cells are marginal zone B cells [99].

It becomes increasingly clear that SLE is a heterogeneous disease and that different B-cell populations may be responsible for the majority of autoantibody production in different patients.

TABLE 2 Antigens That Cross-React with Anti-DNA Antibodies

Antigen	Cross-reacts with anti-dsDNA	Cross-reacts with anti-ssDNA
Polynucleotides		
Z-DNA	+	+
Phospholipids		
Cardiolipin	+	+
Phosphatidyl glycerol		+
Bacterial antigens		
Bacterial phospholipids	+	+
Pneumococcal phosphorylcholine	+	
Mycobacterial glycolipid	+	+
Polysacharides of		
Klebsiella	+	+
Meningococci	+	+
Pneumococci	+	
E. coli	+	+
Extracellular matrix components		
Hyaluronic acid	+	
Chondroitin sulfate	+	
Heparan sulfate	+	
Laminin	+	+
Cellular components		
Cell surface proteins	+	
Vimentin	+	+
Ribosomal protein S1	+	
A and D SnRNP	+	

STRUCTURE OF ANTI-DNA ANTIBODIES

The anti-DNA antibody response resembles a normal antibody response in its shift from IgM to IgG [100] and in its pattern of somatic mutation [101, 102]. Both IgM and IgG anti-DNA antibodies [103] have features suggesting selection in response to a specific antigen (i.e., a greater than random ratio of replacement to silent mutations in CDRs). Sequence analysis has demonstrated a pattern of somatic mutation consistent with DNA, alone or in a complex, as the selecting antigen. Furthermore, some of the IgG anti-DNA-producing B cells that have been isolated are derived from the same clonal precursors as the IgM anti-DNA B cells, and progression of the isotype switch is paralleled by a greater affinity for dsDNA among IgG than IgM antibodies.

Extensive genetic characterization of anti-DNA antibodies from both lupus patients and mouse models of lupus has been undertaken in an attempt to understand the molecular basis for DNA binding. In general, the heavy or light chain V genes that are used to produce anti-DNA antibodies are used to encode anti-DNA antibodies that are also used in the response to foreign antigen [104–106]. Restriction fragment length polymorphism analysis comparing the Ig loci of lupus patients with those of nonautoimmune individuals shows that there are no apparent differences, such as gene loss or gene duplication, that distinguish the two groups [107]. Similar conclusions have been reached in mice [108], and it is clear from mouse studies that Ig genes of a nonautoimmune strain can encode pathogenic anti-DNA antibodies. When a mouse from the nonautoimmune SWR strain is mated to a mouse from the NZB autoimmune strain, the offspring produce pathogenic anti-DNA antibodies encoded by SWR Ig genes [109]. Similarly, when C57Bl/6 mice carry three lupus susceptibility loci derived from NZM mice, they develop anti-DNA antibodies and nephritis [110]. Two studies, however, suggest that polymorphisms in the Ig loci, although clearly not required for disease expression, may predispose to SLE. One study suggests that the heavy chain locus of 20% of individuals with SLE lacks a particular V_H3 gene [111], and the second study demonstrates that expression of the V_K gene, A30, seems to be unique to the antibodies of SLE patients with lupus nephritis [112]. Patients who do not express A30-encoded light chains, perhaps because they lack a functional A30 gene, have a lower incidence of lupus nephritis. Although genetic susceptibility to lupus has not been mapped to the Ig locus, the finding that there is preferential expression of some anti-DNA associated idiotypes in family members of patients with SLE also demonstrates that genetic factors may influence variable gene usage [113].

The process of Ig gene rearrangement is similar in autoimmune and nonautoimmune individuals. One notable exception is the suggestion that an uncommon reading frame of the D segment, D fusions and D segment inversions, may occur at a higher frequency in anti-DNA antibodies than in protective antibodies [114, 115]. These abnormal D regions display an increased number of arginines in CDR3 of the heavy chain. Arginine, along with other basic amino acids, is a potential DNA contact residue (discussed later). Finally, investigation of the process of somatic mutation in anti-DNA antibodies has led to conflicting data. Analysis of somatic mutation in 10 human anti-DNA antibodies showed no abnormality in the frequency of somatic mutation nor in the distribution of mutations in CDRs or FRs [116]. However, less targeting to mutational hot spots was found in the V_H genes encoding anti-DNA antibodies than in antibodies from nonautoimmune individuals encoded by the same V_H genes [116]. Decreased targeting of somatic mutations to hot spots was also detected in anti-DNA antibodies obtained from mice transgenic for the antiapoptotic gene bcl-2 [117]. This observation may suggest that B cells in SLE undergo somatic mutation at a different state of cellular activation. Other studies suggest other potential abnormalities in this process. Analysis of somatic mutations in the V_κ gene repertoire of B cells from one lupus patient showed an increased number of mutations and targeting of mutations to hot spots of FRs [118].

A large number of heavy and light chain variable genes can potentially encode anti-DNA antibodies [119], but some appear to dominate the response [120, 121]. Although IgM anti-DNA antibodies in humans are encoded by members of all seven V_H families [122], pathogenic IgG anti-DNA antibodies are biased toward members of the V_H3 and V_H4 families [123]. Although the overexpression of V_H3 family members may simply be a reflection of the large size of this V_H family, preference for the small V_H4 family is intriguing [123].

Light chain V gene use in both murine and human anti-DNA antibodies is more diverse than heavy chin V gene use [124]. There seems to be a preference for light chains that have long CDR1 loops containing basic amino acids, such as $V_\kappa1A$, $V_\kappa8A$, and Vk8B in the mouse [120]. The wider repertoire of light chains used to encode anti-DNA antibodies may be a reflection of the proposed dominance of the heavy chain in determining DNA reactivity [125] and may explain the polyreactivity of anti-DNA antibodies [126]. However, experiments pairing a murine anti-DNA heavy chain with different light chains demonstrate that light chain

identity can greatly influence the fine specificity of dsDNA binding [125].

Examination of the protein sequence of murine and human anti-DNA antibodies [120] has been fruitful in delineating features that are specific to these antibodies. Several basic amino acids, namely arginine, histidine, and lysine, and some polar amino acids, such as glutamine, tyrosine, and asparagine, have been found to contribute to DNA binding [127]. Arginine is the most versatile amino acid for DNA binding. It is a frequently used residue in protein–nucleic acid interactions [128] due to its potential to form electrostatic or hydrogen bonds with the DNA backbone and to its ability to interact with aromatic DNA bases [129]. Its importance in determining DNA reactivity is illustrated by the fact that somatic mutations to arginine are common among anti-DNA antibodies [130]. N base addition or unusual VDJ rearrangements that shift or invert the D reading frame are additional mechanisms by which anti-DNA antibodies may be enriched for arginine residues in CDR3 [129, 130]. Mutational analyses testing the relevance of arginine residues to DNA binding have yielded variable results, suggesting that other motifs as well play a significant role in DNA binding. The substitution of arginine 96 by glycine in one anti-DNA antibody resulted in complete loss of DNA binding; the addition of arginine at position 31 of CDR1, 56 and 64 of CDR2, and 76 of FR3 of this same antibody contributed to DNA binding both independently and additively [131]. In other anti-DNA antibodies, however, the mutation of arginine does not alter DNA binding or the removal of an arginine leads to an increase in DNA binding [132].

Molecular modeling has reinforced data obtained from anti-DNA antibody sequences. An early study investigated a complex of the anti-DNA antibody, D42, and poly(dT) and showed that the cleft of the antibody-binding site contains many arginine and tyrosine residues that make electrostatic and stacking interactions with phosphates and nucleic bases, respectively [114]. Homology modeling and molecular dynamic simulation of the antigen-binding site of anti-dsDNA monoclonal antibodies generated from a NZB/W mouse surprisingly suggest a significant increase in the flexibility of the antigen-binding loops in affinity-matured anti-DNA antibodies [133]. Only one anti-DNA antibody, BV04-01, has been crystallized both alone and in complex with ssDNA [134]. In complex with the trinucleotide, this antibody shows local changes in the conformation in CDR-H3, CDR-L1, and CDRl-L3 compared to its uncomplexed form; the interaction with DNA is achieved by four hydrogen bonds and an ion pair. The recently determined crystal structure of the complex of a murine anti-ssDNA antibody, DNA-1, with dT_5 shows that the structural stability of the antibody-binding site is maintained by the arginine side chains of the heavy chain CDR3 [135]. This observation extends previous information that ion pairs involving arginine are important for DNA recognition [129]. These few available crystal structures of anti-DNA antibodies have suggested that anti-ssDNA and anti-dsDNA antibodies may have distinct DNA-binding sites [136]; antibodies to ssDNA contain a deep cleft, whereas antibodies to dsDNA usually have a planar binding site [137, 138].

IDIOTYPES OF ANTI-DNA ANTIBODIES

Idiotypes are antigenic determinants located on an antibody molecule to which anti-idiotypic antibodies can bind. Idiotypes shared by multiple antibodies with distinct antigenic specificities are called cross-reactive idiotypes and may reflect the use of common germline genes used to encode these antibodies. Private idiotypes are unique to specific antibodies. Idiotypes can be antigenic epitopes formed by the heavy or light chain independently or may reflect an epitope formed by the association of both chains [139]. The idiotype network is a complex cascade of antibodies. Normally, after antibodies are produced in response to antigen, anti-idiotypic antibodies arise. These anti-idiotypes can bind to all antibodies bearing the specific idiotypic determinant, independent of their antigenic specificity. In this fashion, a network of interacting antibodies is produced [140].

Anti-DNA antibodies found in sera of lupus patients and lupus-prone mice have been demonstrated to express cross-reactive idiotypes [141–144]. These idiotypes are also present on serum Igs of nonautoimmune hosts, but in lupus patients they are present at increased titer. Some are directly correlated with disease activity and some are found in immune deposits in nephritic kidneys [145, 146].

The 16/6 idiotype, one of the most studied anti-DNA idiotypes, is expressed in a significant number of patients with SLE and is prevalent in patients with anti-cardiolipin antibodies [147]. It has been found at high titer in 50% of patients with active disease and in 40% of renal Ig isolates, and is associated with mesangial deposits [148]. The 9G4 idiotype is present at increased titer in the serum of 40% of SLE patients and correlates with cardiovascular, respiratory, and hematological disease manifestations, as well as with decreased complement component 3 [149]. 8.12 is a light chain-specific idiotype found in 50% of lupus patients with anti-DNA activity and is expressed on up to 30% of their serum

anti-DNA antibodies. 3I recognizes k light chains and, like 8.12, is elevated in a majority of SLE patients with anti-DNA activity and is present on a high percentage of anti-DNA antibodies. Both 8.12 and 3I idiotypes exist at low titer in normal individuals and both are elevated in relatives of SLE patients who have no evidence of clinical disease [113, 150].

Idiotypic analyses are of potential utility to the clinician, as anti-idiotypes may distinguish between pathogenic and nonpathogenic subsets of autoantibodies. For example, 3I, 8.12, 16/6, and 9G4 positive antibodies are all pathogenic. In a multicenter study of 19 anti-DNA associated idiotypes, an elevated expression of 3I positive antibodies correlated with active lupus and with nephritis [151].

Anti-DNA associated idiotypes are also present on antibodies that bind to microbial antigens. Both 3I and 8.12 are present on the antipneumococcal antibodies that arise in nonautoimmune individuals following vaccination with pneumococcal polysaccharide [152, 153]. Titers of 16/6 positive antibodies are elevated in patients with leprosy and with *Klebsiella* infection [154]. These observations have led to the suggestion that anti-DNA antibodies might arise as a response to bacterial antigen, either by somatic mutation of the antibacterial antibody or through an idiotypic network [155].

Immunization with the anti-idiotype 16/6 induces disease in mice [156]. This observation, along with multiple studies in mice showing that anti-idiotypic antibodies can regulate the expression of idiotype bearing antibodies, led to the hypothesis that the idiotypic network may play a role in triggering SLE or regulating disease activity and to the suggestion that therapy of autoimmune disease can be achieved through manipulation of idiotype–anti-idiotype interactions. An anti-idiotype can either mimic DNA and function as antigen by binding to the antigen-binding site or it can bind outside the antigen-binding site as a superantigen would. Like antigen, an anti-idiotype can induce or suppress an antibody response. One study has demonstrated that a SLE patient in remission has anti-idiotypic antibodies to anti-DNA antibodies, whereas the same patient experiencing flares lacks anti-idiotypic antibody [157]. In mouse studies, it has been suggested that NZB/W lupus-prone mice develop disease at the time they no longer produce anti-idiotypes to anti-DNA antibodies [158]. Furthermore, passive administration of anti-idiotypic antibodies has been shown to decrease anti-DNA antibody production and attenuate disease in mice for a short period of time [159]. There are also studies underscoring the importance of anti-idiotypic antibodies as stimulators of autoantibody production. Antibodies to the Ku protein, for example, which binds DNA and functions in the repair of DNA double-stranded breaks [160], are found with increased frequency among African-American patients with SLE [161] and are anti-idiotypic to anti-DNA antibodies [162]. Furthermore, immunization of mice with an anti-idiotypic antibody to an anti-DNA antibody induces autoantibody production [163]. An anti-idiotypic response to antibodies against p53, which acts as a tumor suppressor protein and binds DNA [164], leads to the production of antibodies to both DNA and p53. A specific class of anti-idiotypic antibodies that display specificity for both dsDNA and anti-DNA antibody has been identified. These anti-idiotypic antibodies arise naturally in SLE, may significantly stimulate production of anti-dsDNA antibodies, and consequently contribute to the development of lupus nephritis [165]. While these studies are provocative, the role of the idiotype network in SLE remains uncertain.

ANTIGENIC CROSS-REACTIVITY OF ANTI-DNA ANTIBODIES

The diversity of the autoreactivities found in lupus sera differentiates SLE from many other autoimmune disorders [166]. Although such diversity has been attributed to a polyclonal activation of B cells [167], studies show that the diversity of antigenic specificities may be due, at least partially, to the cross-reactivity of anti-DNA antibodies with a wide variety of molecules (Table 2). IgG anti-DNA-secreting B cells derived from SLE patients are much more cross-reactive than IgG anti-DNA-secreting B cells from nonautoimmune individuals and fail to show a normal decrease in cross-reactivity compared to IgM anti-DNA B cells [168].

Polynucleotides and phospholipids were the first molecules found to cross-react with anti-DNA antibodies. Immunization of autoimmune MRL mice with β2 glycoprotein I induced anti-DNA antibodies that could be inhibited with cardiolipin micelles, demonstrating that these antibodies bind both DNA and cardiolipin [169]. Using hybridoma technology as a means to isolate individual antibody molecules, it was demonstrated that monoclonal anti-DNA antibodies from lupus-prone mice bind not only to cardiolipin, but also to other phosphorylated molecules [170]. Analysis of the specificities of these antibodies, as well as these of human anti-DNA antibodies [171, 172], suggests that phosphodiester groups common to both DNA and phospholipids constitute the shared epitope recognized by these cross-reactive antibodies. Phospholipids lacking regularly spaced phosphate groups are not bound by anti-DNA antibodies.

Anti-DNA antibodies derived from naive lupus-prone mice can bind to the cell surfaces of murine

endogenous microbial flora [173]. This binding, which is unaffected by DNase treatment and is competitively inhibited by DNA, is due in part to the phosphodiester groups of bacterial cell walls. The human monoclonal IgM anti-DNA antibody DJ binds to human commensal bacteria, such as *L. acidophyllus*, *B. bifidum*, and *L. plantarum*; the cross-reactive epitope is phosphodiester groups [174]. Structural similarities between the sugar-phosphate backbone of DNA and lipid A, a common constituent of gram-negative bacteria [175], provide a possible explanation for the cross-reactivity of human IgM anti-DNA antibodies to these antigens. Antibodies binding both DNA and pneumococcal cell wall and capsular polysaccharide have been isolated from mice [176, 177] and humans [178]. Finally, a human monoclonal antibody that reacts with bacterial capsular polysaccharides of the human pathogens, group *B Meningococcus*, and *Escherichia coli K1*, also recognizes DNA [179]. Although many cross-reactivities of anti-DNA antibodies can be explained by their recognition of the phosphodiester groups common to the molecules they bind, the basis for other cross-reactivities is less apparent. For example, monoclonal anti-DNA antibodies derived from mice and humans with SLE bind to the glycolipid components of the mycobacterial cell wall [180].

Two explanations can account for the cross-reactivity observed between DNA and carbohydrate antigens. Either the binding site of anti-DNA antibodies may be able to accommodate a variety of antigenic structures or an as yet unidentified tertiary structure of these molecules may be the common epitope recognized by anti-DNA antibodies. Studies suggesting that negative charge distribution may be the key component responsible for the cross-reactivity between DNA and bacterial capsular polysaccharide support the latter possibility.

Anti-DNA antibodies have been shown to cross-react with a variety of extracellular matrix components, including proteoglycans. These molecules are rich in repeating, negatively charged units deriving from their glycosaminoglycan constituents hyaluronic acid, chondroitin sulfate, and heparan sulfate. Analysis of the interaction of several murine monoclonal anti-DNA antibodies with extracellular structures within both glomeruli and tubules of kidneys reveals cross-reactivity with laminin, a glycoprotein constituent of basement membranes [181]. This binding is inhibited by DNA and cannot be abolished by DNase treatment.

Anti-DNA antibodies have also been shown to interact with cell surface molecules expressed on several human and mouse cell types, including Raji cells [182], B cells [183], T cells, erythrocytes [184], neurons [185], epithelial cell cultures from human kidney and skin [183], and embryonal cells [186]. In general, pretreatment of target cells with proteases abolishes anti-DNA antibody binding whereas degradative enzymes targeting sugar moieties have no effect on binding [183], suggesting that the reactive cell surface components are proteins, not the sugar moieties of glycoproteins and proteoglycans. The identity of a majority of these proteins has not been determined yet, although studies have shown cross-reactivity with laminin [181]. Both murine and human anti-DNA antibodies cross-react with the NR2 subunit of *N*-methyl-D-asparate (NMDA) glutamate receptor on neurons, and cerebrospinal fluid of a lupus patient containing anti-DNA anti-NR2 cross-reactive antibodies induces neuronal apoptosis [185]. Because NMDA receptors are expressed on many cell types, this subset of anti-DNA antibodies may mediate injury in a variety of organs.

Anti-DNA antibodies can cross-react with intracellular proteins as well. Monoclonal human and mouse anti-DNA antibodies react with the intermediate filament vimentin, and possibly other cytoskeletal proteins, such as α-actinin [187] and tubulin [188]. Anti-dsDNA antibodies bind the ribosomal proteins S1 [189], P0 [190], and P1 [191] and the SnRNP polypeptides, A and D [192,193]. Phenylalanine in the C-terminal hydrophobic region of ribosomal protein P1 appears crucial for cross-reactivity with anti-dsDNA antibodies, which may be due to structural similarities between the aromatic ring of phenylalanine and the cyclic forms of a DNA molecule, such as nucleotide bases or pentoses in the backbone [191]. However, the shared epitope between intracellular proteins and nucleic acid is not always apparent. The cross-reactive epitope may also arise from the conformational apposition of negative charges in the tertiary structure of the cross-reactive proteins. For example, the S1 protein is devoid of stretches of negatively charged segments in its primary structure, but may resemble DNA in its tertiary structure [189]. Human anti-DNA antibodies have also been found to cross-react with lamin in the nuclear envelope [194].

The search for cross-reactive antigens continues, as these antigens may yield clues to the origin and pathogenesis of anti-DNA antibodies.

PATHOGENIC POTENTIAL OF ANTI-DNA ANTIBODIES

The presence of anti-dsDNA antibodies is virtually diagnostic of SLE and occurs only rarely in other conditions [195]. Over the past several years, it has been clearly demonstrated that anti-DNA antibodies have pathogenic potential. Clinical data demonstrate that anti-DNA antibody titers correlate with the disease activity in a significant number of patients with lupus

nephritis, and glomerular eluates from patients with active lupus nephritis contain anti-DNA antibodies [196]. Experiments in mice have also confirmed the pathogenicity of anti-DNA antibodies. For example, administration of anti-DNA antibodies into nonautoimmune mice has been shown to produce nephritis [197, 198], and transgenic mice expressing only the secreted form of an anti-DNA antibody develop lupus nephritis [199]. Moreover, perfusion of rat kidney with monoclonal mouse and polyclonal human IgG anti-DNA antibodies leads to proteinuria and decreased renal function [200]. However, not all anti-DNA antibodies are pathogenic. Although about two-thirds of lupus patients demonstrate anti-DNA antibodies in their serum, not all of them develop clinical manifestations related to these autoantibodies. The pathogenic potential of anti-DNA antibodies depends on their structural and molecular properties [197–201]. Nephritogenic anti-DNA antibodies are predominantly high-avidity, IgG, cationic antibodies that fix complement and show preferential reactivity to dsDNA [202, 203].

Within the kidneys of both SLE patients and mice with lupus-like disease, anti-DNA antibody deposition can be found at multiple sites, within the subendothelial and subepithelial spaces, in the mesangium, and along the basement membrane and tubules. These differing sites of antibody deposition result in variable disease severity among patients and murine lupus strains [204] and reflect a complicated disease process. Although studies on the mechanisms of pathogenesis by anti-DNA antibodies agree that the formation of glomerular immune complexes is the initial event in the inflammatory process [204, 205], there are different explanations for the origin of these immune complexes. There are, at present, three hypothesized mechanisms to explain the deposition of anti-DNA immune complexes in the kidney. The circulating immune complex hypothesis states that immune complexes preformed in the periphery are passively trapped within glomeruli. The second model suggests that direct binding of cross-reactive anti-DNA antibodies to glomerular antigens is the leading mechanism of immune deposit formation. The third is the "planted" antigen hypothesis that suggests that intracellular antigens released into the circulation after cell death bind to certain sites within the glomerulus and serve as antigenic determinants to which anti-DNA antibodies can bind, initiating immune deposit formation.

It has been demonstrated that some anti-DNA antibodies can penetrate living cells [206, 207], but whether these antibodies are pathogenic and how often they arise remains to be determined.

Hypothesis 1: Circulating Immune Complexes

Sera from patients with lupus nephritis contain high levels of circulating immune complexes [208–211]. Because anti-DNA antibodies are one of the major pathogenic antibodies in lupus nephritis, it was hypothesized that DNA–anti-DNA antibody complexes preformed in the circulation deposit in the renal parenchyma causing disease. Although it has been reported that the presence of DNA–anti-DNA antibody complexes in lupus sera correlates with disease activity [212, 213], the pathogenicity of such complexes has not been confirmed [214]. DNA–anti-DNA complexes formed *in vitro* and injected into mice are cleared rapidly by the liver [215] and show no affinity for the glomerulus [216], whereas unbound anti-DNA antibodies injected into mice deposit in glomeruli. Nevertheless, data that anti-DNA circulating immune complexes are present preferentially in lupus patients with nephritis and are associated with immunoglobulin deposition in the subendothelial area of the renal glomeruli [217] cannot be ignored.

To reconcile these findings, it has been suggested that circulating anti-DNA antibodies are bound to histones or nucleosomes rather than naked DNA [218, 219]. When perfused into a rat kidney, nucleosome–antibody complexes are able to deposit in kidneys and fix complement following their deposition [220], suggestive of the nephritogenic potential of such immune complexes. Finally, nucleosomes complexed with anti-DNA antibodies bind to heparan sulfate, a constituent of the glomerular basement membrane (GBM) [221].

Hypothesis 2: Cross-Reactivity with Renal Antigen

Nephritogenic anti-DNA antibodies are highly polyreactive. A comparison of anti-DNA antibodies derived from normal individuals, patients with active lupus, or renal eluates of patients with active lupus nephritis showed that anti-DNA antibodies eluted from the kidney were the most cross-reactive, binding to polynucleotides, phospholipids, and the SmRNP complex [222]. Similar observations were also made in a lupus mouse model [223]. The observation that anti-DNA antibodies are cross-reactive for cardiolipin and other negatively charged phospholipids [170–172] gave rise to the suggestion that anti-DNA antibodies may exert their pathogenic effect by reacting with renal tissue antigens that share common epitopes with DNA. Support for this hypothesis came from studies showing that an exogenously administered murine monoclonal

anti-DNA antibody formed immune deposits by binding directly to the GBM antigens and that this binding was not inhibited by DNase [224]. In addition, mouse and human anti-DNA antibodies cause renal dysfunction in the perfused rat kidney through direct interaction with glomeruli and subsequent fixation of complement [200]. Finally, observations on mutated anti-DNA antibodies have suggested that binding to DNA is not the sole determinant of pathogenicity. Mutants of anti-DNA antibodies with decreased binding to DNA are nevertheless pathogenic [225]. Furthermore, small numbers of amino acid changes in the V region of a monoclonal anti-DNA antibody that increase DNA binding can be associated with a decrease in glomerular binding.

Clues to the identity of the glomerular antigens to which anti-DNA antibodies bind have come from several investigators who found anti-DNA antibodies to be cross-reactive with important constituents of the GBM and the mesangium, including hyaluronic acid, chondroitin sulfate, heparan sulfate [226], laminin [181], and α-actinin [187, 227]. Heparan sulfate is the major glaycosaminoglycan constituent of GBM and has been shown to play a leading role in the maintenance of the charge [228] and size-selective barrier of the GBM [229]. Masking of glycosaminoglycan polyanionic sites occurs following the deposition of cross-reactive anti-DNA antibodies, resulting in proteinuria [228] and influencing the intraglomerular handling of circulating antigens, antibodies, and immune complexes [230]. This observation, therefore, presents at least two possible mechanisms by which the cross-reaction of anti-DNA antibodies with glomerular antigens might lead to lupus nephritis. Binding of anti-DNA antibodies to renal antigens may disrupt the normal physiologic function of the kidney, may trigger local inflammation and tissue damage, or both.

In addition to reacting with extracellular components of the kidney, direct cell membrane binding of anti-DNA antibodies has been shown to be a potential mechanism of immune deposition. For example, human and mouse anti-DNA antibodies that cross-react with A and D SnRNP polypeptides exert their deleterious effects by binding directly to kidney cells. Some of these antibodies have been demonstrated to penetrate the cells and localize to the cytoplasm or nucleus [192, 207]. Whether they interfere with intracellular processes *in vivo* in SLE patients is not known.

Hypothesis 3: Planted Antigen

In contrast to the cross-reactivity theory of anti-DNA antibody-induced nephritis, the planted antigen hypothesis states that the major mechanism by which anti-DNA antibodies form immune deposits is by binding to autoantigens, either DNA or nucleosomes, that have been bound previously to renal tissue [219]. Both DNA and nucleosomes have been shown to have an affinity for the GBM [231] and glomeruli [232, 233]. Specifically, DNA has been shown to interact with collagen [234], fibronectin [235], and laminin [181], whereas nucleosomes have an affinity for the GBM component heparan sulfate [221].

The observation that DNA already bound to collagen can be recognized by anti-DNA antibodies and lead to the formation of immune complexes, whereas preformed DNA-antiDNA complexes do not bind to collagen to the same extent, provided a rationale for the local formation of DNA–antiDNA complexes in lupus nephritis [231]. Further support came from *ex vivo* perfusion studies showing that anti-DNA antibodies do not bind directly to renal antigens, but that binding to the GBM is mediated through histones and DNA [233]. Furthermore, some IgG from patients with SLE nephritis bound human glomerular extracts only in the presence of either DNA or nucleosomes [236].

Although the apparent disparity in data supporting each of the aforementioned hypotheses may at first be disconcerting, it must be kept in mind that all the studies have been performed with distinct sets of antibodies. Therefore, these seemingly contradictory results may simply be a reflection of the diversity of the anti-DNA antibodies present in the sera of lupus patients and mouse models, in addition to antigenic differences in the target organs in each assay. The three models of pathogenesis presented earlier may not necessarily be mutually exclusive. Rather, it is possible that different pathogenic mechanisms may be operative simultaneously or successively in the same lupus patient or different mechanisms may be at work in different patients. This may explain the observation that the glomerular lesions of SLE represent several distinct forms of nephritis that often differ from patient to patient.

ACTIVATION OF ANTI-dsDNA B CELLS

In order to understand how the production of anti-DNA antibodies might occur, it is necessary to briefly review the process of B-cell activation. B cells are activated when they encounter an antigen that cross-links membrane Ig. In addition, an activated T cell must provide costimulatory signals to the activated B cell.

Costimulation occurs through several different pathways, such as the interaction of CD40 on the B cell with the CD40 ligand on the T cell, B7 or ICOS on the B cell, and CD28 or CTLA-4 on the T cell, as well as BAFF and its B-cell receptors, TACI, BAFF-R, and BCMA (Fig. 3). Interaction of the B cell and T cell is further dependent on the T-cell receptor-binding major histocompatiblity complex (MHC) class II molecules on the B cell. These class II molecules are complexed to a peptide from self or foreign proteins that have been bound and internalized by the B cell. Thus, the B cell binds antigen and presents peptides from that antigen to T cells. The T cells that recognize a class II-peptide complex on the B cell then activate that B cell. In this way, B and T cells interact in the presence of antigen and costimulatory signals to generate an immune response. Generally, when B or T cells encounter antigen in the absence of costimulatory molecules, as is often the case with self-antigens, tolerance is induced [8].

Although it is clear that anti-dsDNA antibodies are involved in disease pathogenesis, it is not known how they arise and whether they are elicited by self or foreign antigens. A number of theories have been proposed to explain their induction and it is likely that more than one event can trigger their production. Some investigators have suggested that self-antigens presented in an immunogenic manner induce SLE, whereas many believe that the initial event is a response to foreign antigen. Multiple models thus exist to account for anti-DNA antibody production: (1) a novel association between DNA and a foreign antigen such as a viral protein may establish a hapten–carrier complex that can trigger a T-cell-dependent anti-DNA response; (2) an antibody elicited in response to a microbial antigen may cross-react with DNA because of certain shared epitopes between the microbial antigen and DNA (molecular mimicry); (3) somatic mutation of an antimicrobial antibody may give rise to an autoantibody; (4) cells undergoing apoptosis and releasing enzymatically degraded DNA and nucleosomal complexes may present novel (cryptic) epitopes of self-proteins that may be immunogenic [237]. These may activate T cells that in turn provide help to anti-DNA B cells; and [5] polyclonal activation of either T or B cells may induce anti-DNA antibody production. The first three models propose a role for foreign antigen, which are described in detail later. If the response to foreign antigen activates T or B cells that are cross-reactive with self-antigen, an immunologic cascade, termed epitope spreading can ensue, leading to autoantibodies against multiple self-antigens. This secondary amplification of target antigens is also discussed.

Immunogenicity of DNA

Hypothesis 1: DNA as a Hapten

In order to understand the etiology of the pathogenic anti-dsDNA antibodies found in SLE, several laboratories have tried to induce similar anti-dsDNA antibodies in mice. Early attempts to generate anti-dsDNA antibodies by immunizing mice with native eukaryotic B-DNA were unsuccessful [238]. Immunization with Z-DNA can induce anti-dsDNA antibodies [239], but these antibodies are specific for Z-DNA and do not cross-react with native B-DNA [238]. Bacterial DNA complexed with BSA is more immunogenic than eukaryotic DNA. When autoimmune NZB/W mice are immunized with *Escherichia (E.) coli* DNA complexed with methylated bovine serum albumin they develop antibodies that bind both *E. coli* and mammalian DNA [240]. These antibodies, like those that arise spontaneously in this strain, display similar V_H CDR3 arginine content and V gene utilization, and bind preferentially to adenosine thymidine (AT)-rich sequences [241]. These studies have led to speculations that *E. coli* DNA can elicit an anti-DNA response because it bears unique antigenic epitopes. It has been suggested that bacterial DNA may be more immunogenic because of a high guanosine cytodine (GC) content, the presence of methylated adenosine residues [242, 243], or the abundance of unmethylated cytodine guanosine dimers [244]. However, immunizaton of nonautoimmune mice with *E. coli* DNA results in an antibody response restricted to bacterial DNA and rarely cross-reactive with eukaryotic DNA [245]. These data indicate that bacterial DNA can elicit lupus-like antibodies in a host lacking intact mechanisms of self-tolerance, but will only induce antibodies specific for bacterial DNA in a host maintaining self-tolerance.

Based on the poor immunogenicity of DNA, it has been hypothesized that the immunogen in lupus is not naked DNA but consists of DNA complexed to DNA-binding proteins [246]. To determine whether DNA in association with protein might induce anti-dsDNA antibodies, nonautoimmune mice were immunized with a highly immunogenic peptide, Fus 1 (derived from the protozoan *Trypanosoma cruzi*), complexed with calf thymus DNA [246]. In this model it is helpful to consider DNA as a hapten recognized by B cells and the peptide as a carrier recognized by T cells. Mice developed low titers of antibodies to calf thymus DNA, suggesting that DNA can be somewhat immunogenic when presented in a complex with a highly immunogenic carrier. DNA coupled to viral proteins can also induce anti-DNA antibodies. Nonautoimmune mice hyperimmunized with polyoma BK virus T antigen develop antibodies to both mammalian and viral DNA. The

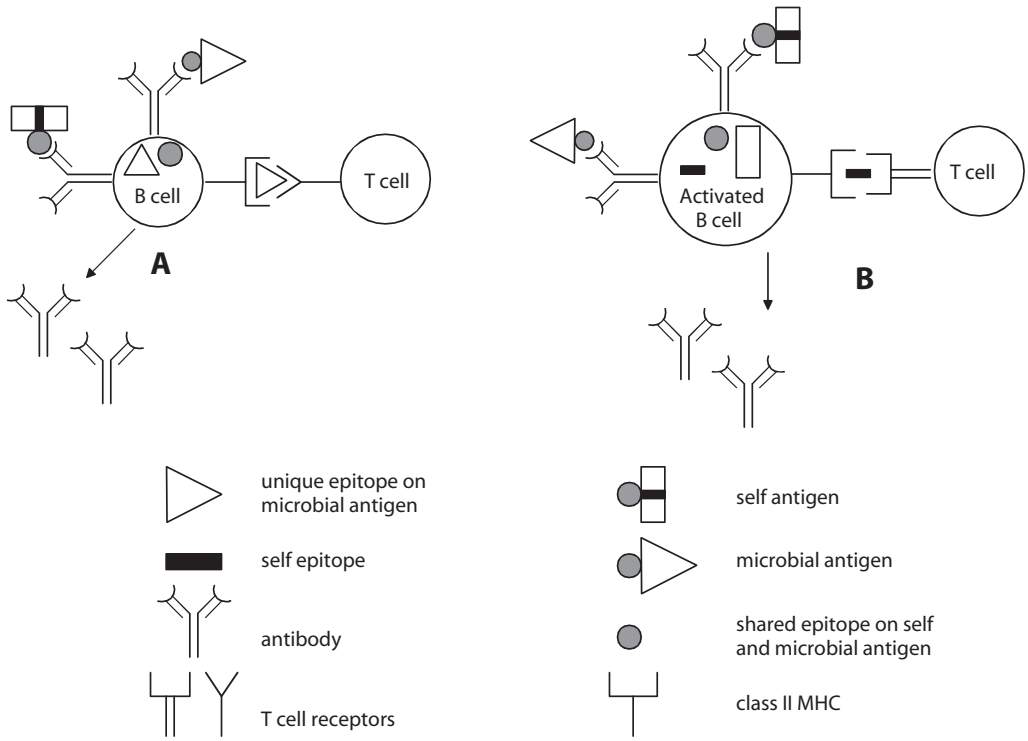


FIGURE 1 Molecular mimicry and presentation of self-epitopes can perpetuate an autoimmune response. (A) A B cell specific for a determinant shared by a microbial and self-antigen is triggered by a T cell specific for a unique determinant on the foreign antigen. (B) The activated B cell internalizes, processes, and presents epitopes of the self-antigen. A T cell that has never seen this epitope before, and has therefore not been tolerized to it, is now activated and continues to provide help to the autoreactive B cell and to other B cells with this autospecificity.

anti-dsDNA response has been shown to depend on the DNA-binding property of the T antigen, which serves as the carrier protein for the DNA hapten [247–249]. By establishing hapten–carrier complexes with DNA, viruses may initiate the production of anti-dsDNA antibodies *in vivo* and persistent viral infection may lead to autoimmunity.

Hypothesis 2: Molecular Mimicry

Environmental pathogens have long been suspected to be inducers of autoimmune disease. Autoimmune NZB mice raised in a germ-free environment develop reduced titers of autoantibodies, and elevated titers of anti-DNA antibodies can be found in patients with microbial infections, suggesting that microbial stimulation of the immune system helps stimulate the induction of autoantibodies in some way [250, 251]. Similarly, mice transgenic for an antibody to red blood cells do not develop autoimmune hemolytic anemia if they are bred in pathogen-free conditions. In conventional breeding conditions, however, many develop anemia, demonstrating that bacterial exposure plays a role in activating the autoreactive B cells [252, 253]. Studies of human and murine monoclonal anti-DNA antibodies have demonstrated extensive cross-reactivity with bacterial antigens (discussed earlier), suggesting that bacterial antigens may elicit these antibodies. This cross-reactivity, known as molecular mimicry, offers a potential explanation for the development of autoimmunity (Fig. 1). Alternatively, T cells cross-reactive with self-antigen may be activated during the response to microbial antigen and subsequently provide help to B cells specific for the self-antigen (Fig. 2).

Hypothesis 3: B-Cell Somatic Mutation

The majority of pathogenic anti-dsDNA antibodies in human and murine lupus are encoded by somatically mutated Ig genes [254–260]. The importance of somatic mutation in generating pathogenic anti-DNA antibodies is illustrated by experiments that show that transgenes coding for anti-ssDNA antibody can gain specificity for dsDNA by somatic mutation. Also, treatment with a costimulatory blockade in NZB/W mice leads to a decreased frequency of somatic mutations in

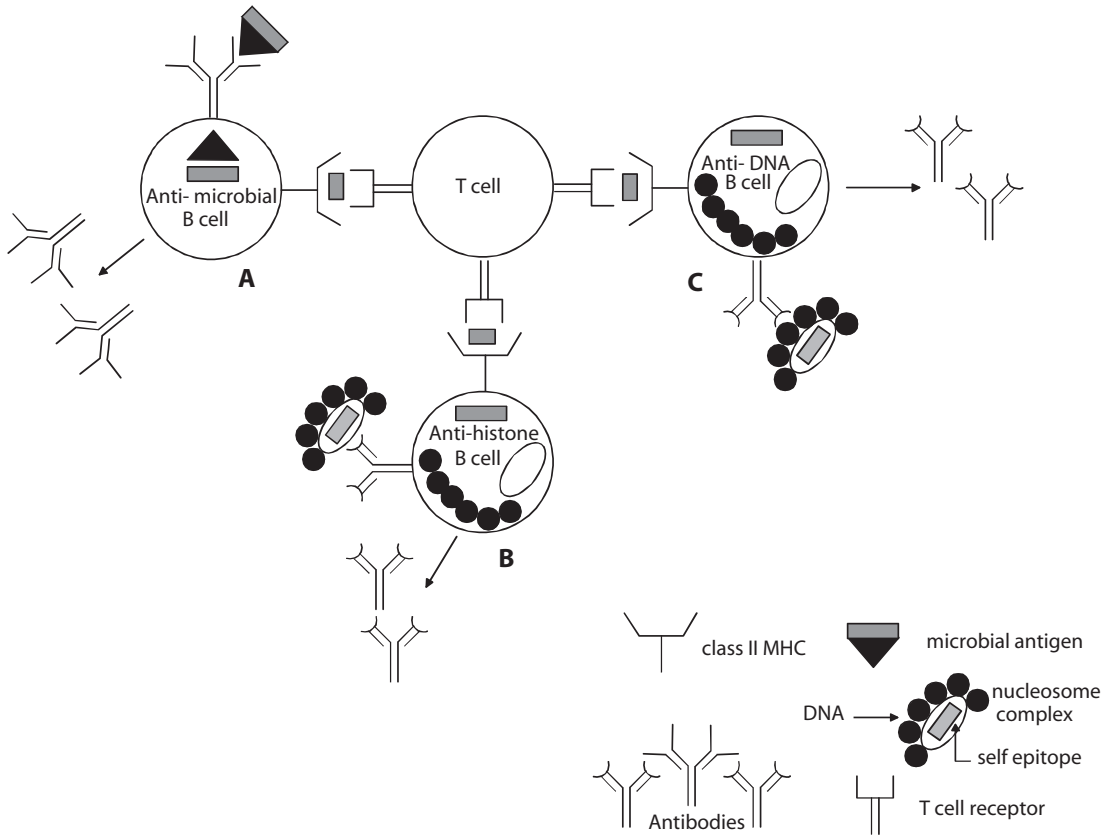


FIGURE 2 Epitope spreading leads to the production of anti-dsDNA antibodies. (A) A B cell specific for a microbial antigen internalizes, processes, and presents epitopes from the foreign antigen to T cells. (B) A T cell recognizing an epitope on a microbial antigen cross-reacts with a self-epitope and therefore activates both antimicrobial- and antihistone-specific B cells, causing them to secrete antibodies. (C) An anti-dsDNA B cell recognizes DNA in the nucleosome complex and presents the same histone epitope that has been presented by the antihistone B cells to activated T cells. These T cells can now activate anti-dsDNA B cells, causing them to secrete anti-dsDNA antibodies.

the VHBW-16 gene that encodes pathogenic anti-DNA antibodies, contributing to decreased anti-dsDNA antibody titer and prolonged survival [92].

The observation that many anti-DNA antibodies cross-react with microbial antigens suggests that antibodies to dsDNA may arise from antimicrobial antibodies undergoing somatic mutation. Support for this hypothesis was obtained in an *in vitro* culture system in which spontaneous mutation of an antibody to phosphorylcholine (PC) resulted in an antibody with specificity for dsDNA [53]. To determine if anti-dsDNA antibodies routinely derive from antimicrobial antibodies following mutation, efforts were made to generate anti-dsDNA hybridomas from nonautoimmune mice immunized with PC. Only rarely is it possible to obtain anti-dsDNA antibodies that cross-react with PC [176] unless splenocytes from PC-immunized mice are fused with a B-cell fusion partner transfected with the anti-apoptic gene bcl-2 [54]. Bcl-2 promotes B-cell survival and is upregulated in B cells destined to become plasma cells or memory B cells. Many hybridomas generated from these latter fusions cross-react with dsDNA [54]. These display somatically mutated genes, suggesting that autoreactive B-cell clones arise routinely following exposure to a bacterial antigen, but are deleted in normal mice.

Perhaps the most convincing data suggesting that anti-dsDNA antibodies can arise by somatic mutation following a response to bacterial antigen come from a study of human antibodies. Nonautoimmune patients given a polyvalent pneumococcal polysaccharide vaccine developed antipneumococcal antibodies bearing the anti-DNA-associated idiotypes 3I and 8.12 [104, 152]. In contrast, 3I+ and 8.12+ antibodies obtained from a combinatorial library derived from a lupus patient given pneumovax prior to splenectomy bind

both pneumococcal polysaccharide and dsDNA [177]. DNA binding appears to correlate with an increased frequency of somatic mutation, supporting the hypothesis that a bacterial antigen can trigger the development of anti-DNA antibodies through somatic mutation. In normal individuals, these antibodies are downregulated, but may escape regulation in autoimmune individuals.

There are conflicting data about the process of somatic mutation in SLE. An analysis of 10 human antibodies with a F4 idiotype that is highly associated with specificity for dsDNA demonstrated a decrease in targeting of mutations to mutational hot spots, but no change in the number of somatic mutations or their distribution in CDRs or FRs [116]. Analysis of V genes from B cells from one lupus patient demonstrated an increased frequency of mutation in both CDRs and FRs, with targeting to hot spots in FRs [118].

Epitope Spreading

The production of a diverse repertoire of autoantibodies can begin with the activation of a single autoreactive lymphocyte, either a B cell (Fig. 1) or a T cell (Fig. 2), followed by the diversification of the initial immune response. Native DNA is a component of many multimolecular complexes whose subunits are often targets for autoantibody production in SLE. DNA associates with histone proteins to form a nucleosome, and antibodies to nucleosomes and histones as well as DNA are found frequently in patients with lupus [261–263]. DNA also associates with replication/repair proteins, the Ku protein, and transcription factors. Antibodies to several of these DNA-binding proteins are also often present in SLE patients [261]. Cryptic or novel epitopes derived from the protein subunits in DNA protein complexes may be presented by B cells, activate autoreactive T cells, and so perpetuate and expand an autoantibody response (Fig. 1). This process is termed epitope spreading.

Evidence for epitope spreading exists in animal models [264–267]. It has been observed for some time now that lupus patients who have antibodies to Sm often develop antibodies to RNP and DNA over time [193, 266, 268]. Both RNP and Sm are components of the spliceosomal complex. To test whether epitope spreading occurs between Sm and RNP, rabbits and mice were immunized with peptides derived from the Sm B protein and control peptides. Animals immunized with the Sm peptides developed antibodies to Sm as well as to other components of the splicesome, including U RNAs and nRNPs [266, 269]. In fact, a few rabbits and mice also developed antibodies to dsDNA, exemplifying how anti-dsDNA antibodies may arise even when DNA is not the eliciting autoantigen as long as there is physical linkage between the immunizing protein and nucleic acid.

T CELLS INVOLVED IN ANTI-DNA ANTIBODY PRODUCTION

Most pathogenic anti-dsDNA antibodies in lupus are IgG and are somatically mutated. Because isotype switching and somatic mutation require T-cell help, it is clear that T cells have an important function in the production of autoantibodies [270–273]. The role of T cells in lupus has been demonstrated in autoimmune mouse models, and interfering with T-cell–B-cell interactions has been successful in ameliorating disease activity [274–276].

T cells from SLE patients display an activation defect that arises from altered expression of different signaling molecules. For example, an aberration in the protein kinase C- mediated activation pathway has been identified [277]; lupus T cells have an impaired protein kinase C response to integrin-mediated activation, diminished proliferation in response to anti-CD3 and anti-integrin antibody, and diminished adhesion to fibronectin [278]. Studies have also shown that lupus T cells exhibit a decreased expression of the TCR ζ chain, a crucial signaling molecule, caused by an increased frequency of mutations and polymorphisms in the TCR ζ chain-untranslated region [279]. These T cells also display increased phosphorylation of downstream signaling molecules and increased free intracellular calcium concentrations, demonstrating that alternative signaling pathways substitute for the deficient TCR ζ chain. It has been demonstrated that lupus T cells have an increased expression of the Fc ε receptor γ chain on both $CD4^+$ and $CD8^+$ T cells; its increased expression may contribute to the abnormal T-cell activation in SLE patients [280].

In recent years, attention has focused on the antigenic specificity of T cells involved in murine and human lupus, and it has been observed that autoreactive T cells may recognize either self-peptides that derive from a nucleoprotein complex or peptides derived from the autoantibodies themselves. An analysis of 15 autoreactive T-cell clones derived from five patients with SLE demonstrated that autoreactive T cells support the polyclonal activation of B cells [281]. Autoantibody secretion did not occur when antigen presentation was blocked with anti-HLA class II antibodies, demonstrating that T-cells engage in cognate interactions with autoreactive B cells. Histone-specific T-cell clones generated from autoimmune mice are able to augment the production of anti-DNA antibodies *in*

vitro and induce anti-dsDNA production when transferred into autoimmune hosts prior to disease onset [264]. Because DNA and histones are components of the same nuclear complex, a histone-specific T cell can activate anti-DNA B cells. Similarly, in a peptide-induced model of SLE, activation of class II-restricted $CD4^+$ T cells is necessary for the breakdown of tolerance and autoantibody production [282]. B cells can process their own immunoglobulin molecules and present immunoglobulin-derived peptides in the context of MHC class II molecules [283–286]. Peptides derived from anti-dsDNA antibodies can prime autoreactive T cells in autoimmune NZB/W mice, lead to activation of anti-dsDNA B cells, and hasten the onset of disease [287]. Although some studies have suggested that autoreactive T cells have anionic residues in the CDR3 of their T-cell receptor that are capable of binding to cationic residues present in nucleosomes [266], others have not confirmed the preferential usage of anionic receptors [287].

The majority of T cells express a T-cell receptor composed of an α chain and β chain, which are covalently linked to one another. These T cells are required for the development of autoantibodies and the onset of autoimmunity. A small subset of T cells (less than 5% in humans) expresses a T-cell receptor consisting of a γ chain and a δ chain. Some studies suggest that γδ T cells may play an important regulatory role in SLE. Autoimmune MRL/lpr mice deficient in γδ T cells have higher titers of autoantibodies and exacerbated disease compared to mice in which both αβ and γδ T cells are present [288]. The importance of γδ cells in SLE is also supported by human data. A decreased number of γδ T cells has been reported in SLE patients and correlates inversely with markers of inflammation [289].

REGULATION OF ANTI-DSDNA B CELLS

Tolerance Induction

Autoreactive B cells can be downregulated by one of three mechanisms of tolerance: deletion, anergy, or receptor editing. Deletion refers to the death of the cell through apoptosis or programmed cell death. Anergy is a state of functional paralysis in which the normal signals for B-cell activation will no longer activate the cell. Anergy can be reversed by exposure to B-cell mitogens, such as bacterial lipopolysaccharide (LPS). Receptor editing is a mechanism by which a B cell can attempt to change its specificity and avert autoreactivity by undergoing additional V region rearrangements.

The advent of transgenic technology has enabled investigators to follow the fate of autoreactive B cells and to learn more about the maintenance of tolerance. A number of laboratories have generated transgenic mouse models to study the regulation of anti-DNA B cells [290–292]. These studies have led to the development of a model for tolerance induction. B-cell activation depends on stimulation by two signals: antigen and a costimulatory molecule. B-cell tolerance occurs when the cell encounters antigen in the absence of costimulatory molecules. As inflammation is needed to induce the expression of costimulatory molecules, an encounter with antigen in a noninflammatory setting will, in general, lead to tolerance. Because most encounters with self-antigen occur under noninflammatory conditions, B cells reactive with self-antigen are generally tolerized. When a B cell encounters self-antigen in the bone marrow, RAG genes are reexpressed and receptor editing occurs. If this process does not lead to successful production of a new nonautoreactive antibody, then deletion or anergy will ensue with deletion occurring under conditions of extensive cross-linking of membrane Ig and anergy under conditions of more modest cross-linking. Some autoreactive B cells will have an affinity for antigen below a threshold for tolerance induction or will encounter too little antigen to trigger tolerance induction and willl remain activatable in the periphery [293].

Anti-DNA Antibody Transgenic Mice

Two transgenic mouse models expressing either the heavy chain of an anti-DNA antibody or both the heavy and the light chain have been analyzed in some detail. In one transgenic system, nonautoimmune BALB/c mice were made transgenic for the heavy chain of an IgM anti-DNA antibody that arises spontaneously in MRL/lpr lupus-prone mice. Pairing of the heavy chain with different endogenous light chains resulted in either antibodies that recognized ssDNA only or those that also recognized dsDNA. Analysis of anti-DNA B cells suggested that anti-ssDNA B cells are anergized, whereas B cells with an affinity for dsDNA are deleted [290]. Subsequent studies using mice transgenic for IgM heavy chain, as well as particular transgenic light chains, confirmed these results [294, 295]. Transgenic anti-ssDNA B cells display functional defects, such as decreased total surface Ig and suboptimal response to T-dependent and T-independent stimuli, but have a normal life span compared to nontransgenic B cells. Further analysis revealed that receptor editing was responsible for a shift in specificity of the B-cell receptor from dsDNA binding to non-dsDNA binding [295, 296]. Definitive studies have not yet been performed to

determine whether receptor editing is less efficient or defective in human SLE. Of interest, however, is one study suggesting that B cells from several patients with SLE were unable to edit a particular V_κ gene that is frequently associated with a cationic anti-DNA antibody whereas normal B cells appear capable of editing this light chain [297].

In another transgenic system, both nonautoimmune BALB/c mice and autoimmune NZB/W mice were made transgenic for the heavy chain of an IgG anti-dsDNA antibody [126, 291]. In these mice, the transgenic heavy chain can pair with the spectrum of endogenous light chains to produce B cells with DNA-binding and non-DNA-binding specificities. Although nonautoimmune mice display negligible titers of transgene-encoded anti-dsDNA antibody due to the induction of tolerance, the autoimmune mice secrete elevated titers of transgene-encoded anti-dsDNA antibody. Analysis of autoimmune and nonautoimmune mice revealed the presence of three populations of anti-dsDNA B cells, each of which is subject to a different mechanism of regulation in nonautoimmune hosts. One population with high affinity for dsDNA is anergized; a second population that also has high affinity for dsDNA is deleted; and a third population producing low-affinity anti-dsDNA antibodies escapes regulation and is termed indifferent. It has been demonstrated that some members of the low-affinity subset of anti-dsDNA B cells differ from the high-affinity subsets by a single base substitution, resulting in a single amino acid replacement [298]. Thus high-affinity pathogenic anti-DNA B cells may arise when low-affinity anti-dsDNA B cells, making "natural autoantibody," undergo somatic mutation.

Genes That Regulate B-Cell Tolerance

Several molecules are involved in setting thresholds for the survival, activation, or tolerance of autoreactive B cells. The MRL/lpr mouse model of lupus has a defect in the Fas gene [299–301] that inhibits activation-induced T- and B-cell death, which results in severe lymphoproliferation. MRL/lpr mice transgenic for an IgM anti-DNA antibody fail to display tolerance and do not delete anti-dsDNA B cells [302]. These mice allow anti-DNA B cells to enter the B-cell follicles, from which they are normally excluded [303]. The lpr mutation does not require the MRL background for the induction of anti-dsDNA antibodies; C57BL/6J, AKR/J, and C3H/HeJ strains of mice bearing the lpr mutation are all capable of producing anti-DNA antibodies; however, they develop lower titers of autoantibodies and less severe glomerulonephritis than MRL mice [304]. Although the Fas mutation leads to a lupus-like syndrome in mice, the same mutation in humans leads to lymphoproliferation without the autoantibody specificities that characterize lupus [305].

The bcl-2 gene product promotes B-cell survival in both the bone marrow and the periphery [306–308]. Overexpression of bcl-2 in nonspontaneously autoimmune mice transgenic for the heavy chain of a pathogenic anti-DNA antibody leads to survival of anti-dsDNA B cells that normally would be deleted [309] and "rescue" of cross-reactive anti-DNA/anti-pneumococcal B cells in nonautoimmune mice during a primary response to PC [310, 311]. The hormones estrogen [312, 313] and prolactin (E. Peeva, unpublished data) promote the survival of anti-dsDNA B cells and upregulation of the bcl-2 gene. The phenotypic impact of bcl-2 overexpression depends on the genetic background; bcl-2 overexpression induces a lupus-like syndrome in some mouse strains [309], but not in others [314]. A spontaneous overexpression of bcl-2 has not been observed in patients with lupus.

Several regulatory molecules are involved in signaling B cells to proliferate and secrete antibody or undergo apoptosis. These molecules include cell surface receptors, such as CD19, CD45, and CD22, and their intracellular components, such as Lyn, Fyn, Btk, and the tyrosine phosphatase SHP-1. Alterations in expression or function of any of these molecules can be associated with abnormal B-cell function and development of a lupus-like phenotype. Motheaten mice spontaneously develop an autoimmune syndrome characterized by the production of anti-dsDNA as well as other autoantibodies [315]. These mice have a mutation that ablates the activity of SHP-1 phosphatase, which helps regulate B-cell activation [316–318]. B cells producing autoantibodies in motheaten mice are $CD5^+$ B1 cells. It has been shown that a death signal can be delivered through ligation of the CD5 molecule [319, 320]. CD5 is, therefore, thought to play a role in negative signaling, and it has been suggested that its mechanism of action requires the recruitment of SHP-1 phosphatase. Hence, in SHP-1-deficient mice the absence of a CD5–SHP-1 interaction may lead to the accumulation of $CD5^+$ B cells. Lyn is a tyrosine kinase associated with membrane Ig that downmodulates B-cell responses. Lyn deficiency results in increased anti-DNA titers and immune complex deposits in the glomeruli [321]. Lyn is also important in determining B-cell development, as demonstrated by the absence of marginal zone B cells in Lyn-deficient mice [322, 323]. CD22 is another transmembrane molecule that associates with SHP-1 phosphatase that can negatively regulate antigen receptor signaling [324]. Mice deficient in CD22 expression also display elevated titers of anti-DNA antibodies due to an expanded B1 cell population [325–328]. In some SLE patients, a sig-

nificant decrease in the expression of Lyn in resting as well as in anti-IgM-stimulated B cells was observed, but the expression of CD22 was intact [329]. A newly discovered molecule called BAFF plays a pivotal role in B-cell activation, maturation, and proliferation. BAFF transgenic mice develop an increased number of mature B cells and T cells, and hypergamaglobulinemia. These mice have a lupus-like illness characterized by anti-DNA antibodies and Ig deposits in the kidney [330, 331]. NZB/W lupus-prone mice exhibit increased levels of BAFF.

CD45 or B220 is a transmembrane tyrosine phosphatase involved in B-cell activation [332]. Mice deficient in CD45 display a hyporesponsiveness to antigen receptor cross-linking and display diminished signaling in response to an autoantigen. This leads to a need for more autoantigen to be present to signal the elimination of autoreactive B cells. When the concentration of autoantigen is low, only those B cells with the highest affinity for autoantigen are eliminated. CD45-deficient mice, therefore, have an increased number of autoreactive B cells and an enhanced incidence of autoimmunity [333]. Human studies also suggest a decreased expression of CD45 on B cells of SLE patients [334]. The cell surface molecule CD19 has also been observed to alter signaling thresholds, and mice with an increase in CD19 expression have elevated titers of anti-dsDNA antibodies that are predominantly of the IgG isotype. B1 cells are upregulated in these mice, but it is not clear whether these cells or conventional cells are the source of anti-DNA antibodies [335]. Even 15–29% increases in CD19 expression in C57Bl/6 mice can induce autoantibody production, suggesting that subtle changes in the levels of expression of signaling molecules may lead to autoreactivity [336].

Expression of molecules that are involved in the costimulation of B cells may also influence the induction of anti-DNA antibodies. As stated earlier, two signals are required for B-cell activation: (1) ligation of surface immunoulobulin with antigen leading to BCR signaling and (2) engagement of costimulary receptors on the surface of B and T lymphocytes. Costimulatory signals are delivered by the binding of the B-cell surface molecules CD40, B7, or TACI/BCMA/BAFF-R with the T-cell surface molecules CD40 ligand (CD40L), CD28/CTLA-4, or secreted molecule BAFF, respectively (Fig. 3). Lpr/lpr mice deficient in CD40L have been observed to have lower titers of IgG anti-DNA antibodies [337], and BAFF-deficient mice display a severe B-cell depletion [83]. Studies such as these have led to speculations that costimulatory molecules are upregulated in SLE patients and lupus-prone mice, resulting in an increased production of autoantibodies. Indeed, there is an increased expression of CD40 ligand on both B and T lymphocytes [338] and an increased expression of CD86 on peripheral blood cells in lupus patients [339]. Cytokines and hormones may also play a role in the development of lupus. Interleukin (IL)-10, for example, has been shown to activate B cells to secrete autoantibodies, and treatment with the anti-IL-10 antibody delays the appearance of autoantibodies in lupus-prone NZB/W mice [340] and provides some therapeutic benefit in humans [341]. Mice lacking expression of transforming growth factor-β produce anti-DNA antibodies and develop glomerulonephritis [342]. Finally, elevated estrogen and prolactin levels lead to elevated titers of anti-DNA antibodies and a lupus-like phenotype in certain genetic backgrounds.

The importance of dysregulated innate immunity has been emphasized as a factor in the etiopathogenesis of SLE. Impaired clearance of apoptotic material from the circulation plays a significant role in the breakdown of tolerance and induction of anti-DNA antibodies [343]. Apoptotic cells given intravenously induce anti-DNA antibodies in nonautoimmune mice [344], whereas an increased expression of the soluble complement inhibitor prolongs survival in autoimmune MRL/lpr mice [345]. The lupus susceptibility locus Sle1c includes genes encoding complement receptors CD21/CD35; a single nucleotide polymorphism of CD21 that affects the glycolysation and dimerization of the molecule that is necessary for the binding of C3d results in a lupus-like phenotype [346]. A decreased expression of both CD21 and CD35 has been observed in MRL/lpr lupus-prone mice [347]. The importance of complement in the maintenance of tolerance is further supported by the association of lupus and deficiency of the early complement components. C1q deficiency accelerates disease progression in MRL/lpr mice [348], whereas C4-deleted lpr mice and mice lacking complement receptors 1 and 2 demonstrate high titers of antinuclear and anti-DNA antibodies and severe glomerulonephritis [349]. Clearance of apoptoic debris through Fcγ receptors also seems to regulate the development of autoimmunity. Fcγ receptors bind pentraxins involved in the clearance of nuclear proteins, serum amyloid protein (SAP), and C-reactive protein (CRP), allowing phagocytes that express these receptors to ingest SAP and CRP-coated apoptotic material directly [350]. Utations of FcRIIIA causing decreased uptake of immune complexes are observed in patients with SLE [351]. Toll-like receptors (TLR) are also implicated in autoimmunity. Cross-linking of the BCR and TLR9 activates B cells; thus, chromatin alone can activate anti-DNA B cells [352].

Dysregulation of either the innate or the adaptive immune system may lead to the development of autoantibodies against nuclear components, including DNA.

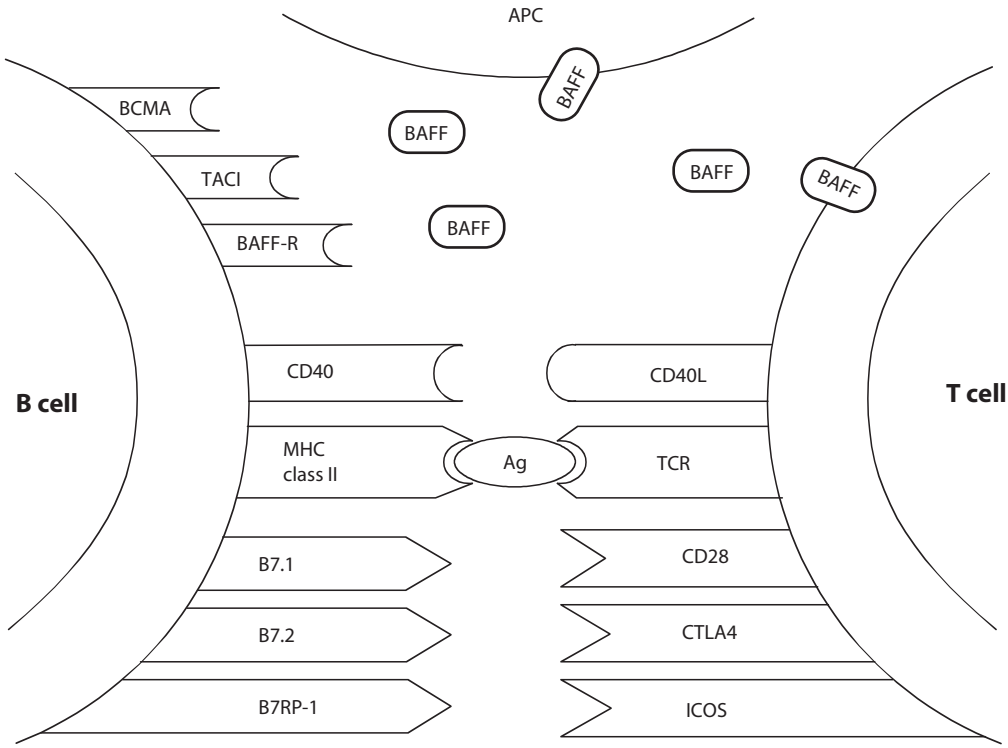


FIGURE 3 B-cell–T-cell costimulation is dysregulated in SLE and leads to enhanced autoantibody production. An antigen-presenting cell (APC) processes antigen (Ag) and presents it in context of MHCII to a $CD4^+$ T cell via an interaction with the T-cell receptor (TCR). Costimulatory molecules including B7 family members (B7.1, B7.2, and B7RP-1) on the B cell bind to T-cell receptors CD28 and ICOS, and tumor necrosis factor family members that include CD40L and BAFF bind to B-cell surface molecules CD40 and TACI, BAFF-receptor and BCMA, respectively. B-cell/T-cell costimulatory augmentation is negatively regulated by the interaction of B7 molecules with CTLA4 on $CD4^+$ T cells. Aberrant costimulatory processes appear to exist in SLE. For example, there is an increased expression of soluble and T-cell membrane-expressed CD40L and BAFF in lupus patients, as well as an increased expression of B7.1/2 on the surface of lupus B cells.

It is clear that aberrant expression of a variety of different molecules may result in the production of anti-DNA antibodies and in the development of a lupus-like phenotype in mice with a particular genetic background. Defects in expression of more than one of these molecules are required to sustain the autoimmune response.

THERAPY

A major goal of understanding the structure, origin, and pathogenicity of anti-DNA antibodies is to develop therapies that will be less harmful than the current nonselective immunosuppressive regimens used to treat lupus patients. Several novel approaches to therapy of SLE have been proposed based on our growing understanding of B-cell activation. This section briefly mentions only the novel therapeutic approaches that are specific to anti-DNA antibodies.

The association of anti-DNA antibodies with pathologic events in SLE, especially with lupus nephritis, suggests that removal of anti-DNA antibodies, or blocking their production or activity, may be beneficial. Many anti-DNA antibodies in SLE arise from T-cell dependent responses in which costimulation between B and T cells is mediated through the CD40-CD40 ligand and B7-CD28 pathway (Fig. 3). Blocking the costimulatory pathways, independently or collectively, prevents the generation of a second signal, which is necessary for B-cell proliferation, differentiation, and antibody production. Monoclonal antibody to the CD40 ligand leads to improved survival in the NZB/W and SNF mouse models of SLE. Inhibition of the B7-CD28 costimulatory pathway by CTLA-4 Ig, a fusion molecule constructed of CTLA-4 and the Fc portion of an

immunoglobulin, also blocks anti-DNA antibody production and prolongs life in NZB/W mice [353]. Simultaneous treatment with anti-CD40 and CTLA-4 Ig or cyclophophosphamide and CTLA-4 Ig provides greater benefit than either intervention alone [92, 354]. However, clinical trials with anti-CD40 ligand in patients were less successful. It remains a question whether blockade of the CD40-CD40 ligand or B7-CD28 costimulatory pathways or other costimulatory pathways will be useful therapeutic modalities for SLE.

Idiotypic interventions may be used to deplete specific antibodies or specific B-cell populations. For example, patients have been subjected to the extracorporeal depletion of antibodies bearing the 3I idiotype that are present on anti-DNA antibodies and associated with lupus nephritis. These patients showed a marked diminution in DNA binding. The patient number was, however, too small and the duration of the study too short to determine if there was a lasting effect of this therapy [355]. Two new techniques to remove anti-DNA antibodies from the blood of lupus patients based on immunoadsorbtion of DNA have been developed [356, 357]. Both techniques lead to significant declines in the anti-DNA titer, but neither has entered into a clinical trial.

It has been suggested that anti-idiotype administration *in vivo* might alter the disease course. Preparations of IVIG containing anti-idiotypic activity to anti-DNA-associated idiotypes were reported to be more likely to induce disease remission than IVIG preparations that lack anti-idiotypic activity [358]. It has also been suggested that anti-idiotypic antibodies can be complexed to cytotoxic drugs to target specifically anti-DNA B cells [359].

Understanding mechanisms of tolerance induction in B cells has led to the design of a novel therapeutic for SLE, LJP 394, a conjugate constructed from a polyethylene glycol and tetrameric oligonucleotides [360]. This reagent is designed to provide antigen signaling through the BCR in the absence of costimulation, thus leading to B-cell deletion or anergy. In BXSB male lupus-prone mice, treatment with LJP 394 led to a significant decrease in proteinuria and increased survival [361], but results in a preliminary study in patients showed only a modest, if any, benefit [362]. A second antigen-based heteropolymer has been developed to bind anti-DNA antibodies [363], but it has not been tested in animal studies yet.

Another novel therapeutic approach to SLE in mice is based on the observation that peptides derived from anti-DNA antibodies can activate T cells that regulate autoantibody production. Treatment of NZB/W mice with peptides derived from an anti-DNA antibody demonstrated a beneficial effect with a delay in the development of autoantibodies and prolonged survival [364].

SUMMARY

All individuals have the potential to produce high-affinity potentially pathogenic anti-DNA antibodies. Anti-DNA antibodies are encoded by genes that are present in nonautoimmune individuals and that are used routinely to encode protective antibodies. The mechanisms of somatic diversification of the immunoglobulin repertoire appear to be normal in lupus patients. It has been demonstrated that anti-DNA antibodies arise in nonautoimmune hosts during microbial infection, but that tolerance is restored when microbial antigens are eliminated. Therefore, it appears that the critical defect in SLE is not in the formation of the B-cell repertoire, but in the regulation of autoreactive B cells.

The antigenic stimulus for anti-DNA antibody production is not known, nor is it clear whether a self or foreign antigen begins the process. There are probably several triggers for anti-DNA antibody production. Just as the antigenic trigger for anti-DNA antibody production may not always be DNA, the antigenic target may also not be DNA. It is apparent that at least some anti-DNA antibodies can cross-react directly with tissue antigens and so mediate tissue damage. On the near future, transgenic models and human genetic studies in combination should elucidate the defects in B-cell repertoire selection and tolerance induction that can lead to SLE. Major questions need to be addressed in future studies: (1) What abnormalities of B-cell function alter tolerance induction in SLE? (2) What genetic defects underlie these abnormalities? (3) How do anti-DNA antibodies cause tissue injury, (4) What differences in target tissue regulate susceptibility to anti-DNA-mediated tissue damage? Such studies in turn will suggest new and specific therapies.

References

1. Tan, E. M. (1982). Autoantibodies to nuclear antigens (ANA): Their immunobiology and medicine. *Adv. Immunol.* **33**, 167–240.
2. Pisetsky, D. S. (1993). Autoantibodies and their significance. *Curr. Opin. Rheumatol.* **5**, 549–556.
3. Reichlin, M. (1998). Antibodies to Ro and La. *Ann. Med. Interne* (*Paris*) **149**, 34–41.
4. Gharavi, A. E., Harris, E. N., Lockshin, M. D., *et al.* (1988). IgG subclass and light chain distribution of anticardiolipin and anti-DNA antibodies in systemic lupus erythematosus. *Ann. Rheum. Dis.* **47**, 286–290.

5. Bootsma, H., Spronk, P. E., Ter Borg, E. J., *et al*. (1997). The predictive vaule of fluctuations in IgM and IgG class anti-dsDNA antibodies for relapses in SLE. A prospective long term observation. *Ann. Rheum. Dis*. **56**, 661–666.
6. Tan, E. M., Chan, E. K. L, Sullivan, K. F., *et al*. (1988). Short analytical review: Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. *Clin. Immunol. Immunopathol*. **47**, 121–141.
7. Winfield, J. B., Faiferman, I., and Koffler, D. (1977). Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus: Association of high avidity anti-native DNA antibody with glomerulonephritis. *J. Clin. Invest*. **59**, 90–96.
8. Abbas, A. K., Lichtman, A. H., and Pober, J. S. (1997). "Cellular and Molecular Immunolog," 3rd Ed. Saunders, Philadelphia.
9. Kabat, E. A., and Wu, T. T. (1971). Attempts to locate complementarity determining residues in the variable positions of light and heavy chains. *Ann. N. Y. Acad. Sci*. **190**, 382–393.
10. Poljak, R. J., Anzel L. M., Avey, H. P., *et al*. (1973). Three dimensional structure of the Fab fragment of a human immunoglobulin at 2.8 A. *Proc. Natl. Acad. Sci. USA* **70**, 3305–3310.
11. Padlan, E. A. (1994). Anatomy of the antibody molecule. *Mol. Immunol*. **31**, 169–217.
12. Capra, J. D., Kehoe, J. M., Winchester, R. J., *et al*. (1971). Structure-function relationship among anti-gamma globulin antibodies. *Ann. N. Y. Acad. Sci*. **190**, 371–381.
13. Oudin, J., and Cazenave, P. A. (1971). Similar idiotypic specificities in immunoglobulin fractions with different antibody functions or even without detectable antibody function. *Proc. Natl. Acad. Sci. USA* **68**, 2616–2620.
14. Schiff, C., Milili, M., Hue, I., *et al*. (1986). Genetic basis for expression of the idiotypic network: One unique Ig VH germ line gene accounts for the major family of Ab1 and Ab3 (Ab1) antibodies of the GAT system. *J. Exp. Med*. **163**, 573–587.
15. Early, P., Huang, H., Davis, M., *et al*. (1980). An immunoglobulin heavy chain variable region is generated from three segments of DNA: VH, D, and JH. *Cell* **19**, 981–992.
16. Kurosawa, Y., and Tonegawa, S. (1982). Organization, structure and assembly of immunoglobulin heavy chain diversity DNA segments. *J. Exp. Med*. **155**, 201–218.
17. Meindl, A., Klobeck, H. G., Ohnheiser, R., *et al*. (1990). The V kappa gene repertoire in the human germ line. *Eur. J. Immunol*. **20**, 1855–1863.
18. Chuchana, P., Blancher, A., Brockly, F., *et al*. (1990). Definition of the human immunoglobulin variable lambda (IGLV) gene subgroup. *Eur. J. Immunol*. **20**, 1317–1325.
19. Kodaira, M., Kinashi, T., Umemura, I., *et al*. (1986). Organization and evolution of variable region genes of the human immunoglobulin heavy chain. *J. Mol. Biol*. **190**, 529–533.
20. Pascual, V., and Capra, J. D. (1991). Human immunoglobulin heavy chain variable region genes: Organization, polymorphism, and expression. *Adv. Immunol*. **49**, 1–5.
21. Cook, G. P., and Tomlinson, I. M. (1995). The human immunoglobulin VH repertoire. *Immunol. Today* **16**, 37–42.
22. Berman, J., Mellis, S., Pollack, R., *et al*. (1988). Content and organization of the human Ig VH locus: Definition of this new VH families and linkage to the Ig CH locus. *EMBO J*. **7**, 727–738.
23. Lieber, M. (1996). Immunoglobulin diversity-rearranging by cutting and repairing. *Curr. Biol*. **6**, 134–136.
24. Honjo, T., and Habu, S. (1985). Origin of immune diversity: Genetic variation and selection. *Annu. Rev. Biochem*. **54**, 803–830.
25. Lewis, S. M. (1994). The mechanism of V(D)J joining: Lessons from molecular, immunological and comparative analysis. *Adv. Immunol*. **56**, 27–150.
26. Fugmann, S. D., Lee, A. I., Shockett, P. E., *et al*. (2000). The RAG proteins and V(D)J recombination: Complexes, ends, and transposition. *Annu. Rev. Immunol*. **18**, 495–499.
27. Paull, T. T., and Gellert, M. (1998). The 3′ to 5 exonuclease activity of Mre 11 facilitates repair of DNA ds breaks. *Mol. Cell* **1**, 969–979.
28. Jeggo, P. A., Taccioli, G. E., and Jackson, S. P. (1995). Menage a trois: Double strand break repair, V(D)J recombination and DNA-PK. *Bioessays* **17**, 949–957.
29. Weaver, D. T. (1995). What to do at an end: DNA double strand break repair. *Trends Genet*. **11**, 388–392.
30. van Gent, D. C., Hiom, K., Paull, T. T., *et al*. (1997). Stimulation of V(D)J cleavage by high mobility group proteins. *EMBO J*. **16**, 2665–2670.
31. Desiderio, S. (1984). Insertion of N regions into heavy chain genes is correlated with expression of terminal deoxytransferase in B cells. *Nature* **311**, 752–755.
32. Klobeck, H. G., Meindl, A., Combriato, G., *et al*. (1985). Human immunoglobulin kappa light chain genes of subgroups II and III. *Nucleic Acids Res*. **13**, 6499–6514.
33. Klobeck, H. G., Bornkamm, G. W., Cabriato, G., *et al*. (1985). Subgroup IV of human immunoglobulin K light chains is encoded by a single germline gene. *Nucleic Acids Res*. **13**, 6515–6519.
34. Jaenichen, H. R., Pech M., Lindenmaier, W., *et al*. (1984). Composite human VK genes and a model of their evolution. *Nucleic Acids Res*. **12**, 5249–5263.
35. Solomon, A., and Weiss, D. T. (1987). Serologically defined V region subgroups of human lambda light chains. *J. Immunol*. **139**, 824–830.
36. Chang, L. Y., Yen, C. P., Besl, L., *et al*. (1994). Identification and characterization of a functional human Ig V lambda VI germline gene. *Mol. Immunol*. **31**, 531–536.
37. Frippiat, J. P., and Lefranc, M. P. (1994). Genomic organization of 34 kb of the human immunoglobulin lambda locus (IGLV): Restriction map and sequences of new V lambda III genes. *Mol. Immunol*. **31**, 657–670.
38. Heller, M., Owens, J. O., Mushinski, J. F., *et al*. (1987). Amino acids at the site of VKJK recombination not

encoded by germline sequences. *J. Exp. Med.* **166**, 637–646.
39. Matsuoka, M., Yoshida, K., Maeda, T., *et al.* (1990). Switch circular DNA formed in cytokine-treated mouse splenocytes: Evidence for intramolecular DNA deletion in immunoglobulin class switching. *Cell* **62**, 135–142.
40. Shimuzu, A., and Honjo, T. (1984). Immunoglobulin class switching. *Cell* **36**, 801–803.
41. Davis, M. M., Kim, S. K., and Hood, L. E. (1980). DNA sequences mediating class switching in immunoglobulins. *Science* **209**, 1360–1365.
42. Korsmeyer, S. J. (1981). Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B cells. *Proc. Natl. Acad. Sci. USA* **78**, 7096–7100.
43. Heiter, P., Korsmeyer, S. J., Waldmann, T. A., *et al.* (1981). Human immunoglobulin kappa light chain genes are deleted or rearranged in lambda producing B cells. *Nature* **290**, 368–372.
44. Retter, M. W., and Namazee, D. (1998). Receptor editing occurs frequently during normal B cell development. *J. Exp. Med.* **188**, 1231–1238.
45. Hikida, M., Mori, M., Takai, T., *et al.* (1996). Reexpression of RAG-1 and RAG-2 in activated mature B cells. *Science* **274**, 2092–2094.
46. Han, S., Dillon, S. R., Zheng, B., *et al.* (1997). V(D)J recombinase activity in a subset of germinal center B lymphocytes. *Science* **278**, 301–305.
47. Papavasiliou, F. N., and Schatz, D. G. (2002). Somatic hypermutation in immunoglobulin genes: Merging mechanisms for genetic diversity. *Cell* **109**, S35–44.
48. Hikida, M., Nakayama, Y., Yamashita, Y., *et al.* (1998). Expression of recombination activating genes in germinal center B cells: Involvement of interleukin 7 (IL-7) and IL-7 receptor. *J. Exp. Med.* **188**, 365–370.
49. French, D. L., Laskov, R., and Scharff, M. D. (1989). The role of somatic hypermutation in the generation of antibody diversity. *Science* **244**, 1152–1157.
50. Rogozin, I. B., and Kolchanov, N. A. (1992). Somatic hypermutagenesis in immunoglobulin genes. II. Influence of neighboring base sequences on mutagenesis. *Biochem. Biophys. Acta.* **117**, 1:11.
51. Wabl, M., and Steinberg, C. (1996), Affinity maturation and class switching. *Curr. Opin. Immunol.* **8**, 89–92.
52. Levy, Y., Gupta, N., Le Deist, F., *et al.* (1998). Defect in Ig V gene somatic hypermutation in common variable immunodeficiency syndrome. *Proc. Natl. Acad. Sci. USA* **95**, 35–40.
53. Diamond, B., and Scharff, M. D. (1984). Somatic mutation of the T15 heavy chain gives rise to an antibody with autoantibody specificity. *Proc. Natl. Acad. Sci. USA* **81**, 5841–5844.
54. Ray, S. K., Putterman, C., and Diamond, B. (1996). Pathogenic antibodies are routinely generated during the response to foreign antigen: a paradigm for autoimmune disease. *Proc. Natl. Acad. Sci. USA* **93**, 2019–2024.
55. Frey, S., Bertocci, B., Delbos, F., *et al.* (1998). Mismatch repair deficiency interferes with the acumulation of mutations in chronically stimulated B cells and not with the hypermutation process. *Immunity* **9**, 127–134.
56. Rada, C., Ehrenstein, M. R., Neuberger, M. S., *et al.* (1998). Hot spot focusing of somatic mutation in MSH2-deficient mice suggests two stages of mutational targeting. *Immunity* **9**, 135–141.
57. Wiesendanger, M., Kneitz, B., Edelmann, W., *et al.* (2000). Somatic hypermutation in Mut homologue (MSH)3-, NSH6-, and MSH3/MSH6-deficient mice reveals a role for the MSH2-MSH6 heterodimer in modulating the base substitution pattern. *J. Exp. Med.* **197**, 579–584.
58. Kim, N., Bozek, G., Lo, J. C., *et al.* (1999). Different mismatch repair deficiencies all have the same effect on somatic hypermutation: Intact primary mechanism accompanied by secondary modifications. *J. Exp. Med.* **190**, 21–30.
59. Winter, D. B., Phung, Q. H., Umar, A., *et al.* (1998). Altered spectra of hypermutation in antibodies of mice deficient for the mismatch repair protein PMS2. *Proc. Natl. Acad. Sci. USA* **95**, 6953–6958.
60. Ehrenstein, M. R., Rada, C., Jones, A. M., Milstein, C., *et al.* (2001). Switch junction sequences in PMS2-deficient mice reveal a microhomology-mediated mechanism of Ig class switch recombination. *Proc. Natl. Acad. Sci. USA* **98**, 14553–14558.
61. Muramatsu, M., Sankaranand, V. S., Anant, S., *et al.* (1999). Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J. Biol. Chem.* **274**, 18470–18476.
62. Kinoshita, K., and Hanjo, T. (2001). Linking class-switch recombination with somatic hypermutation. *Nature Rev. Mol. Cell. Biol.* **2**, 493–503.
63. Martin, A., Bardwell, P. D., Woo, C. J., *et al.* (2002). Activation-induced cytidine deaminase turns on somatic hypermutation in hybridomas. *Nature* **415**, 802–806.
64. Kocks, C., and Rajewsky, K. (1988). Stepwise intraclonal maturation of antibody affinity through somatic mutation. *Proc. Natl. Acad. Sci. USA* **85**, 8206–8210.
65. Diamond, B., Katz, J. B., Paul, E., *et al.* (1992). The role of somatic mutation in the pathogenic anti-DNA response. *Annu. Rev. Immunol.* **10**, 731–757.
66. Ginsberg, B., and Keiser, H. (1973). A millipore filter assay for antibodies to native DNA in sera of patients with systemic lupus erythematosus. *Arthritis Rheum.* **16**, 199–207.
67. Smeenk, R., and Hylkema, M. (1992). Detection of antibodies to DNA: A technical assessment. *Mol. Biol. Rep.* **17**, 71–79.
68. Smeenk, R., Van de Brink, H. G., Brinkman, K., *et al.* (1991). Anti-dsDNA: Choice of assay in relation to clinical value. *Rheumatol. Int.* **11**, 101–107.
69. Takeuchi, Y., Ishikawa, O., and Miyachi, Y. (1997). The comparative study of anti-double-stranded DNA antibody levels measured by radioimmunoassay and enzyme-linked immunosorbent assay in systemic lupus erythematosus. *J. Dermatol.* **24**, 297–300.

70. Bootsma, H., Spronk, P., Derksen, R., *et al*. (1995). Prevention of relapses in systemic 1upus erythematosus. *Lancet* **345**, 1595–1599.
71. Hayakawa, K. (1990). Autoreactivity and CD5+ B cells. *Curr*. *Opin*. *Immunol*. **2**, 582–587.
72. Hardy, R. R., and Hayakawa, K. (1994). CD5 B cells, a fetal B cell lineage. *Adv*. *Immunol*. **55**, 297–339.
73. Hayakawa, K., and Hardy, R. R. (2000). Development and function of B-1 cells. *Curr*. *Opin*. *Immunol*. **12**, 346–353.
74. Murakami, M., Yoshioka, H., Shirai, T., *et al*. (1995). Prevention of autoimmune symptoms in autoimmune-prone mice by elimination of B-1 cells. *Int*. *Immunol*. **7**, 877–882.
75. Casali, P., Burastero, S. E., Balow, J. E., *et al*. (1989). High affinity antibodies to ssDNA are produced by CD5– B cells in SLE patients. *J*. *Immunol*. **143**, 3476–3483.
76. Casali, P., and Notkins, A. L. (1989). Probing the human B cell repertoire with EBV: Polyreactive antibodies and CD5+ B lymphocytes. *Annu*. *Rev*. *Immunol*. **7**, 513–535.
77. Suzuki, N., Sakane, T., and Engleman, E. G. (1990). Anti-DNA antibody production by CD5+ and CD5– B cells of patients with SLE. *J*. *Clin*. *Invest*. **85**, 238–247.
78. Kipps, T. J. (1989). The CD5 B cell. *Adv*. *Immunol*. **47**, 117–185.
79. Bikah, G., Carey, J., Ciallella, J. R., *et al*. (1996). CD5-mediated negative regulation of antigen receptor-induced growth signals in B-1 B cells. *Science* **274**, 1906–1909.
80. Berland, R., and Wortis, H. H. (2002). Origins and functions of B-1 cells with notes on the role of CD5. *Annu*. *Rev*. *Imunol*. **20**, 253–300.
81. Cariappa, A., and Pillai, S. (2002). Antigen-dependent B cell development. *Curr*. *Opin*. *Immunol*. **14**, 241–249.
82. Schneider, P., Takatsuka, H., Wilson, A., Mackay, F., Tardivel, A., *et al*. (2001). Maturation of marginal and follicular B cells requires B cell activating factor of the tumor necrosis factor family and is independent of B cell maturation antigen. *J*. *Exp*. *Med*. **194**, 1691–1697.
83. Schiemann, B., Gommerman, J. L., Vora, K. *et al*. (2001) An essential role for BAFF in development of B cells through BCMA-independent pathway. *Science* **293**, 2111–2114.
84. Hayakawa, K., Hardy, R. R., Honda, M., *et al*. (1989). Ly-1 B cells: Functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc*. *Natl*. *Acad*. *Sci*. *USA* **81**, 2494–2498.
85. Baccala, R., Quang, T. V., Gilbert, M., *et al*. (1989). Two murine natural polyreactive autoantibodies are encoded by non-mutated germ-line genes. *Proc*. *Natl*. *Acad*. *Sci*. *USA* **86**, 4624–4628.
86. Trepicchio, W., Maruya, A., and Barett, K. J. (1987). The heavy chain genes of a lupus, anti-DNA autoantibody are encoded in the germline of a nonautoimmune strain of mouse and conserved in strains of mice polymorphic for this gene locus. *J*. *Immunol*. **139**, 3139–3145.
87. Ternyck, T., and Avrameas, S. (1986) Murine natural monoclonal autoantibodies: A study of their polyspecificities and their affinities. *Immunol*. *Rev*. **94**, 99–112.
88. Adib, M., Ragimbeau, J., Avrameas, S., *et al*. (1990). IgG autoantibody activity in normal mouse serum is controlled by IgM. *J*. *Immunol*. **145**, 3807–3813.
89. Marion, T. N., Bothwell, A. L. M., Briles, D. E., *et al*. (1989). IgG anti-DNA autoantibodies within an individual autoimmune mouse are the products of clonal selection. *J*. *Immunol*. **142**, 4269–4274.
90. Schlomchik, M. J., Aucoin, J. A. H., Pisetsky, D. S., *et al*. (1987). Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. *Proc*. *Natl*. *Acad*. *Sci*. *USA* **84**, 9150–9154.
91. Wofsy, D., and Seaman, W. E. (1985). Successful treatment of autoimmunity in NZB/NZW F1 mice with a monoclonal antibody to L3T4. *J*. *Exp*. *Med*. **171**, 378–391.
92. Wang, X., Huang, W., Mihara, M., *et al*. (2002). Mechanism of action of combined short-term CTLA4Ig and anti-CD40ligand in murine systemic lupus erythematosus. *J*. *Immunol*. **168**, 2046–2053.
93. Wither, J. E., Roy, V., and Brennan, L. A. (2000). Activated B cells express increased levels of costimulatory molecules in young autoimmune NZB and (NZB/NZW)F1 mice. *Clin*. *Immunol*. **94**, 51–63.
94. Dammeres, P. M., Visser, A., Popa, E. R., *et al*. (2000). Most marginal zone B cells in rat express germline encoded Ig VH genes and are ligand selected. *J*. *Immunol*. **165**, 6156–6159.
95. Roark, J. H., Park, S. H., Jayawardena, J., *et al*. (1998). CD1.1 expression by mouse-antigen presenting cells and marginal zone B cells. *J*. *Immunol*. **160**, 3121–3127.
96. Muller, C. G., Cremer, I., Paulet, P. E., *et al*. (2001). Mannose receptor ligand-positive cells express the metalloprotease decysin in the B cell follicle. *J*. *Immunol*. **167**, 5052–5060.
97. Baumgarth, N. (2000). A two phase model of B cell activation. *Immunol*. *Rev*. **176**, 171–180.
98. Wither, J. E., Paterson, A. D., and Vucusic, B. (2000). Genetic dissection of B cell traits in New Zealand black mice: The expanded population of B cells expressing upregulated costimulatory molecules show linkage to Nba2. *Eur*. *J*. *Immunol*. **30**, 356–365.
99. Grimaldi, C. M., Michael, D. J., and Diamond, B. (2001). Expansion and activation of a population of autoreactive marginal zone B cells in a model of estrogen-induced lupus. *J*. *Immunol*. **167**, 1886–1890.
100. Steward, M. W., and Hay, F. C. (1976). Changes in immunoglobulin class and subclass on anti-DNA antibodies with increasing age in NZB/W F1 hybrid mice. *Clin*. *Exp*. *Immunol*. **26**, 363–370.
101. Schlomchik, M. J., Aucoin, J. A. H., Pisetsky, D. S., *et al*. (1987). Structure and function of anti-DNA autoantibodies derived from a single autoimmune mouse. *Proc*. *Natl*. *Acad*. *Sci*. *USA* **84**, 9150–9154.
102. Schlomchik, M. J., Marshak-Rothstein, A., Wolfovkicz, C. B., *et al*. (1987). The role of clonal selection and somatic mutation in autoimmunity. *Nature* **328**, 805–811.
103. Tillman, D. M., Jou, N., Hill, R. J., *et al*. (1992). Both IgM, and IgG anti-DNA antibodies are the products of clonally selective B cell stimulation in (NZB X NZW) F1 mice. *J*. *Exp*. *Med*. **176**, 761–779.

104. Livneh A., Gazit E., and Diamond B. (1994) The preferential expression of the anti-DNA associated 8.12 idiotype in lupus is not genetically controlled. *Autoimmunity* **18**, 1–6.
105. Datta, S. K., Stollar, B. D., and Schwartz, R. S. (1983). Normal mice express idiotypes related to autoantibody idiotypes of lupus mice. *Proc. Natl. Acad. Sci. USA* **80**, 2723–2727.
106. Kofler, R., Noonan, D. J., Levy, D. E., *et al.* (1985). Genetic elements used for a murine lupus anti-DNA autoantibodies are closely related to those for antibody to exogenous antigens. *J. Exp. Med.* **161**, 805–815.
107. Zouali, M., Madaio, M., Canoso, R., *et al.* (1989). Restriction fragment length polymorphism of the V kappa locus in humans. *Eur. J. Immunol.* **19**, 1757–1760.
108. Kofler, R., Duchosal, M. A., Johnson, M. E., *et al.* (1987). The genetic origin of murine lupus-associated autoantibodies. *Immunol. Lett.* **16**, 265–271.
109. Gavalchin, J., Nicklas, J. A., Eastcott, A. V., *et al.* (1985). Lupus prone (SWR X NZB) F1 mice produce potentially nephritogenic autoantibodies inherited from the normal SWR parent. *J. Immunol.* **134**, 885–894.
110. Morel, L., Mohan, C., Croker, B. P., *et al.* (1997). Functional dissection of systemic lupus erythernatosus using congenic mouse strains. *J. Immunol.* **158**, 6019–6028.
111. Olee, T., Yang, P. M., Siminovitch, Y. A., *et al.* (1991). Molecular basis of an autoantibody-associated restriction fragment length polymorphism that confers susceptibility to autoimmune diseases. *J. Clin. Invest.* **88**, 193–203.
112. Suzuki, N., Harada, T., Mihara, S., *et al.* (1996). Characterization of a germline V_{κ} gene encoding cationic anti-DNA antibody and role of receptor editing for development of the autoantibody in patients with systemic lupus erythematosus. *J. Clin. Invest.* **98**, 1843–1850.
113. Halpern, R., Davidson, A., Lazo, A., *et al.* (1985). Familial systemic lupus erythematosus: Presence of a cross-reactive idiotype in healthy family members. *J. Clin. Invest.* **76**, 731–736.
114. Eilat, D., Webster, D. M., and Rees, A. R. (1988). V region sequences of anti-DNA and anti-RNA autoantibodies from NZB/NZW F1 mice. *J. Immunol.* **141**, 1745–1753.
115. Eilat, D., and Fischel, R. (1991). Recurrent utilization of genetic elements in V regions of antinucleic acid antibodies from autoimmune mice. *J. Immunol.* **147**, 361–368.
116. Manheimer-Lory, A. J., Zandman-Goddard, G., Davidson, A., *et al.* (1970). Lupus-specific antibodies reveal an altered pattern of somatic mutation. *J. Clin. Invest.* **100**, 2538–2546.
117. Kuo, P., Alban, A., Gephard, D., *et al.* (1997). Overexpression of bcl-2 alters usage of mutational hot spots in germinal center B cells. *Mol. Immunol.* **34**, 1011–1018.
118. Dorner, T., Heimbacher, C., Farnel, N. L., *et al.* (1999). Enhanced mutation activity of V kappa gene rearrangements in systemic lupus erythematosus. *Clin. Immunol.* **92**, 188–196.
119. Marion, T. N., Tillman, D. M., Jou, N. T., *et al.* (1992). Selection of immunoglobulin variable regions in autoimmunity to DNA. *Immunol. Rev.* **218**, 123–149.
120. Radic, M. Z., and Weigert, M. (1994). Genetic and structural evidence for antigen selection of anti-DNA antibodies. *Annu. Rev. Immunol.* **12**, 487–520.
121. Foster, M. H., MacDonald, M., Barrett, K. J., *et al.* (1991). VH gene analysis of spontaneously activated B cells in adult MRL lpr/lpr mice. J558 bias is not limited to classic lupus autoantibodies. *J. Immunol.* **147**, 1504–1511.
122. Isenberg, D. A., Ehrenstein, M. R., Longhurst, C., *et al.* (1994). The origin, sequence, structure, and consequences of developing anti-DNA antibodies. A human perspective. *Arthritis Rheum.* **37**, 169–180.
123. Zouali, M. (1997). The structure of human lupus anti-DNA antibodies. *Methods* **11**, 27–35.
124. Kofler, R., Strohal, R., Balderas, R. S., *et al.* (1988). Immunoglobulin kappa light chain variable region gene complex organization and immunoglobulin genes encoding anti-DNA autoantibodies in lupus mice. *J. Clin. Invest.* **82**, 852–860.
125. Radic, M. Z., Mascelli, M. A., Erikson, J., *et al.* (1991). IgH and L chain contributions to autoimmune specificities. *J. Immunol.* **146**, 176–182.
126. Spatz, L., Saenko, V., Iliev, A., *et al.* (1997). Light chain usage in anti double-stranded DNA B cell subsets: Role in cell fate determination. *J. Exp. Med.* **185**, 1317–1326.
127. Padlan, E. A. (1990). On the nature of the antibody combining sites: Unusual structural features that may confer on these sites an enhanced capacity for binding legends. *Proteins* **7**, 112–124
128. Pavletich, N. P., and Pabo, C. O. (1991). Zinc finger-DNA recognition: Crystal structure of a Zif268-DNA complex at 2.1 A. *Science* **252**, 809–817.
129. Seeman, N. C., Rosenberg, J. M., and Rich, A. (1976). Sequence-specific recognition of double helical nucleic acids by proteins. *Proc. Natl. Acad. Sci. USA* **73**, 804–808.
130. Radic, M. Z., Mascelli, M. A., Erikson, J., *et al.* (1989). Structural patterns in anti-DNA antibodies from MRL/1pr mice. *Cold Spring Harb. Symp. Quant. Biol.* **54**, 933–946.
131. Radic, M. Z., Mackle, J., Erikson, J., *et al.* (1993). Residues that mediate DNA binding of autoimmune antibodies. *J. Immunol.* **150**, 4966–4977.
132. Katz, J. B. I., Limpanasithikul, W., and Diamond, B. (1994). Mutational analysis of an antibody: Differential binding and pathogenicity. *J. Exp. Med.* **180**, 925–932.
133. Miyazaki, S., Shimura, J., Hirose, S., *et al.* (1997). Is structural flexibility of antigen-binding loops involved in the affinity maturation. *Int. Immunol.* **9**, 771–777.
134. Herron, J. N., He, X. M., Ballard, D. W., *et al.* (1991). An autoantibody to single-stranded DNA: Comparison of the three-dimensional structures of the unliganded Fab and a deoxynucleotide-Fab complex. *Proteins* **11**, 159–175.
135. Tanner, J. J., Komissarov, A. A., and Deutscher, S. L. (2001). Crystal structure of antigen-binding fragment bound to single-stranded DNA. *J. Mol. Biol.* **314**, 807–822.

136. Eliat, D., and Anderson, W. F. (1994). Structure function correlates of autoantibodies to nucleic acids: Lessons from immunochemical, genetic and structural studies. *Mol. Immunol.* **31**, 1377–1390.
137. Ravirajan, C. T., Rahman, M. A., Papadaki, L., *et al.* (1998). Genetic, structural and functional properties of an IgG DNA-binding monoclonal antibody from a lupus patient with nephritis. *Eur. J. Immunol.* **28**, 339–350.
138. Kalsi, J. K., Martin, A. C., Hirabayashi, Y., *et al.* (1996). Functional and modeling studies of the binding of human monoclonal anti-DNA antibodies to DNA. *Mol. Immunol.* **33**, 471–483.
139. Hahn, B. H., Ando, D., Ebling, F. M., *et al.* (1988). T cell upregulation of B cells via their idiotypes contributing to the development of systemic lupus erythematosus: A hypothesis. *Am. J. Med.* **8**, 32–34.
140. Jerne, N. K. (1984). Idiotypic networks and other preconceived ideas. *Immunol. Rev.* **79**, 5–24.
141. Solomon, G., Schiffenbauer, H., Keiser, D., *et al.* (1983). Use of monoclonal antibodies to identify shared idiotypes on human antibodies to native DNA from patients with SLE. *Proc. Natl. Acad. Sci. USA* **80**, 850–854.
142. Shoenfeld, Y., Isenberg, D. A., Rauch, J., *et al.* (1983). Idiotypic cross reactions of monoclonal human lupus autoantibodies. *J. Exp. Med.* **185**, 718–730.
143. Eilat, D., Fischel, R., and Zlotnick, A. (1985). A central anti-DNA idiotype in human and murine systemic lupus erythernatosus. *Eur. J. Immunol.* **15**, 368–375.
144. Zhang, W., Winkler, T., Kalden, J. R., *et al.* (2001). Isolation of human anti-idiotypes broadly cross reactive with anti-dsDNA antibodies from patients with systemic lupus erythematosus. *Scand. J. Immunol.* **53**, 192–197.
145. Isenberg, D. A., and Collins, C. (1985). Detection of cross-reactive anti-DNA antibody idiotypes on renal tissue bound imunoglobulins from lupus patients. *J. Clin. Invest.* **76**, 287–294.
146. Paul, E., Manheimer-Lory, A., Livneh, A., *et al.* (1990). Antibodies in SLE: Idiotypic families and genetic origins. *Int. Rev. Immunol.* **5**, 295–313.
147. Galeazzi, M., Bellisai, F., Sebastiani, G. D., *et al.* (1988). Association of 16/6 and SA1 anti-DNA idiotypes with anticardiolipin antibodies and clinical manifestations in a large cohort of SLE patients: European Concerted Action on the Immunogenetics of SLE. *Clin. Exp. Rheumatol.* **16**, 717–720.
148. Abu-Shakra, M., and Shoenfeld, Y. (1990). Human anti-DNA idiotype (16/6 idiotype): Pathogenic role in autoimmunity. *Hum. Antibodies Hybridomas* **1**, 10–14.
149. Isenberg, D. A., McClure, C., Farewell, V., *et al.* (1998). Correlation of 9G4 idiotype with disease activity in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **57**, 566–570.
150. Dang, H., Takei, M., Isenberg, D. A., *et al.* (1988). Expression of an interspecies idiotype in sera of SLE patients and their first degree relatives. *Clin. Exp. Immunol.* **71**, 445–449.
151. Isenberg, D., Williams, W., Axford, J., *et al.* (1990). Comparision of DNA antibody idiotypes in human sera: An international collaborative study of 19 idiotypes from 11 different laboratories. *J. Autoimmun.* **3**, 393–414.
152. Grayzel, A., Solomon, A., Aranow, C., *et al.* (1991). Antibodies elicited by pneumococcal antigens bear an anti-DNA-associated idiotype. *J. Clin. Invest.* **87**, 842–846.
153. Livneh, A., Or, G., Many, A., *et al.* (1993). Anti-DNA antibodies secreted by peripheral B cells of 1upus patients have both normal and lupus-specific features. *Clin. Immunol. Immunopathol.* **68**, 68–73.
154. Mackworth-Young, C., Sabbaga, J., and Schwartz, R. S. (1987). Idiotypic markers of polyclonal B cell activation: Public idiotypes shared by monoclonal antibodies derived from patients with systemic lupus erythematosus or leprosy. *J. Clin. Invest.* **79**, 572–581.
155. Dayan, M., Segal, R., Sthoeger, Z., *et al.* (2000). Immune response of SLE patients to peptides based on the complimentarity determining regions of a pathogenic anti-DNA monoclonal antibody. *J. Clin. Immunol.* **20**, 187–194.
156. Mendlovic, S., Brocke, Y., Shoenfeld, Y., *et al.* (1988). Induction of a systemic lupus erythematosus-like disease in mice by a common human anti-DNA idiotype. *Proc. Natl. Acad. Sci. USA* **85**, 2260–2264.
157. Zouali, M., and Eyquem, A. (1983). Idiotypic/anti-idiotypic interactions in systemic lupus erythematosus: Demonstration of oscillating levels of anti-DNA autoantibodies and reciprocal antiidiotypic activity in a single patient. *Ann. Immunol.* **134**, 377–391.
158. Matsiota, P., Druet, P., Dosquet, P., *et al.* (1987). Natural autoantibodies in systemic lupus erythematosus. *Clin. Exp. Immunol.* **69**, 79–88.
159. Hahn, B. H., and Ebling, F. M. (1983). Suppression of NZB/NZW murine nephritis by administration of a syngeneic monoclonal antibody to DNA. *J. Clin. Invest.* **71**, 1728–1736.
160. Mimori, T., and Hardin, J. A. (1986). Mechanism of interaction between Ku protein and DNA. *J. Biol. Chem.* **261**, 10375–10379.
161. Wang, J., Satoh, M., Kabir, F., *et al.* (2001). Increased prevalence of autoantibodies to Ku antigen in African Americans versus white patients with SLE. *Arthritis Rheum.* **44**, 2367–2370.
162. Reeves, W. H., and Chiorazzi, N. (1986). Interaction between anti-DNA and anti-DNA binding protein autoantibodies in cryoglobulins from sera of patients with systemic lupus erythematosus. *J. Exp. Med.* **164**, 1029–1042.
163. Mendlovic, S., Fricke, H., Shoenfeld, Y., *et al.* (1989). The role of anti-idiotypic antibodies in induction of experimental SLE. *Eur. J. Immunol.* **19**, 729–734.
164. Erez-Alon, N., Herkel, J., Wolkowicz, R., *et al.* (1998). Immunity to p53 induced by an idiotypic network of anti-p53 antibodies: Generation of sequence specific anti-DNA antibodies and protection from tumor metastasis. *Cancer Res.* **58**, 5447–5452.
165. Eivazova, E. R., McDonnel, J. M., Sutton, B. J., *et al.* (2000). Cross-reactivity of antiidiotypic antibodies with DNA in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 429–545.

166. Koffler, D., Carr, R., Agnella, V., *et al.* (1971). Antibodies to polynucleotides in human sera: Antigenic specificity and relation to disease. *J. Exp. Med.* **134**, 294–439.
167. Decker, J. L., Steinberg, A. D., Reinertsen, J. L., *et al.* (1979). Systemic lupus erythematosus: Evolving concepts. *Ann. Intern. Med.* **91**, 587–604.
168. Klinman, D. M., Shirai, A., Conover, J., *et al.* (1994). Cross-reactivity of IgG anti-DNA secreting cells in patients with systemic lupus erythematosus. *Eur. J. Immunol.* **24**:53–58.
169. Aron, A. L., Cuellar, M. L., Brey, R. L., *et al.* (1995). Early onset of autoimmunity in MRL/++ mice following immunization with beta 2 glycoprotein I. *Clin. Exp. Immunol.* **101**, 78–81.
170. Lafer, E. M., Rauch, J., Andrzejewski, C. J. R., *et al.* (1981). Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J. Exp. Med.* **153**, 897–909.
171. Koike, T., Tomioka, H., and Kumagai, A. (1982). Antibodies cross-reactive with DNA and cardiolipin in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **50**, 298–302.
172. Shoenfeld, Y., Rauch, J., Massicotte, M., *et al.* (1983). Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N. Engl. J. Med.* **308**, 414–420.
173. Carroll, P., Stafford, D., Schwartz, R. S., *et al.* (1985). Murine monoclonal anti-DNA autoantibodies bind to endogenous bacteria. *J. Immunol.* **135**, 1086–1090.
174. Dimitrijevic, L. A., Radulovic, M. I., Ciric, B. P., *et al.* (1999). Human monoclonal IgM DJ binds ssDNA and human commensal flora. *Hum. Antibodies* **9**, 37–45.
175. Spellerberg, M. B., Chapman, C. J., Mockridge, C. I., *et al.* (1995). Dual recognition of lipid A and DNA by human antibodies encoded by the V114–21 gene: A possible link between infection and lupus. *Hum. Antibodies Hybridomas* **6**, 52–56.
176. Limpanasithikul, W., Ray, S., and Diamond, B. (1995). Cross-reactive antibodies have both protective and pathogenic potential. *J. Immunol.* **155**, 967–973.
177. Kowal, C., Weinstein, A., and Diamond, B. (1999). Molecular mimicry between bacterial and self antigen in a patient with SLE. *Eur. J. Immunol.* **29**, 1901–1911.
178. Sharma, A., Isenberg, D. A., and Diamond, B. (2001). Crossreactivity of human anti-dsDNA antibodies to phosphorycholine: Clues to their origin. *J. Autoimmun.* **16**, 479–484.
179. Kabat, E. A., Nickerson, K. G., Liao, J., *et al.* (1986). A human monoclonal macroglobulin with specificity for a (2-8)-linked poly-N-acetyl neuraminic acid, the capsular polysaccharide of group B meningococci and Escherichia coli K1, which crossreacts with polynucleotides and with denatured DNA. *J. Exp. Med.* **164**, 642–654.
180. Shoenfeld, Y., Vilner, Y., Coates, A. R. M., *et al.* (1986). Monoclonal antituberculosis antibodies react with DNA, and monoclonal anti-DNA autoantibodies react with *Mycobacterium tuberculosis*. *Clin. Exp. Immunol.* **66**, 255–261.
181. Sabbaga, J., Line, S. R. P., Potocnjak, P., *et al.* (1989). A murine nephritogenic monoclonal anti-DNA autoantibody binds directly to mouse laminin, the major non-collagenous protein component of the glomerular basement membrane. *Eur. J. Immunol.* **19**, 137–143.
182. Tron, F., Jacob, L., and Bach, J. F. (1984). Binding of a murine monoclonal anti-DNA antibody to Raji cells: Implications for the interpretation of the Raji cell assay for immune complexes. *Eur. J. Immunol.* **14**, 283–286.
183. Raz, E., Ben-Bassat, H., Davidi, T., *et al.* (1993). Crossreactions of anti-DNA autoantibodies with cell surface proteins. *Eur. J. Immunol.* **23**, 383–388.
184. Jacob, L., Tron, F., Bach, J. F., *et al.* (1984). A monoclonal anti-DNA antibody also binds to cell surface protein(s). *Proc. Natl. Acad. Sci. USA* **81**, 3843–3845.
185. DeGiorgio, L. A., Konstantinov, K. N., Lee, S. C., *et al.* (2001). A subset of lupus anti-DNA antibodies cross-react with NR2 glutamate receptor in SLE. *Nature Med.* **11**, 1189–1193.
186. Putterman, C., Ulmansky, R., Rasooly, L., *et al.* (1998). Down-regulation of surface antigens recognized by systemic lupus erythematosus antibodies on embryonal cells following differentiation and exposure to corticisteroids. *Eur. J. Immunol.* **28**, 1656–1662.
187. Mostoslavsky, G., Fischel, R., Yachimovich, N., *et al.* (2001). Lupus anti-DNA autoantibodies cross-react with a glomerular structural protein: A case for tissue injury by molecular mimicry. *Eur. J. Immunol.* **31**, 1221–1227.
188. Andre-Schwartz, J., Datta, S. K., Shoenfeld, Y., *et al.* (1984). Binding of cytoskeletal proteins by monoclonal anti-DNA lupus autoantibodies. *Clin. Immunol. Immunopathol.* **31**, 261–271.
189. Tsuzaka, K., Leu, A. K., Frank, M. B., *et al.* (1996). Lupus autoantibodies to double-stranded DNA cross-react with ribosomal protein S1. *J. Immunol.* **156**, 1668–1675.
190. Singh, S., Chatterjee, S., Sohoni, R., *et al.* (2001). Sera from lupus patients inhibit growth of *P. falciparum* in culture. *Autoimmunity* **33**, 253–263.
191. Sun, K., Hong, C., Tang, S., *et al.* (1999). Anti-dsDNA autoantibody cross-reacts with c–terminal hydrophobic cluster region containing phenylalanines in the acidic ribosomal phosphoprotein P1 to exert a cytostatic effect on the cells. *Biochem. Biophys. Res. Commun.* **263**, 334–339.
192. Koren, E., Koscec, M., Wolfson-Reichlin, M., *et al.* (1995). Murine and human antibodies to native DNA that cross-react with the A and D SnRNP polypeptides cause direct injury of cultured kidney cells. *J. Immunol.* **154**, 4857–4864.
193. Reichlin, M., Martin, A., Taylor-Albert, E., *et al.* (1994). Lupus autoantibodies to native DNA cross-react with the A and D SnRNP polypeptides. *J. Clin. Invest.* **93**, 443–449.
194. Herrera-Diosdado, R., Avalos, D., and Herrera-Esparza, R. (1997). Cross-reactivity of anti-nDNA antibodies with nuclear envelope proteins: Isolation of a cDNA encoding the 70 kDa annular protein recognized by autoanti-

bodies from patients with systemic lupus erythematosus. *Rev. Rheum. Engl. Ed.* **64**, 82–88.
195. Hecht, B., Siegel, N., Adler, N. J., *et al.* (1976). Prognostic indices in lupus nephritis. Medicine **55**, 163–170.
196. Pisetsky, D. S. (1992). Anti-DNA antibodies in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **18**, 437–454.
197. Vlahakos, D. V., Foster, M. H., Adams, S., *et al.* (1992). Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. *Kidney Int.* **41**, 1690–1700.
198. Gilkeson, G. S., Bernstein, K. A., Pippin, A. M., *et al.* (1995). The influence of variable region somatic mutations on the specificity and pathogenicity of murine monoclonal anti-DNA antibodies. *Clin. Immunol. Immunopathol.* **76**, 59–67.
199. Tsao, B. P., Ohnishi, K., Cheroutre, H., *et al.* (1992). Failed self-tolerance and autoimmunity in IgG anti-DNA transgenic mice. *J. Immunol.* **140**, 350–358.
200. Raz, E., Brezis, M., Rosenmann, E., *et al.* (1989). Anti-DNA antibodies bind directly to renal antigens and induce kidney dysfunction in the isolated perfused rat kidney. *J. Immunol.* **142**, 3076–3082.
201. Ebling, F., and Hahn, B. H. (1980). Restricted subpopulations of DNA antibodies in kidneys of mice with systemic lupus: Comparison of antibodies in serum and renal eluates. *Arthritis Rheum.* **23**, 392–403.
202. Hahn, B. H. (1982). Characteristics of pathogenic subpopulations of antibodies to DNA. *Arthritis Rheum.* **25**, 747–752.
203. Foster, M. H., Cizma, B., and Madaio, M. P. (1993). Biology of disease. Nephritogenic auto-antibodies in systemic lupus erythematosus: Immunochemical properties, mechanisms of immune deposition, and genetic origin. *Lab. Invest.* **69**, 494–507.
204. Couser, W. C., Salant, D. J., Madaio, M. P., *et al.* (1982). Factors influencing glomerular and tubulointerstitial patterns of injury in SLE. *Am. J. Kidney Dis.* **2**, 126–132.
205. Glassock, R. J., Cohen, A. H., Adler, S. G., *et al.* (1991). Secondary glomerular diseases. *In* "The Kidney" (B. Brenner and F. C. Rector eds.), Vol. I, pp. 1280–1368. Saunders, Philadelphia.
206. Yanase, K., Smith, R. M., Puccetti, A., Jarett, L., and Madaio, M. P. (1997). Receptor-mediated cellular entry of nuclear localizing anti-DNA antibodies via myosin1. *J. Clin. Invest*. **100**, 25–31.
207. Vlahakos, D., Foster, M. H., Ucci, A. A., *et al.* (1992). Murine monoclonal anti-DNA antibodies penetrate cells, bind to nuclei, and induce glomerular proliferation and proteinuria in-vivo. *J. Am. Soc. Nephrol.* **2**, 1345–1354.
208. Nydegger, U. E., Lambert, P. H., Gerber, H., *et al.* (1974). Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen: Quantation by binding to radio-labeled C1q. *J. Clin. Invest.* **54**, 297–309.
209. Zubler, R. H., Lange, G., Lambert, P. H., *et al.* (1976). Detection of immune complexes in unheated sera by a modified labelled C1q binding test: Effect of heating on the binding of C1q by immune complexes and application of the test to systemic lupus erythematosus. *J. Immunol.* **116**, 232–235.
210. Woodruffe, A. J., Border, W. A., Theofilopoulos, A. N., *et al.* (1977). Detection of circulating immune complexes in patients with glomerulonephritis. *Kidney Int.* **12**, 268–278.
211. Casali, P., Bossus, A., Carpentier, N. A., *et al.* (1977). Solid-phase enzyme immunoassay or radioimmunoassay for the detection of immune complexes based on their recognition by conglutinin: Conglutinin-binding test. A comparative study with 125 I-labelled Clq binding and Raji cell RIA tests. *Clin. Exp. Immunol.* **29**, 342–352.
212. Harbeck, R. J., Bardana, E. J., Kohler, P. F., *et al.* (1973). DNA: AntiDNA complexes: Their detection in systemic lupus erythematosus sera. *J. Clin. Invest.* **52**, 789–795.
213. Bruneau, C. D., Edmonds, J. P., Hughes, G. R. V., *et al.* (1977). Detection and characterization of DNA-anti-DNA complexes in a patient with systemic lupus erythematosus. *J. Exp. Immunol.* **28**, 433–436.
214. Izui, S., Lambert, P. H., and Miescher, P. A. (1977). Failure to detect circulating DNA-anti-DNA complexes by four radioimmunological methods in patients with systemic lupus erythernatosus. *Clin. Exp. Immunol.* **30**, 384–392.
215. Emlen, W., and Mannik, M. (1982). Clearance of circulating DNA-antiDNA immune complexes in mice. *J. Exp. Med.* **155**, 1210–1215.
216. Emlen, W., and Mannik, M. (1984). Effect of DNA size and strandedness on the *in vitro* clearance and organ localization of DNA. *Clin. Exp. Immunol*. **56**, 185–192.
217. Sasaki, T., Muryoi, T., Hatakeyama, A., *et al.* (1991). Circulating anti-DNA immune complexes in active lupus nephritis. *Am. J. Med.* **91**, 355–362.
218. Van, Bruggen, M. C. J., Kramers, C., and Berden, J. H. M. (1996). Autoimmunity against nucleosomes and lupus nephritis. *Ann. Med. Int.* **147**, 485–489.
219. Lefkowith, J. B., and Gilkeson, G. S. (1996). Nephritogenic autoantibodies in lupus. *Arthritis Rheum.* **39**, 894–903.
220. Kramers, C., Hylkema, M. N., Van Bruggen, M. C. J., *et al.* (1994). Antinucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. *J. Clin. Invest.* **94**, 568–577.
221. Termaat, R. M., Brinkman, K., Van Gompel, L. F., *et al.* (1990). Cross-reactivity of monoclonal anti-DNA antibodies with heparan sulfate is mediated via bound DNA/histone complexes. *J. Autoimmun.* **3**, 531–545.
222. Sabbaga, J., Pankewycz, O. G., Lufft, V., *et al.* (1990). Cross-reactivity distinguishes serum and nephritogenic anti-DNA antibodies in human lupus from their natural counterparts in normal serum. *J. Autoimmun.* **3**, 215–235.
223. Pankewycz, O. G., Migliorini, P., and Madaio, M. (1987). Polyreactive autoantibodies are nephritogenic in murine lupus nephritis. *J. Immunol.* **139**, 3287–3294.

224. Madaio, M. P., Carlson, J., Cataldo, J., *et al.* (1987). Murine monoclonal anti-DNA antibodies bind directly to glomerular antigens and form immune deposits. *J. Immunol.* **138**, 2883–2889.
225. Putterman, C., Limpanasithikul, W., Edelman, M., and Diamond, B. (1996). The double edged sword of the immune response. Mutational analysis of a murine anti-pneumococcal, anti-DNA antibody. *J. Clin. Invest.* **97**, 2251–2259.
226. Faaber, P., Rijke, T. P. M., Van de Putte, L. B. A., *et al.* (1986). Cross-reactivity of human and murine anti-DNA antibodies with heparan sulfate, the major glycosaminoglycan in glomerular basement membranes. *J. Clin. Invest.* **77**, 1824–1830.
227. Deocharan, B., Qing, X., Lichauco, J., *et al.* (2002). α–Actinin is a cross-reactive renal target for pathogenic anti-DNA antibodies. *J. Immunol.* **168**, 3072–3078.
228. Farquhar, M. G., Courtoy, P. J., Lemkin, M. C., *et al.* (1982). Current knowledge of the functional architecture of the glomerular basement membrane. *In* "New Trends in Basement Membrane Research" (R. Kuehn, H.-H. Schoene, and R. Timpl, eds.), pp. 9–29. Raven Press, New York.
229. Barnes, J. L., Radnick, R. A. I., Gilchrist, E. P., *et al.* (1984). Size and charge selective permeability defects induced in the glomerular basement membrane by a polycation. *Kidney Int.* **25**, 11–19.
230. Border, W. A., Ward, H. J., Karnil, E. S., *et al.* (1982). Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen: Demonstration of a pathogenic role for electrical charge. *J. Clin. Invest.* **69**, 451–461.
231. Izui, S., Lambert, P. H., and Miescher, P. H. (1976). In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA: A possible role for local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. *J. Exp. Med.* **144**, 428–443.
232. Coritsidis, G. N., Beers, P. C., and Rumore, P. I. (1995). Glomerular uptake of nucleosomes: Evidence for receptor-mediated mesangial cell binding. *Kidney Int.* **47**, 1258–1265.
233. Termaat, R. M., Assmann, K. J. M., Dijkman, H. B. P., *et al.* (1992). Anti-DNA antibodies can bind to the glomerulus via two distinct mechanisms. *Kidney Int.* **42**, 1363–1371.
234. Gay, S., Losman, M., Koopman, W., *et al.* (1985). Interaction of DNA with connective tissue matrix proteins reveals preferential binding to type V collagen. *J. Immunol.* **135**, 1097–1100.
235. Lake, R. A., Morgan, A., Henderson, B., *et al.* (1985). A key role for fibronectin in the sequential binding of dsDNA and monoclonal anti-DNA antibodies to components of the extracellular matrix: Its possible significance in glomerulonephritis. *Immunology* **54**, 389–395.
236. Lefkowith, J. B., Kiehl, M., Rubenstein, J., *et al.* (1996). Heterogeneity and clinical significance of glomerular-binding antibodies in systemic lupus erythematosus. *J. Clin. Invest.* **98**, 1373–1380.
237. Casciola-Rosen, L. A., Anhalt, G., and Rosen, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317–1330.
238. Madaio, M. P., Hodder, S., Schwartz, R. S., *et al.* (1984). Responsiveness of autoimmune and normal mice to nucleic acid antigens. *J. Immunol.* **132**, 872–876.
239. Lafer, E. M., Moller, A., Nordheim, A., *et al.* (1981). Antibodies specific for left handed Z-DNA. *Proc. Natl. Acad. Sci. USA* **78**, 3546–3550.
240. Gilkeson, G. S., Pippen, A. M. M., and Pisetsky, D. S. (1995). Induction of crossreactive anti-dsDNA antibodies in preautoimmune NZB/NZW mice by immunization with bacterial DNA. *J. Clin. Invest.* **95**, 1398–1402.
241. Wloch, M. K., Alexander, A. L., Pippen, A. M., *et al.* (1997). Molecular properties of anti-DNA induced in preautoimmune NZB/W mice by immunization with bacterial DNA. *J. Immunol.* **158**, 4500–4506.
242. Impraim, C. C., Conner, B. J., Klotz, J. L., *et al.* (1985). A method for binding specificity analysis of anti-DNA autoantibodies in SLE. *J. Immunol. Methods* **78**, 191–198.
243. Schwartz, R. S., and Stollar, B. D. (1985). Origin of anti-DNA antibodies. *J. Clin. Invest.* **75**, 321–327.
244. Krieg, A. M., Yi, A.-K., Matson, S., *et al.* (1995). CpG motifs in bacterial DNA trigger direct B cell activation. *Nature* **374**, 546–549.
245. Gilkeson, G. S., Grudier, J. P., Karounos, D. G., *et al.* (1989). Induction of anti-double stranded DNA antibodies in normal mice by immunization with bacterial DNA. *J. Immunol.* **142**, 1482–1486.
246. Desai, D. D., Krishnan, M. R., Swindle, J. T., *et al.* (1993). Antigen specific induction of antibodies against native mammalian DNA in nonautoimmune mice. *J. Immunol.* **151**, 1614–1626.
247. Rekvig, O. P., Fredriksen, K., Hokland, K., *et al.* (1995). Molecular analyses of anti-DNA antibodies induced by polyomavirus BK in BALB/c mice. *Scand J. Immunol.* **41**, 593–602.
248. Moens, U., Seternes, O. M., Hey, A. W., *et al.* (1995). In vivo expression of a single viral DNA-binding protein generates systemic lupus erythematosus-related autoimmunity to double-stranded DNA and histones. *Proc. Natl. Acad. Sci. USA* **92**, 12393–12397.
249. Bredholt, G., Olaussen, E., Moens, U., *et al.* (1999). Linked production of antibodies to mammalian DNA and to human polyomavirus large T antigen: Footprints of a common molecular and cellular process? *Arthritis Rheum.* **42**, 2583–2592.
250. El-Roiey, A., Sela, O., Isenberg, D. A., *et al.* (1987). The sera of patients with Klebsiella infections contain a common anti-DNA idiotype (16/6) Id and anti-polynucleotide activity. *Clin. Exp. Immunol.* **67**, 507–515.
251. Unni, K. K., Holley, K. E., McDuffie, F. C., *et al.* (1975). Comparative study of NZB mice under germ-free and conventional conditions. *J. Rheumatol.* **2**, 36–44.
252. Okamoto, M., Murakami, M., Shimizu, A., *et al.* (1992). A transgenic model of autoimmune hemolytic anemia. *J. Exp. Med.* **175**, 71–79.

253. Murakami, M., Nakajima, K., Yamazaki, K., *et al.* (1997). Effect of breeding environments on generation and activation of autoreactive B-1 cells in anti-red blood cell autoantibody transgenic mice. *J. Exp. Med.* **185**, 791–794.
254. Winkler, T. H., Fehr, H., and Kalden, J. R. (1992). Analysis of immunoglobulin variable region genes from human IgG anti-DNA hybridomas. *Eur. J. Immunol.* **22**, 1719–1728.
255. van Es, J. H., Aanstoot, H., Gmelig-Meyling, *et al.* (1992). A human systemic lupus erythematosus-related anticardiolipin single stranded DNA autoantibody is encoded by a somatically mutated variant of the developmentally restricted 51P1 VH gene. *J. Immunol.* **149**, 2234–2240.
256. Song, Y. W., Kim, H. A., Lee, E. B., *et al.* (1992). Molecular and genetic characterization of two anti-DNA antibodies derived from patients with SLE. *Rheumatol. Int.* **17**, 223–228.
257. Munakata, Y., Saito, S., Hoshino, A., *et al.* (1998). Somatic mutation in autoantibody-associated VH genes of circulating IgM+IgD+ B cells. *Eur. J. Immunol.* **28**, 1435–1444.
258. Brard, F., Shannon, M., Prak, E. L., *et al.* (1999). Somatic mutation and light chain rearrangement generate autoimmunity in anti-single-stranded DNA transgenic MRL/lpr mice. *J. Exp. Med.* **190**, 691–704.
259. Kuo, P., Kowal, C., Tadmor, B., *et al.* (1997). Microbial antigens can elicit autoantibody production. *Ann. N.Y. Acad. Sci.* **815**, 230–236.
260. Dorner, T., Heimbacher, C., Farner, N. L., *et al.* (1999). Enhanced mutation activity of V kappa gene rearrangements in systemic lupus erythematosus. *Clin. Immunol.* **92**, 188–196.
261. Reeves, W. H., Satoh, M., Wang, J., *et al.* (1994). Antibodies to DNA, DNA-binding proteins, and histones. *Rheum. Dis. Clin. North Am.* **20**, 1–28.
262. van, Venrooij, W. J., and Pruijn, G. J. M. (1995). Ribonucleoprotein complexes as autoantigens. *Curr. Biol. Immunol.* **7**, 819–824.
263. Mohan, C., Adams, S., Stanik, V., *et al.* (1994). Nucleosome: A major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J. Exp. Med.* **177**, 1367–1381.
264. Fatenejad, S., Mamula, M. J., and Craft, J. (1994). Role of intermolecular/intrastructural B- and T-cell determinants in the diversification of autoantibodies to ribonucleoprotein particles. *Proc. Natl. Acad. Sci. USA* **90**, 12010–12040.
265. Bockenstedt, L. K., Gee, R. J., and Mamula, M. J. Self-peptides in the initiation of lupus autoimmunity. *J. Immunol.* **4**, 3516–3524.
266. James, J. A., Gross, T., Scofield, R. H., *et al.* (1995). Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B′ derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. *J. Exp. Med.* **181**, 453–461.
267. Topfer, F., Gordon, T., and McCluskey, J. Intra- and intermolecular spreading of autoimmunity involving the nuclear self-antigens La (SS-B) and Ro (SS-A). *Proc. Natl. Acad. Sci. USA* **92**, 875–879.
268. Fisher, D. E., Reeves, W. H., Wismiewolski, R., *et al.* (1985). Temporal shifts from Sm to ribonucleoprotein reactivity in systemic lupus erythematosus. *Arthritis Rheum.* **28**, 1348–1873.
269. James, J. A., and Harley, J. B. (1998). A model of peptide-induced lupus autoimmune B cell epitope spreading is strain specific and is not H-2 restricted in mice. *J. Immunol.* **160**, 502–508.
270. Laskin, C. A., Haddad, G., and Solonika, C. A. (1986). The regulatory role of NZB T lymphocytes in the production of anti-DNA antibodies *in vitro*. *J. Immunol.* **137**, 1867–1873.
271. Sekigawa, I., Ishida, Y., Hirose, S., *et al.* (1986). Cellular basis of *in vitro* anti-DNA antibody production, evidence for T cell dependence of IgG-class anti-DNA antibody synthesis in (NZB × NZW) F1 hybrid. *J. Immunol.* **136**, 1247–1252.
272. Datta, S. K., Patel, H., and Berry, D. (1987). Induction of a cationic shift in IgG anti-DNA autoantibodies: Role of T helper cells with classical and novel phenotypes in three murine models of lupus nephritis. *J. Exp. Med.* **165**, 1252–1268.
273. Ando, D. G., Sercarz, E. E., and Hahn, B. H. (1987). Mechanisms of T and B cell collaboration in the *in vitro* production of anti-DNA antibodies in the NZB/NZW F1 murine SLE model. *J. Immunol.* **138**, 3185–3190.
274. Mohan, C., Shi, Y., Laman, J. D., *et al.* (1995). Interaction between CD40 and its ligand gp39 in the development of murine lupus nephritis. *J. Immunol.* **154**, 1470–1480.
275. Finck, B. K., Linsley, P. S., and Wofsy, D. (1994). Treatment of murine lupus with CTLA4 Ig. *Science* **265**, 1225–1227.
276. Ruiz, P. J., Zinger, H., and Mozes, E. (1996). Effect of injection of anti-CD4 and anti-CD8 monoclonal antibodies on the development of experimental systemic lupus erythematosus in mice. *Cell. Immunol.* **167**, 30–37.
277. Tada, Y., Nagasawa, K., Yamauchi, *et al.* (1991). A defect in protein kinase C system in T cells from patients with systemic lupus erythematosus in mice. *Clin. Immunol. Immunopathol.* **60**, 220–231.
278. Ng, T. T., Collins, I. E., Kanner, S. B., *et al.* (1999). Integrin signalling defect in T-lymphocytes in SLE. *Lupus* **8**, 39–51.
279. Nambiar, M. P., Enyedy, E. J., Warke, V. G., *et al.* (2001). Polymorphisms/mutations of TCR-zeta-chain promoter and 3′ untranslated region and selective expression of TCR zeta-chain with an alternatively spliced 3′untranslated region in patients with systemic lupus erythematosus. *J. Autoimmun.* **16**, 133–142.
280. Enyedy, E. J., Nambiar, M. P., Liossis, S. N., *et al.* (2001). Fc epsilon receptor type I gamma chain replaces the deficient T cell receptor zeta chain in T cells of patients with SLE. *Arthritis Rheum.* **44**, 1114–1121.
281. Takeno, M., Nagafuchi, H., Kaneko, S., *et al.* (1997). Autoreactive T cell clones from patients with SLE support poyclonal autoantibody production. *J. Immunol.* **158**, 3529–3538.
282. Khalil, M., Inaba, K., Steinman, R., *et al.* (2001). T cell studies in a peptide-induced model of SLE. *J. Immunol.* **166**, 1667–1674.

283. Goud, B., and Antoine, J. C. (1984). Emergence of a surface immunoglobulin recycling process during B lymphocyte differentiation. *J. Cell. Biol.* **98**, 1238–1246.
284. Weiss, S., and Bogen, B. (1989). B-lymphoma cells process and present their endogenous immunoglobulin to major histocompatibility complex-restricted T cells. *Proc. Natl. Acad. Sci. USA* 282–286.
285. Davidson, H. W., West, M. A., and Watts, C. (1990). Endocytosis, intracellular trafficking, and processing of membrane IgG and monovalent antigen/membrane IgG complexes in B lymphocytes. *J. Immunol.* **144**, 4101–4109.
286. Yurin, V. L., Rudensky, A. Y., Mazel, S. M., *et al.* (1989). Immunoglobulin-specific T-B cell interaction. II. T cell clones recognize the processed form of B cells own surface immunoglobulin in the context of the major histocompatibility complex class II molecule. *Eur. J. Immunol.* **19**, 1685–1691.
287. Ebling, F. M., Tsao, B. P., Singh, R. R., *et al.* (1993). A peptide derived from an autoantibody can stimulate T cells in the (NZB × NZW) F 1 mouse model of systemic lupus erythematosus. *Arthritis Rheum.* **36**, 355–364.
288. Peng, S. L., Madaio, M. P., Hayday, A. C., *et al.* (1996). Propagation and regulation of systemic autoimmunity by gamma/delta T cells. *J. Immunol.* **157**, 5689–5698.
289. Riccieri, V., Spadaro, A., Parisi, G., *et al.* (2000). Down-regulation of natural killer cells and of gamma/delta T cells in SLE. Does it correlate with autoimmunity and to laboratory indices of disease activity? *Lupus* **9**, 333–337.
290. Erikson, J., Radic, M. Z., Camper, S. A., *et al.* (1991). Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. *Nature* **349**, 331–334.
291. Offen, D., Spatz, L., Escowitz, H., *et al.* (1992). Induction of tolerance to an IgG autoantibody. *Proc. Natl. Acad. Sci. USA* **89**, 8332–8336.
292. Tsao, B. P., Chow, A., Cheroutre, H., *et al.* (1993). B cells are anergic in transgenic mice that express IgM anti-DNA antibodies. *Eur. J. Immunol.* **23**, 2332–2337.
293. Fulcher, D. A., and Basten, A. Whither the anergic B cell? *Autoimmunity* **19**, 135–140.
294. Chen, C., Radic, M. Z., Erikson, J., *et al.* (1994). Deletion and editing of B cells that express antibodies to DNA. *J. Immunol.* **152**, 19–70.
295. Chen, C., Nagy, Z., Radic, M. Z., *et al.* (1995). The site and stage of anti-DNA B-cell deletion. *Nature* **373**, 252–255.
296. Chen, C., Nagy, Z., Prak, E. L., *et al.* (1995). Immunoglobulin heavy chain gene replacement: A mechanism of receptor editing. *Immunity* **3**, 747–755.
297. Suzuki, N., Mihara, S., and Sakane, T. (1997). Development of pathogenic anti-DNA antibodies in patients with systemic lupus erythematosus. *FASEB J.* **11**, 1033–1038.
298. Bynoe, M. S., Spatz, L., and Diamond, B. (1999). Charcterization of anti-DNA B cells that escape negative selection. *Eur. J. Immunol.* **29**, 1304–1313.
299. Chu, J. L., Drappa, J., Parriassa, A., *et al.* (1993). The defect in Fas mRNA expression in MRL/lpr mice is associated with insertion of the retrotransposon. *J. Exp. Med.* **178**, 723–730.
300. Adachi, M., F. R. W., and Nagata, S. (1993). Aberrant transcription caused by the insertion of an early transposable element in an intron of the Fas antigen gene of lpr mice. *Proc. Natl. Acad. Sci.* (*USA*) **90**, 1756–1760.
301. Wu, J., Zhou, T., He, J., *et al.* (1993). Autoimmune disease in mice due to integration of an endogenous retrovirus in an apoptosis gene. *J. Exp. Med.* **178**, 461–468.
302. Roark, J. H., Kuntz, C. L., Nguyen, K. A., *et al.* (1995). Breakdown of B cell tolerance in a mouse model of systemic lupus erythematosus. *J. Exp. Med.* **181**, 1157–1167.
303. Mandik-Nayak, L., Seo, S., Sokol, C., *et al.* (1999). MRL-lpr/lpr mice exhibit a defect in maintaining developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J. Exp. Med.* **189**, 1799–1814.
304. Izui, S., Kelley, V. E., Masuda, K., *et al.* (1984). Induction of various autoantibodies by mutant gene lpr in several strains of mice. *J. Immunol.* **133**, 227–233.
305. McNally, J., Yoo, D. H., Drappa, J., *et al.* (1997). Fas ligand expression and function in systemic lupus erythematosus. *J. Immunol.* **159**, 4628–4636.
306. Vaux, D. L., Cory, S., and Adams, J. M. (1988). Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**, 440–442.
307. Moudgil, K. D., and Sercarz, E. E. (1994). The T cell repertoire against cryptic self determinants and involvement in autoimmunity and cancer. *Clin. Immunol. Immunopathol.* **73**, 283–289.
308. Liu, Y. J., Mason, D. Y., Johnson, G. D., *et al.* (1991). Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. *Eur. J. Immunol.* **21**, 1905–1910.
309. Strasser, A., Whittingham, S., Vaux, D. L., *et al.* (1991). Enforced Bcl-2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Natl. Acad. Sci. USA* **88**, 8661–8665.
310. Kuo, P., Bynoe, M. S., Wang, C., *et al.* (1999). Bcl-2 leads to expression of anti-DNA B cells, but not nephritis: a model for a clinical subset. *Eur. J. Immunol.* **29**, 3168–3178.
311. Kuo, P., Bynoe, M., and Diamond, B. (1999). Crossreactive B cells are present during primary, but not secondary response in BALB/c mice expressing a bcl-2 transgene. *Mol. Immunol.* **36**, 471–479.
312. Bynoe, M. S., Grimaldi, C. M., and Diamond, B. (2000). Estrogen up-regulates bcl-2 and blocks tolerance induction of naïve B cells. *Proc. Natl. Acad. Sci. USA* **97**, 2703–2708.
313. Peeva, E., Grimaldi, C., Spatz, L., and Diamond, B. (2000). Bromocriptine restores tolerance in estrogen-treatred mice. *J. Clin. Invest.* **11**, 1373–1379.
314. McDonnell, T. J., Deane, N., Platt, F. M., *et al.* (1989). Bcl-2 immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell* **57**, 78–88.
315. Westhoff, C. M., Whittier, A., Kathol, S., *et al.* (1997). DNA-binding antibodies from viable motheaten mutant mice: Implications for B cell tolerance. *J. Immunol.* **159**, 3024–3033.

316. Shultz, L. D., and Green, M. C. (1976). Motheaten, an immunodeficient mutant of mouse II Depressed immune competence and elevated serum immunoglobulins. *J. Immunol.* **116**, 936–943.
317. Sidman, C. L., Shultz, L. D., and Unanue, E. R. (1978). The mouse mutant "motheaten." I. Development of lymphocyte populations. *J. Immunol.* **121**, 2392–2399.
318. Cyster, J. G., and Goodnow, C. C. (1995). Protein tyrosine phosphatase I C negatively regulates antigen receptor signaling in B lymphocytes and determines thresholds for negative selection. *Immunity* **2**, 13–24.
319. Morris, D. L., and Rothstein, T. L. (1996). Abnormal transcription factor induction through the surface immunoglobulin M receptor of B-1 lymphocytes. *J. Exp. Med.* **177**, 857–861.
320. Bikah, G., Carey, J., Ciallella, J. R., *et al.* (1996). CD5-mediated negative regulation of antigen receptor-induced growth signals in B-1 B cells. *Science* **274**, 1906–1909.
321. Chan, V. W., Meng, F., Soriano, P., *et al.* (1997). Characterization of the B lymphocyte populations in Lyn deficient mice and the role of Lyn in signal initiation and down-regulation. *Immunity* **7**, 69–81.
322. Smith, K. G. C., Tarlinton, D. M., Doody, G. M., *et al.* (1998). Inhibition of the B cell by CD22: A requirement for Lyn. *J. Exp. Med.* **187**, 807–811.
323. Seo, S., Buckler, J., and Erikson, J. (2001). Novel roles for Lyn in B cell migration and lipopolysaccharide responsiveness revealed using anti-double-stranded DNA Ig transgenic mice. *J. Immunol.* **166**, 3710–3716.
324. Cyster, J. G., and Goodnow, C. C. (1997). Tuning antigen receptor signaling by CD22: Integrating cues from antigens and the microenvironment. *Immunity* **6**, 509–517.
325. Sato, S., Miller, A. S., Inaoki, M., *et al.* (1996). CD22 is both a positive and negative regulator of B lymphocyte antigen receptor signal transduction: Altered signaling in CD22-deficient mice. *Immunity* **5**, 551–562.
326. O'Keefe, T. L., Williams, G. T., Dades, S. L., *et al.* (1996). Hyperresponsive B cells in CD22-deficient mice. *Science* **274**, 798–801.
327. Otipoby, K. L., Andersson, K. B., Draves, K. E., *et al.* (1996). CD22 regulates thymus independent responses and the lifespan of B cells. *Nature* **384**, 634–637.
328. Nitschke, L., Carsetti, R., Ocker, B., *et al.* (1997). CD22 is a negative regulator of B-cell receptor signalling. *Curr. Biol.* **7**, 133–134.
329. Liossis, S. N., Solomou, E. E., Dimpoulos, M. A., *et al.* (2001). B-cell kinase lyn deficiency in patients with systemic lupus erythematosus. *J. Invest. Med.* **49**, 157–165.
330. Khare, S. D., Sarosi, I., Xia, X. Z., *et al.* (2000). Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. *Proc. Natl. Acad. Sci. USA* **97**, 3370–3375.
331. Zhang, J., Roschke, V., Baker, K. P., *et al.* (2001). Cutting edge: A role for B lymphocyte stimulator in SLE. *J. Immunol.* **166**, 6–10.
332. Justement, L. B., Campbell, K. S., Chien, N. C., *et al.* (1991). Regulation of B cell antigen receptor signal transduction and phosphorylation by CD45. *Science* **25**, 1839–1842.
333. Cyster, J. G., Healy, J. I., Kishihara, K., *et al.* (1996). Regulation of B-lymphocyte negative and positive selection by tyrosine phosphatase CD45. *Nature* **381**, 325–328.
334. Huck, S., Le, Corre, R., Youinou, P., *et al.* (2001). Expression of B cell receptor-associated signaling molecules in human lupus. *Autoimmunity* **33**, 213–224.
335. Sato, S., Ono, N., Steeber, D. A., *et al.* (1996). CD19 regulates B lymphocyte signaling thresholds critical for the development of B-1 lineage cells and autoimmunity. *J. Immunol.* **157**, 4371–4378.
336. Sato, S., Hasegawa, M., Fugimoto, M., Takehara, K., *et al.* (2000). Quantitative genetic variation in CD19 expression correlates with autoimmunity. *J. Immunol.* **165**, 6635–6643.
337. Ma, J., Xu, J., Madaio, M. P., *et al.* (1996). Autoimmune lpr/lpr mice deficient in CD40 ligand: Spontaneous Ig class switching with dichotomy of autoantibody responses. *J. Immunol.* **157**, 417–426.
338. Desai-Mehta, A., Lu, L., Ramsey-Goldman, R., *et al.* (1996). Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J. Clin. Invest.* **97**, 2063–2073.
339. Bijl, M., Limburg, P. C., and Kallenberg, C. G. M. (2001). Expression of costimulatory molecules on peripheral blood lymphocytes in patients with SLE. *Ann. Rheum. Dis.* **60**, 523–526.
340. Ishida, H., Mucharnuel, T., Sakaguchi, S., *et al.* (1994). Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* **179**, 305–310.
341. Llorente, L., Richaud-Patin, Y., García-Padilla, C., *et al.* (2340). Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 1790–1800.
342. Dang, H., Geiser, A. G., Letterio, J. J., *et al.* (1995). SLE-like autoantibodies and Sjogren's syndrome-like lymphoproliferation in TGF-β knockout mice. *J. Immunol.* **155**, 3205–3212.
343. Prodeus, A. P., Goerg, S., Shen, L. M., *et al.* (1998). A critical role for complement in maintenance of self-tolerance. *Immunity* **9**, 721–731.
344. Mevorach, D., Zhou, J. L., Song, X., *et al.* (1998). Systemic exposure to irradiated apoptotic cells induces autoantibody production. *J. Exp. Med.* **188**, 387–392.
345. Bao, L., Haas, M., Boackle, S. A., *et al.* (2000). Transgenic expression of a soluble complement inhibitor protects against renal disease and promotes survival in MRL/lpr mice. *J. Immunol.* **168**, 3601–3607.
346. Boackle, S. A., Holers, V. M., Chen, X., *et al.* (2001). Cr2, a candidate gene in the murine Sle1c lupus susceptibility locus, encodes a dysfunctional protein. *Immunity* **15**, 775–785.
347. Takahashi, K., Kozono, Y., Waldschmidt, T. J., *et al.* (1997). Mouse complement receptors type 1 (CR1;CD35) and type 2 (CR2;CD21): expression on normal B cell sub-

populations and decreased levels during the development of autoimmunity in MRL/lpr mice. *J. Immunol.* **159**, 1557–1569.
348. Mitchell, D. A., Pickering, M. C., Warren, J., *et al.* (2002). C1q deficiency and autoimmunity: The effects of genetic background on disease expression. *J. Immunol.* **168**, 2538–2543.
349. Chen, Z., Koralov, S. B., and Kelsoe, G. (1998). Complement C4 inhibits systemic autoimmunity through a mechanism indepentent of complement receptors CR1 and CR2. *J. Exp. Med.* **9**, 1339–1352.
350. Bharadwaj, D., Mold, C., Markham, E., *et al.* (2001). Serum amyloid P component binds to Fc gamma receptors and opsonizes particles for phagocytosis. *J. Immunol.* **166**, 6735–6741.
351. Dijstelbloem, H. M., Bijl, M., Fijnheer, R., *et al.* (2000). Fcgamma receptor polymorphisms in systemic lupus erythematosus: Association with disease and in vivo clearance of immune complexes. *Arthritis Rheum.* **43**, 2793–2800.
352. Leadbetter, E. A., Rifkin, I. R., Beaudette, B. C., *et al.* (2002). Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. *Nature* **416**, 603–607.
353. Mihara, M., Tan, I., Chuzin, Y., *et al.* (2000). CTLA4Ig inhibits T cell-dependent B-cell maturation in murine SLE. *J. Clin. Invest.* **106**, 91–101.
354. Daikh, D. I., Finck, B. K., Linsley, P. S., *et al.* (1997). Long-term inhibition of murine lupus by brief simultaneous blockade of B7/CD28 and CD40/gp39 costimulation pathways. *J. Immunol.* **159**, 3104–3108.
355. McLeod, B., Lewis, E., Schnitzer, T., *et al.* (1988). Therapeutic immune adsorption of anti-native DNA antibodies in SLE: Clinical studies with a device utilizing monoclonal anti-idiotypic antibody. *Arthritis Rheum.* **31**, S15. [Abstract]
356. Kong, D. L., Schuett, W., Boeden, H. F., *et al.* (2000). Development of a DNA immunoadsorbent: Coupling DNA on sepharose 4FF by an efficient activation method. *Artif. Organs* **24**, 845–851.
357. Odabasi, M., and Denizli, A. (2001). Polyhydroxyethylmethacrylate-based magnetic DNA-affinity beads for anti-DNA antibody removal from systemic lupus erythematosus patient plasma. *J. Chromatogr. B Biomed. Sci.* **760**, 137–148.
358. Silvestris, F., D'Amore, O., Cafforio, P., *et al.* (1996). Intravenous immune globulin therapy of lupus nephritis: Use of pathogenic anti-DNA-reactive Ig. *Clin. Exp. Immunol.* **104**, 91–97.
359. Thrush, G. R., Lark, L. R., Clinchy, B. C., *et al.* (1996). Immunotoxins: An update. *Annu. Rev. Immunol.* **14**, 49–71.
360. Jones, D. S., Hachmann, J. P., Osgood, S. A., *et al.* (1994). Conjugates of double stranded oligonucleotides with polyethylene glycol and keyhole limpet hemocyanin: A model for treating systemic lupus erythematosus. *Bioconj. Chem.* **5**, 390–399.
361. Coutts, S. M., Plunkett, M. L., Iverson, G. M., *et al.* (1996). Pharmacological intervention in antibody mediated disease. *Lupus* **5**, 158–159.
362. Weisman, M. H., Blustein, H. G., Berner, C. M., *et al.* (1997). Reduction in circulating dsDNA antibody titer after administration of LJP 394. *J. Rheumatol.* **24**, 314–318.
363. Lindorfer, M. A., Schuman, T. A., Craig, M. L., *et al.* (2001). A bispecific dsDNA monoclonal antibody construct for clearance of anti-dsDNA IgG in SLE. *J. Immunol. Methods* **248**, 125–138.
364. Singh, R. R., Ebling, F. M., Sercarz, E. E., *et al.* (1995). Immune tolerance to antibody–derived peptides delay development of autoimmunity in murine lupus. *J. Clin. Invest.* **96**, 2990–2996.

11

ANTICYTOPLASMIC ANTIBODIES IN SLE

Antibodies to Cytoplasmic Antigens

Morris Reichlin

The hallmark of the autoimmune response in patients with systemic lupus erythematosus is the presence of antibodies to nuclear antigens. These include native and denatured DNA, histone, the DNA–histone complex, and some soluble nuclear RNA proteins, termed *Sm* and *nRNP* [1]. As many as 95% of active untreated SLE patients are found to have positive antinuclear antibody tests by indirect immunofluorescence when their sera are tested with rodent liver or kidney tissue as substrate. However, about 5% of the SLE population consistently lack such ANA, but their sera frequently contain antibodies to soluble antigens that are not well represented in the standard substrates used for immunofluorescence. SLE patients also produce antibodies to a number of cytoplasmic antigens, and investigation has sought to ascertain the clinical and biological significance of these reactions.

Antibodies to cytoplasmic constituents were recognized in early studies, but individual antigen–antibody reactions were not defined [1–4]. This chapter describes progress in the immunologic and biochemical study of these systems and the numerous clinical phenomena now recognized as being associated with these immune responses.

Two sets of antibodies are discussed. The first comprises two soluble antigens, *Ro* and *La*, which form precipitates with SLE sera and are RNA protein conjugates. While conflicting data exist regarding their cellular localization, substantial data support a cytoplasmic locus for at least part of their lifetime in cells. The second set of antibodies are those directed to cytoplasmic ribosomal constituents. Finally, the significance and possible origins of these responses as they relate to the spectrum of autoimmune responses exhibited by SLE patients are explored.

ANTIBODIES TO Ro AND La: METHODS OF DETECTION

The initial method used for the detection of antibodies to Ro and La was agar diffusion by Ouchterlony's method. This method and the independence of the two antigen–antibody reactions are illustrated in Fig. 1. In well 1 is a partially purified human spleen extract containing both Ro and La antigens. Well 2 contains a monospecific anti-Ro serum. Well 3 contains an undiluted serum with both anti-Ro and anti-La antibodies. Well 4 contains a one-third dilution of the same serum as in well 3. Note the complete independence of the Ro–anti-Ro and the La–anti-La precipitin lines, which suggests that the two antigens shared no epitopes. Subsequently, both a radioimmunoassay method [5, 6] and an enzyme-linked immunosorbent assay (ELISA) method were developed to measure anti-La.

Antibodies to the soluble antigens Ro and La were originally described in patients with SLE and Sjögren's syndrome [7, 8]. The antigens were thought to be cytoplasmic in origin because they were quantitatively recovered from the cytoplasmic fraction of cells

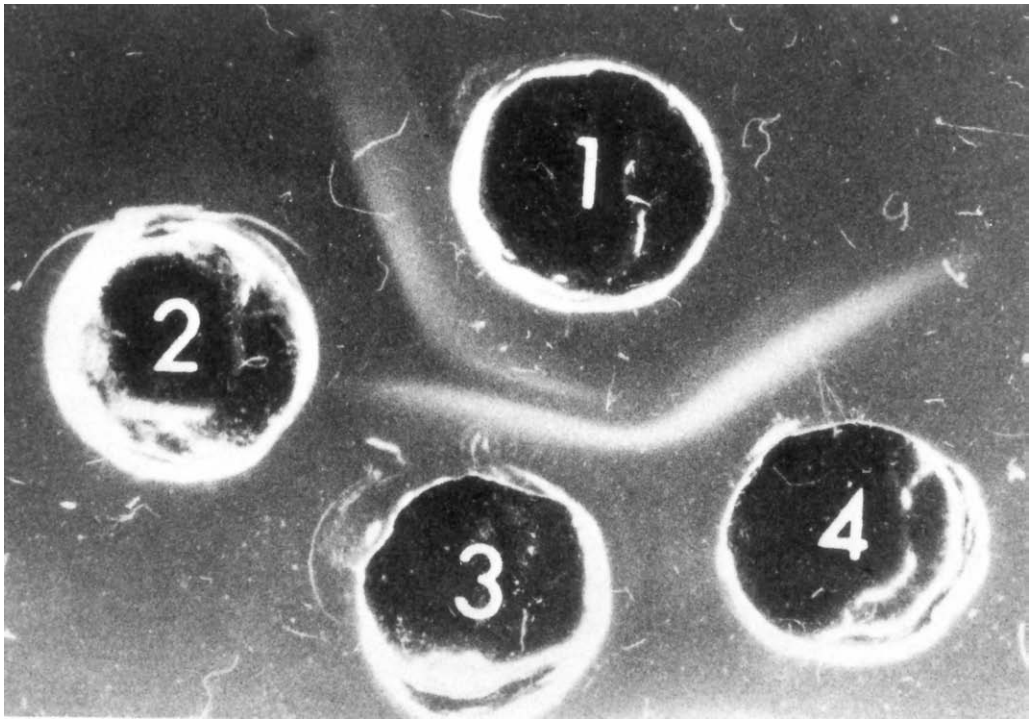


FIGURE 1 Well 1 contains a partially purified human spleen extract containing both Ro/SSA and La/SSB antigens. Well 2 contains a monospecific anti-Ro/SSA serum. Wells 3 and 4 contain a serum with both anti-Ro/SSA and anti-La/SSB undiluted and at one-third dilution, respectively.

prepared in strong sucrose solutions. Moreover, specifically purified antibodies to the Ro antigen stained predominantly the cytoplasm of KB (a human tissue culture line) cells, thymocytes, and Wil_2 (a lymphoblastoid B-cell line) cells, whereas specifically purified antibodies to La stained both the nucleus and the cytoplasm of these cells (P.J. Maddison and M. Reichlin, unpublished data). Alspaugh and Tan [9] described two nuclear antigens extracted from Wil_2 cells, termed *SSA* and *SSB*, that precipitated with the sera of patients with Sjögren's syndrome. Akizuki *et al.* [10] described a soluble nuclear antigen, termed *Ha*, that precipitated with the sera of patients with SLE–Sjögren's syndrome overlap. Interlaboratory exchange of sera and antigen extracts has clearly shown that Ro and SSA, on the one hand, and La, SSB, and Ha, on the other, are antigenically identical. Problems still remain about the cellular localization of Ro/SSA and La/SSB/Ha, but as is elaborated in a later section, these antigens are both RNA protein conjugates [11].

The clinical specificity of antibodies to these antigens in North American populations is that they occur principally in two diseases: SLE and Sjögren's syndrome. Antibodies to Ro/SSA occur in 25–40% of unselected SLE patients when the assay used is the relatively insensitive agar diffusion method. Antibodies to La/SSB/Ha occur in 10–15% of SLE patients, all of whom also have antibodies to Ro/SSA in their sera. In a large survey of hospitalized patients without rheumatic diseases, the false-positive rate for anti-Ro/SSA was found to be approximately 1/1000, as only 6 positive reactions were found in the sera of over 6000 patients [12]. There are differing data about the occurrence of these antibodies: (1) in primary Sjögren's syndrome when keratoconjunctivitis sicca (KCS) occurs alone and (2) in secondary Sjögren's syndrome when KCS occurs with a connective tissue disease, usually rheumatoid arthritis (RA). Studies of Alspaugh *et al.* [13] found anti-Ro/SSA and anti-La/SSB/Ha almost exclusively in primary Sjögren's syndrome. Subsequent studies from Scotland [14] and Baltimore [15] failed to exhibit this specificity; 40–50% of the patients with both primary and secondary Sjögren's syndrome have antibodies to these antigens. A unifying feature noted in the Baltimore patients, which may be relevant in this regard, is that the presence of anti-Ro/SSA is tightly linked to vasculitis and extraglandular disease whether these complications occur in the presence or absence of RA.

When KCS occurs with SLE [15] and scleroderma [16], at least one-half of the patients with anti-Ro/SSA also have anti-La/SSB/Ha in their sera. In patients with primary biliary cirrhosis (PBC) who have Sjögren's

syndrome, only anti-Ro/SSA is found in such sera. Sera from 12 such cases with anti-Ro/SSA were found in a series of 63 PBC patients (E. Penner *et al.*, unpublished data).

Of considerable interest is the vastly different frequency of occurrence of anti-Ro/SSA in Japanese patients with connective tissue diseases. The frequency of occurrence in SLE is 58%, whereas the frequencies in RA, scleroderma, polymyositis, and undifferentiated connective tissue diseases are 24, 29, 15, and 45%, respectively [17]. These frequencies are much higher than those exhibited by North American patients [12] with these same diseases, and the differences are not explained by a more frequent occurrence of Sjögren's syndrome in the Japanese population. Analysis of this difference in anti-Ro/SSA frequency in Japanese versus North American patients with connective tissue diseases should yield important clues to the genesis and regulation of this specific immune response.

CLINICAL ASSOCIATIONS

A number of clinical phenomena have been recognized to be associated with the presence of anti-Ro/SSA in patients with SLE. These are listed in Table 1 and are discussed in the following sections.

ANA-Negative SLE

When the ANA test is performed on cryostat sections of mouse liver or kidney and standardized commercially available fluorescent conjugates of antihuman gammaglobulin are employed, about 95% of active untreated SLE patients will have positive tests. The liters will vary greatly but at least 50% of the liters will exceed 1/100 and a small proportion (≤1/10) will be positive only at the screening dilution (1/10–1/20 in most laboratories). If the screening test is performed on commercially available human tissue culture lines, such as KB or Hep2 cells, the percentage of positive tests approaches 98% of active untreated SLE patients. The difference between the two substrates largely represents the poor representation of the Ro/SSA antigen in mouse or rat liver and kidney sections and its presence in the tissue culture lines.

TABLE 1 Clinical Subsets Associated with Anti-Ro/SSA in SLE and Sjögren's Syndrome[a]

	Anti-Ro/SSA precipitins (%)
SCLE	63
Neonatal SLE[a]	>90
Homozygous C2 and C4 deficiency[b]	85
ANA-negative SLE	65
Vasculitis of Sjögren's syndrome	86
Interstitial lung disease	83
Photosensitivity	90

[a] Prevalence of anti-Ro/SSA in mothers who have children with neonatal SLE.

[b] Prevalence of anti-Ro/SSA in symptomatic patients with lupus-like syndrome.

These sera occur in patients with "ANA-negative SLE," i.e., patients with typical clinical findings of SLE and negative ANA tests on cryostat sections of rodent epithelial tissues [18–20]. Almost two-thirds (41 of 66) of these patients have antibodies to Ro/SSA and/or La/SSB in their sera; of the remainder, the great majority have antibodies to single-stranded DNA (ssDNA) demonstrable by radioimmunoassay. Reexamination of these patients by sensitive ELISA techniques with affinity-purified Ro/SSA reveals that all 66 patients have elevated anti-Ro/SSA levels [21]. These issues have been described and discussed in an analytical review [22]. These negative ANA tests are found in active untreated patients and persist for long periods of clinical follow-up [22]. The clinical picture in these patients is notable for an increased frequency of photosensitive dermatitis and rheumatoid factor tests and a low frequency (10%) of neuropsychiatric and renal disease. It should be noted that some ANA-positive, anti-Ro/SSA-positive SLE patients who enter a steroid-induced remission frequently become ANA negative (on mouse liver or kidney sections) while maintaining substantial titers of anti-Ro/SSA demonstrable by immune precipitation. Indeed, in the author's experience, it is unusual if not rare for anti-Ro/SSA to disappear completely from the sera of SLE patients, whatever the clinical activity of their SLE. The issue of whether anti-Ro/SSA fluctuates with disease activity is still under study in various laboratories, but one report describes a correlation between anti-Ro/SSA titers and disease activity [23].

Differences between SLE Patients with Anti-Ro/SSA and Those with Both Anti-Ro/SSA and Anti-La/SSB

Within the ANA-positive subset of SLE patients who have anti-Ro/SSA, important clinical and serologic differences distinguish patients who produce anti-Ro/SSA alone from those who produce both anti-Ro/SSA and anti-La/SSB [24]. There is a great similarity in nonrenal findings in these two groups of patients but a striking difference in the frequency and severity of renal disease. Thus, 16 of 30 (53%) anti-Ro/SSA patients had one or

more of the following three findings: a serum creatinine level greater than 3.0 mg%, proteinuria exceeding 1.5 g in 24 h, and cellular casts in the urine. Only 2 of 23 (9%) of the anti-Ro/SSA and anti-La/SSB patients had such evidence for nephritis. The serologic correlation of this difference in renal disease was a 77% prevalence of anti-DNA in the anti-Ro/SSA group and a 30% prevalence of such antibodies in the anti-Ro/SSA and anti-La/SSB group. Details of the titers and specificities of these antibodies to DNA have been published, but the titers of both anti-dsDNA and anti-ssDNA were higher in the anti-Ro/SSA-alone group than in patients who had both anti-Ro/SSA and anti-La/SSB in their sera [24]. At least part of the reason for the increased frequency of nephritis in the SLE patients who produce only anti-Ro/SSA is to be found in the usual presence and high titers of antibodies to DNA in this group.

Groups defined by the serologic findings described earlier are stable. Patients producing anti-Ro/SSA alone have never been observed to produce anti-La/SSB. Conversely, patients with both anti-Ro/SSA and anti-La/SSB in their sera have never been observed to lose one or the other of the two. The great difference in frequency of renal disease correlates with a more serious prognosis in the anti-Ro/SSA-alone group compared with patients producing both anti-Ro/SSA and anti-La/SSB. These findings have now been confirmed in two additional cohorts of SLE patients [25, 26].

A possible mechanism for this paucity of anti-dsDNA and nephritis in anti-La/SSB-positive patients has been proposed in which a subset of anti-La/SSB has been shown to be an anti-idiotype to anti-dsDNA [27].

Antibodies to Ro/SSA Are Enriched in Glomerular Eluates from Nephritic SLE Kidneys

Two SLE patients were observed whose progressive renal failure was accompanied by declining serum titers of anti-Ro/SSA [28]. Acid eluates from the postmortem kidneys of these patients revealed enrichment of anti-Ro/SSA by 200- and 12-fold, respectively. These studies provide evidence for the selective deposition of anti-Ro/SSA and participation of these specific antibodies in the nephritis of these patients. The high frequency of nephritis in patients producing anti-Ro/SSA in the absence of anti-La/SSB may, in part, reflect deposition of the Ro/SSA–anti-Ro/SSA immune complexes in renal tissue.

SUBACUTE CUTANEOUS LUPUS ERYTHEMATOSUS

A subset of lupus patients with a characteristic non-scarring dermatitis, subacute cutaneous lupus erythematosus, has been described [29, 30]. The skin disease is intermediate in severity between the acute erythematosus eruption of SLE and the chronic indolent scarring lesions of discoid lupus erythematosus (DLE). Patients exhibit a wide distribution of lesions on the face, arms, and trunk. These lesions are always accompanied by telangiectasia, and there is less hyperkeratosis and follicular plugging than in DLE. There are two morphologic types of lesions: papulosquamous and annular. Primary skin lesions are the same in both, featuring erythematous scaling papules or small plaques. Histopathologically, the two lesions exhibit identical features. Morphologically, the papulosquamous lesions feature merging of the small papules or plaques into large psoriasiform plaques, which can progress to a confluent or retiform array. In annular lesions, the primary active lesions expand peripherally and leave a central inactive zone, resulting in a red scaling ring with a gray–white center. These latter lesions may form a polycyclic array.

Eighty percent of these patients are ANA positive on KB cells and have a mild systemic illness very similar to that described in the so-called ANA-negative SLE patients [22]. When a positive ANA test was taken as a criterion for SLE, 78% of these patients (21 of 27) satisfied at least four American College of Rheumatology criteria for the diagnosis of SLE [31]. The clinical picture is dominated by skin disease, mucosal ulceration, and polyarthritis. Serositis, central nervous system, and renal disease are uncommon. The common factor shared by ANA-negative SLE patients and SCLE patients that may relate to their mild disease and to the low frequency of nephritis is the low frequency (about 10%) of antibodies to dsDNA. Of great interest are two findings that further characterize these patients. One is that 77% of patients with SCLE carry the DR3 antigen, a substantial increase over the 22% frequency found in the control population. The other finding is the presence of antibodies to Ro/SSA, La/SSB, or both in the sera of these patients. While only 25–30% of unselected SLE patients and 3% of DLE patients have anti-Ro/SSA precipitins in their sera, 63% of SCLE patients have anti-Ro/SSA precipitins in their sera. Similar to the findings in ANA-negative SLE, sensitive ELISA tests for anti-Ro/SSA reveal that virtually all are positive for anti-Ro/SSA [21]. Only two SCLE patients had anti-nRNP in their sera, and a single patient had antibodies to Sm. An even tighter linkage of anti-Ro/SSA and DR3 has been observed by Bell and Maddison [32]. In their SLE population, all the anti-Ro/SSA patients (10/10) were positive for the DR3 antigen. The frequency of DR3 in their control populations was 26%. These data suggest that a set of genetic determinants (immune response genes) in the major histocompatibility complex is associated with the production of anti-Ro/SSA.

HOMOZYGOUS C2 = C4 DEFICIENCY

Further data supporting a genetic influence on anti-Ro/SSA production comes from the study of patients with homozygous C2 deficiency. Genes for C2 are known to reside in the major histocompatibility complex on chromosome 6. Antibodies to Ro/SSA were detected in 10 of 20 such C2-deficient sera and were the only precipitins detected. Interestingly, none of these patients produced anti-La/SSB, their sera containing only antibodies to Ro/SSA.

Of even greater interest is that of 12 of these patients with an SLE picture, 9 possessed anti-Ro/SSA and the remaining anti-Ro/SSA-positive patient had DLE. Therefore, all of the 10 homozygous C2-deficient, anti-Ro/SSA-positive patients were symptomatic [33]. Furthermore, 75% of the C2-deficient SLE-like patients were positive for anti-Ro/SSA. No asymptomatic C2-deficient patients were found to have precipitating antibodies to Ro/SSA.

SLE patients with C2 deficiency have a clinical picture very similar to that described for patients with ANA-negative SLE and SCLE described in preceding sections. They have extensive skin disease and polyarthritis; their sera often fail to have demonstrable ANA by indirect immunofluorescence when tested on mouse tissues and only 10% of such patients have antibodies to native DNA. The serologic link of ANA-negative SLE, SCLE, and SLE in C2-deficient patients is anti-Ro/SSA. As it is known that many homozygous C2-deficient patients studied thus far carry the A10, B18, DW2 haplotype, it appears that antibodies to Ro/SSA can be associated with either the DR2 or the DR3 antigen. Although much rarer, homozygous C4 deficiency is also associated with anti-Ro/SSA precipitins. Genes for C4 are also located on chromosome 6 [34].

NEONATAL SLE

It has long been known that in children who develop neonatal SLE, the major clinical features are dermatitis and complete heart block. Studies show that antibodies to Ro/SSA, La/SSB, or both have been found in virtually every child and mother examined in this clinical setting [35–40]. Dermatitis and anti-Ro/SSA disappear with the same time course, suggesting the possible participation of anti-Ro/SSA in the pathogenesis of the dermal lesion. Some of the mothers have not been clinically ill, and anti-Ro/SSA and anti-La/SSB have been demonstrated in their sera in the course of evaluating the clinical and serologic status of the children.

Long-term follow-up of the asymptomatic mothers who gave birth to children with complete congenital heart block (CCHB) reveals that a proportion develop Sjögren's syndrome and a smaller number develop SLE [41].

Three kinds of data implicate placentally transferred anti-Ro/SSA in the pathogenesis of CCHB: (1) the virtual uniform presence in the mothers and children with CCHB, (2) the selective depletion of anti-Ro/SSA in a child with CCHB whose asymptomatic fraternal twin had a serum level identical to the mother, and (3) the enrichment of anti-Ro/SSA in the acid eluate of the heart of a child who died with CCHB [43]. Demonstration of an idiotype characteristic of anti-La in myocardial fibers from a child who died with CCHB suggests that in some cases anti-La/SSB may also participate in the development of CCHB [44].

SEROLOGIC PHENOMENA IN SLE PATIENTS WITH ANTIBODIES TO RO/SSA AND LA/SSB

A number of serologic phenomena have been noted in SLE patients with anti-Ro/SSA, La/SSB, or both; these are listed in Table 2. Rheumatoid factors have been found in 30–40% of unselected SLE patients but are greatly enriched in SLE patients with anti-Ro/SSA, occurring in 75–80% of such patients [16]. This enrichment for rheumatoid factors in anti-Ro/SSA-positive patients may be another manifestation of the linkage antibodies to Ro/SSA form between SLE and Sjögren's syndrome. Further genetic and clinical associations have been noted in a group of patients with SLE–Sjögren's syndrome overlap [45, 46].

A striking linkage of antibody precipitin titers has been noted in SLE patients producing both anti-Ro/SSA and anti-La/SSB. Antibodies to Ro/SSA either equal or exceed La/SSB as determined by counterimmunoelectrophoresis. In 13 instances, the anti-Ro/SSA liters were observed to be greater than the anti-La/SSB titers. Since it has been shown that these precipitins are independent and one can be absorbed out without

TABLE 2 Serologic Phenomena Associated with Anti-Ro/SSA and Anti-La/SSB

1. Rheumatoid factor occurs in 75–80% of anti-Ro/SSA–positive SLE patients
2. In SLE patients producing both anti-Ro/SSA and anti-La/SSB precipitin, titers of anti-Ro/SSA are either equal to or greater than anti-La/SSB titers
3. Homozygous C2-deficient patients [33] and primary biliary cirrhosis patients [24] who produce anti-Ro/SSA have not been observed to have anti-La/SSB in their sera

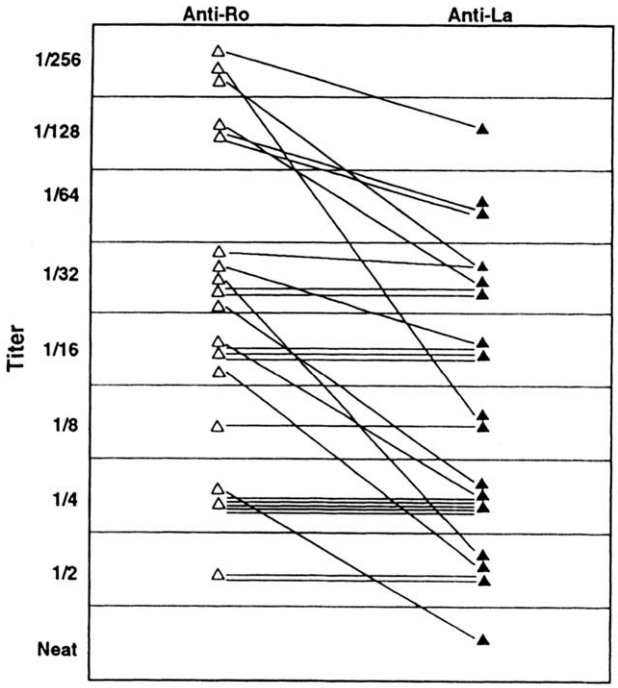


FIGURE 2 Quantitative titers of precipitating antibody to anti-Ro (△) and anti-La (▲) measured by counterimmunoelectrophoresis. Note that the titers to the two antibodies for each patient are connected by a line.

affecting the titer of the other, interesting questions have been raised about the joint regulation of the production of these antibodies. This has also been suggested indirectly by the facts that (1) patients producing both anti-Ro/SSA and anti-La/SSB rarely produce anti-DNA (in contrast to patients producing only anti-Ro/SSA) and (2) both C2-deficient SLE patients and those with primary biliary cirrhosis who produce anti-Ro/SSA (10 and 12 instances, respectively) have rarely been observed to produce anti-La/SSB. These data suggest not only joint regulation of the production of anti-Ro/SSA and anti-La/SSB in patients producing both antibodies, but also independent factors (possibly genetic) controlling the production of anti-Ro/SSA alone as opposed to the joint production of anti-Ro/SSA and anti-La/SSB [25]. This titer linkage is illustrated in Fig. 2.

MOLECULAR BIOLOGY OF RO/SSA AND LA/SSB ANTIGENS

Lerner *et al.* [11] launched a new era in the study of antigens reactive with the sera of patients with SLE and related diseases by a molecular biologic approach. Using intrinsically labeled RNA as the probe, they showed that anti-Ro/SSA and anti-La/SSB are directed to RNA protein particles. Anti-Ro/SSA is directed against cytoplasmic particles containing protein and five small RNAs, designated Y_1–Y_5, containing 80–140 bases. The Ro/SSA particle has been implicated in the discard pathway for 5S RNA [47].

The La/SSB/Ha antigen has proven interesting in at least two regards. Antibodies to La/SSB bind a particle containing a very heterogeneous set of small RNAs and protein from normal cells, but they also bind to an RNA particle containing V_A RNA, encoded by adenovirus, and Epstein–Barr virus-associated RNA particles $EBER_1$ and $EBER_2$, which are encoded by the Epstein–Barr virus [48]. In addition, it appears that anti-La/SSB reacts with predominantly nuclear particles whose RNA components are all products of RNA polymerase III, including pre-tRNA, pre-5S rRNA, and, as mentioned, certain viral transcripts. It may be that the La/SSB protein plays a special role in the processing of RNA that are products of RNA polymerase III [48, 49].

These studies suggest that the La/SSB protein is involved in RNA processing and perhaps that the anti-La/SSB response is a marker for previous viral infection. Of great interest in this regard will be the demonstration that the La/SSB protein is the same or different in normal and virus-infected cells. Should the La/SSB protein be the same in these two circumstances, binding of the relevant RNA protein conjugates in normal and infected cells by anti-La/SSB might simply reflect the uniform role of the La/SSB protein in processing certain RNA classes in both normal and infected cells.

HETEROGENEITY OF Ro/SSA ANTIGEN AND ANTI-Ro/SSA

It has become clear that sera with anti-Ro/SSA precipitins react with at least seven different molecules, some of which bind the HY RNAs and all of which bind autoantibodies in Western blot analysis. These are listed in Table 3, and a detailed discussion of these Ro/SSA molecules is given elsewhere [50]. There appear to be related but different 60-kDa Ro/SSA molecules in lymphocytes and red cells and a completely different set of related smaller molecules of 52 and 54 kDa in lymphocytes and red cells, respectively. In platelets, both 60- and 52-kDa forms of Ro/SSA are present and only HY3 and HY4 Ro/SSA RNA are found. Finally, a 47-kDa Ro/SSA molecule different from all of those just mentioned and related to calreticulin has been reported [51].

It is now thought that only the 60-kDa Ro/SSA molecules within nucleated or nonnucleated cells bind HY RNAs, and past perceptions of such binding by the 52- and 54-kDa forms of Ro/SSA were incorrect [50].

TABLE 3 Molecular Forms of Ro/SSA Antigen[a]

Tissue source	Molecular Mass (kDa)	RNA type
Lymphocytes, HeLa cells	60 52	HY1, 3, 4, 5
Red cells	60 54	HY1, 4
Platelets	60, 52	HY3, 4
Wil_2 cells (calreticulin)	47	HY1, 3, 4, 5

[a] See Reichlin [50] for details.

ANTIBODIES TO RIBOSOMES

Antibodies to ribosomes in SLE patients were first clearly demonstrated by precipitins in agar gel [52]. RNA and protein were both involved in the antigenic determinant(s), and all 11 patients had nephritis and severe disease. In that study, such precipitins were present in 11 of 85 SLE patients. In other studies, antibodies to ribosomes were demonstrated by bentonite flocculation [53] and a fluorescence spot test [54]. In these studies, 25 and 51% of SLE sera contained such antibodies, and in both instances, RNA and protein were both required for full antigenicity. In another study, purified ribosomal RNA was shown to react directly in precipitin reactions with some sera from SLE patients [55].

The most sensitive assay for antibodies to ribosomes has been developed by Koffler *et al*. [56], who reported on the use of intrinsically labeled HeLa cell polysomes in a Farr-type radioimmunoassay. Using this approach, 70% of SLE patients were found to have antibodies to ribosomes [56]. Sera from patients with RA and chronic active hepatitis contained antibodies to ribosomes in 24 and 27%, respectively, and the titers were lower than in SLE patients. More recently, the fine specificity of antibodies to ribosomes was examined by inhibition of the binding of labeled ribosomes to antibody by ribosomal ribonucleoprotein and free RNA [57]. The findings suggested that antibodies to ribosomal ribonucleoprotein correlated most closely with active disease.

ANTIBODIES TO THE RIBOSOMAL P PROTEINS

The first definition of a specific ribosomal polypeptide antigen was reported in 1985 when three related phosphoproteins, P_0, P_1, and P_2 of molecular masses 38, 19, and 17 kDa, were shown to be targets of autoimmunity in SLE patients [58]. An immunodominant 22 amino acid peptide was shown to be shared among these polypeptides [59], and an association was demonstrated between antibodies to the ribosomal P proteins and lupus psychosis [60]. A more recent report utilizing an assay employing the measurement of antibodies to the immunodominant carboxyl-terminal P peptide has shown an even closer association of ribosomal anti-P antibodies with depression in SLE patients [61]. In addition, such autoantibodies to the ribosomal P peptide were shown to occur in 15% of SLE patients [61]. Two additional findings of interest were that antiribosomal P antibodies fluctuated with the clinical neuropsychiatric diseases [60, 61] and that markedly less antiribosomal P antibodies was demonstrated in the cerebrospinal fluid than in the serum [61]. Definition of a specific reaction between a defined ribosomal protein has permitted elucidation of an important link between clinical neuropsychiatric disease and antibody to the ribosomal P proteins. These findings have sparked some controversy, and some studies, but not all, show this linkage between neuropsychiatric disease and anti-P proteins. These matters are the subject of an analytical review [62].

Three studies report new findings suggesting an association of ribosomal P protein with unexplained hepatitis [63, 64] and an even stronger association with lupus nephritis [64–66], an association foreshadowed in 1967 when precipitating antibodies to ribosomes were uniformly associated with the 11 patients who were first described with these precipitins, all of whom undoubtedly had antibodies to the ribosomal P proteins [52].

These latter findings take on added meaning now that it is clear that antiribosomal P antibodies bind to living cells in culture, penetrate these cells, and profoundly affect protein synthesis [67]. Antiribosomal P antibodies are the only specific autoantibodies characteristic of SLE that uniformly behave this way, although a subset of anti-dsDNA antibodies also behave this way [68]. Nonetheless, the ability of anti-P antibodies to bind a variety of living cells may position such autoantibodies uniquely to participate in immunopathogenesis of SLE.

SIGNIFICANCE OF THE AUTOIMMUNE RESPONSE TO CYTOPLASMIC CONSTITUENTS

In the most general sense, antibodies to cytoplasmic constituents in SLE patients are a part of the spectrum of autoimmune responses so characteristic of this disorder. Antibodies to nuclear antigens in SLE have received the greatest attention, but antibodies to both cytoplasmic constituents and lymphocyte membranes occur in a substantial number of patients. The

antibodies discussed in this chapter are part of a general autoimmune response in SLE patients to polynucleotides and protein polynucleotide conjugates. Clearly, however, in the case of antibodies to Ro/SSA and La/SSB, reactivity has only been demonstrated conclusively on the protein moieties of the RNA–protein conjugates known to possess antigenicity. In the case of antibodies to ribosomes, antigenicity resides in free RNA, on RNA–protein conjugates, and on the specific polypeptides P_0, P_1, and P_2.

Genetic factors clearly play a role in the production of anti-Ro/SSA and anti-La/SSB. Strong linkage of these antibodies to DR3 in SLE and to DR2 in homozygous C2 deficiency supports the idea that immune response genes are involved in the production of these antibodies. Are these genes responsible for loss of antigen-specific T suppressor clones, enhancement of antigen-specific T helper clones, or some other as yet unrecognized functional aspect of the D region on chromosome 6 that modulates immune function in SLE patients? Only further research can sort out these possibilities. Finally, the fact that antibodies to La/SSB bind viral-specific RNA–protein conjugates raises anew the possibility that viral infection may provide an etiologic trigger for some patients with SLE. Structural elucidation of the La/SSB protein in antigens derived from normal and virus-infected cells should shed light on this possibility.

There is no doubt that anti-Ro/SSA antibodies are linked to a subset of SLE patients with shared features described previously as ANA-negative SLE, SCLE, the SLE-like disease of homozygous C2 deficiency, and neonatal SLE. In addition, antibodies to ribosomes and Ro/SSA may participate in immune complex disease, and antibodies to the ribosomal P proteins may play a role in the pathogenesis of not only neuropsychiatric disease in SLE patients, but hepatitis and nephritis as well. One can expect further correlations of anticytoplasmic antibodies with disease expression in the future.

Acknowledgment

This work was supported by NIH Grants AR43975 and AR07580.

References

1. Reichlin, M. (1981). Current perspectives on serological reactions in SLE patients. *Clin. Exp. Immunol*. **44**, 1–10.
2. Asherson, G. L. (1959). Antibodies against nuclear and cytoplasmic constituents in systemic lupus erythematosus and other diseases. *Br. J. Exp. Pathol*. **40**, 209–215.
3. Deicher, H. R. G., Holman, H. R., and Kunkel, H. G. (1960). Anti-cytoplasmic factors in the sera of patients with systemic lupus erythematosus and certain other diseases. *Arthritis Rheum.* **3**, 1–15.
4. Wiedermann, G., and Miescher, P. A. (1965). Cytoplasmic antibodies in patients with systemic lupus erythematosus. *Ann. N.Y. Acad. Sci.* **124**, 807–815.
5. Akizuki, M., Boehm-Truitt, M. J., Kassan, S. S., Steinberg, A. D., and Chused, T. M. (1977). Purification of an acidic nuclear protein antigen and demonstration of its antibodies in subsets of patients with sicca syndrome. *J. Immunol*. **119**, 932–938.
6. Venables, P. J., Charles, P. J., Buchanan, R. R., Yi, T., Mumford, P. A., Schrieber, L., Room, G. R., and Maini, R. N. (1983). Quantitation and detection of isotypes of anti-SS-B antibodies by ELISA and assays using affinity purified antigens: An approach to the investigation of Sjögren's syndrome and systemic lupus erythematosus. *Arthritis Rheum.* **26**, 146–155.
7. Clark, G., Reichlin, M., and Tomasi, T. B. (1969). Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythematosus. *J. Immunol.* **102**, 117–122.
8. Mattioli, M., and Reichlin, M. (1974). Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus: Description of a cytoplasmic nonribosomal antigen. *Arthritis Rheum*. **17**, 421–429.
9. Alspaugh, M. A., and Tan, E. M. (1975). Antibodies to cellular antigens in Sjögren's syndrome. *J. Clin. Invest*. **55**, 1067–1073.
10. Akizuki, M., Powers, R., and Holman, H. R. (1977). A soluble acidic protein of the cell nucleus which reacts with serum from patients with systemic lupus erythematosus and Sjögren's syndrome. *J. Clin. Invest*. **59**, 264–272.
11. Lerner, M. R., Boyle, J. A., Hardin, J. A., and Steitz, J. A. (1981). Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* **211**, 400–402.
12. Maddison, P. J., Mogavero, H., Provost, T. T., and Reichlin, M. (1979). The clinical significance of autoantibodies to a soluble cytoplasmic antigen in systemic lupus erythematosus and other connective tissue diseases. *J. Rheumatol.* **6**, 189–195.
13. Alspaugh, M. A., Talal, N., and Tan, E. M. (1976). Differentiation and characterization of autoantibodies and their antigens in Sjögren's syndrome. *Arthritis Rheum.* **19**, 216–222.
14. Alspaugh, M. A., Buchanan, W. W., and Whaley, K. (1978). Precipitating antibodies to cellular antigens in Sjögren's syndrome, rheumatoid arthritis and other organ and nonorgan specific diseases. *Ann. Rheum. Dis.* **37**, 244–246.
15. Alexander, E. L., Hirsch, T. J., Arnett, F. C., Provost, T. T., and Stevens, M. B. (1982). Ro (SSA) and La (SSB) antibodies in the clinical spectrum of Sjögren's syndrome. *J. Rheumatol.* **9**, 239–246.
16. Osial, T. A., Whiteside, T. L., Buckingham, R. B., Singh, G., Barnes, E. L., and Rodnan, G. P. (1982). Antibodies to SS-A, SSB and RANA in progressive systemic sclerosis (PSS) with and without Sjögren's syndrome (SS). *Clin. Res*. **30**, A661.

17. Yamagata, H. (1981). The antibodies to the SS-A antigen in patients with connective tissue diseases. *Keio Igaki J. Med.* **58**, 381.
18. Fessel, W. J. (1978). ANA negative systemic lupus erythematosus. *Am. J. Med*. **64**, 80–86.
19. Gladman, D. D., Chalmers, A., and Urowitz, M. B. (1978). Systemic lupus erythematosus with negative LE cells and antinuclear factor. *J. Rheumatol*. **5**, 142–147.
20. Provost, T. T., Ahmed, A. R., Maddison, P. J., and Reichlin, M. (1977). Antibodies to cytoplasmic antigens in lupus erythematosus: Serological marker for systemic disease. *Arthritis Rheum*. **20**, 1457–1463.
21. Reichlin, M. (2000). ANA negative systemic lupus sera revisited serologically. *Lupus* **9**, 116–119.
22. Maddison, P. J., Provost, T. T., and Reichlin, M. (1981). Serological findings in patients with "ANA-Negative" systemic lupus erythematosus. *Medicine (Baltimore)* **60**, 87–94.
23. Scopelitis, E., Buindo, J. J., Jr., and Alspaugh, M. A. (1980). Anti-SSA antibody and other antinuclear antibodies in systemic lupus erythematosus. *Arthritis Rheum*. **23**, 287–293.
24. Wasicek, C. A., and Reichlin, M. (1982). Clinical and serological differences between systemic lupus erythematosus patients with antibodies to Ro versus patients with antibodies to Ro and La. *J. Clin. Invest.* **69**, 835–843.
25. Hamilton, R. G., Harley, J. B., Bias, W. B., Roebber, M., Reichlin, M., Hochberg, M. C., and Arnett, F. C. (1988). Two Ro (SS-A) autoantibody responses in systemic lupus erythematosus: Correlation of HLA-DR/DQ specificities with quantitative expression of Ro(SS-A) autoantibody. *Arthritis Rheum.* **31**, 496–505.
26. Harley, J. B., Sestak, A. L., Willis, L. G., Fu, S. M., Hansen, J. A., and Reichlin, M. (1989). A model for disease heterogeneity in systemic lupus erythematosus: Relationships between histocompatibility antigens, autoantibodies and lymphopenia or renal disease. *Arthritis Rheum.* **32**, 826–836.
27. Zhang, W., and Reichlin, M. Some autoantibodies to Ro/SS-A and La/SS-B are antiidiotypes to anti-double-stranded DNA. (1996). *Arthritis Rheum.* **39**, 522–531.
28. Maddison, P. J., and Reichlin, M. (1979). Deposition of antibodies to a soluble cytoplasmic antigen in the kidneys of patients with systemic lupus erythematosus. *Arthritis Rheum.* **22**, 858–863.
29. Sontheimer, R. D., Thomas, J. R., and Gilliam, J. N. (1979). Subacute cutaneous lupus erythematosus: A cutaneous marker for a distinct lupus erythematosus subset. *Arch. Dermatol.* **115**, 1409–1415.
30. Sontheimer, R. D., Maddison, P. J., Reichlin, M., Jordan, R. E., Stastny, P., and Gilliam, J. N. (1982). Serologic and HLA associations in subacute cutaneous lupus erythematosus, a clinical subset of lupus erythematosus. *Ann. Intern. Med.* **97**, 664–671.
31. Cohen, A. S., Reynolds, W. E., Franklin, E. C., Kulka, J. P., Ropes, M. W., Shulman, L. E., and Wallace, S. L. (1971). Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21**, 643–648.
32. Bell, D. A., and Maddison, P. J. (1980). Serological subsets in SLE: An examination of autoantibodies in relationship to clinical features of disease and HLA antigens. *Arthritis Rheum.* **23**, 1268–1273.
33. Provost, T. T., Arnett, F. C., and Reichlin, M. (1983). Homozygous C2 deficiency, lupus erythematosus and anti-Ro (SSA) antibodies. *Arthritis Rheum*. **26**, 1279–1282.
34. Meyer, O., Hauptmann, G., and Tappeiner, G. (1985). Genetic deficiency C_4, C_2 or C_{1q} and lupus syndrome. Association with anti-Ro (SS-A) antibodies. *Clin. Exp. Immunol*. **62**, 678–684.
35. Franco, H. L., Weston, W. L., Peebles, C., Forstot, S. L., and Phanuphak, P. (1981). Autoantibodies directed against sicca syndrome antigens in the neonatal lupus syndrome. *Am. Acad. Dermatol*. **4**, 67–72.
36. Kephart, D. C., Hood, A. F., and Provost, T. T. (1981). Neonatal lupus erythematosus: New serological findings. *J. Invest. Dermatol*. **77**, 331–333.
37. Miyagamu, S., Kitamura, W., Yoshioka, J., and Sakamoto, K. (1981). Pacental transfer of anticytoplasmic antibodies in annular erythema of newborns. *Arch. Dermatol*. **117**, 569–572.
38. Taylor, P. V., Taylor, K. F., Norman, A., Griffith, S., and Scott, J. S. (1988). Prevalence of maternal Ro (SS-A) and La(SS-B) autoantibodies in relation to congenital heart block. *Br. J. Rheumatol.* **17**, 128–132.
39. Silverman, E., Mamula, M., Hardin, J. A., and Laxer, R. (1992). Importance of the immune response to the Ro/La particle in the development of congenital heart block and neonatal lupus erythematosus. *J. Rheumatol*. **18**, 120–124.
40. Buyon, J. P., Ben-Chetrit, E., Karp, S., Roubey, R. A., Pompeo, L., Reeves, W. H., Tan, E. M., and Winchester, R. (1989). Acquired congenital heart block: Patterns of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La antibody system in neonatal lupus. *J. Clin. Invest*. **84**, 627–634.
41. Press, J., Uziel, Y., Laxer, R. M., *et al.* (1996). Long term outcome of mothers of children with complete congenital heart block. *Am. J. Med*. **100**, 328–332.
42. Harley, J. B., Kaine, J. L., Fox, O. F., Reichlin, M., and Gruber, B. (1985). Ro/SSA antibody and antigen in a patient with congenital complete heart block. *Arthritis Rheum.* **28**, 1321–1325.
43. Reichlin, M., Brucato, A., Frank, M. B., Maddison, P. J., McCubbin, V. R., Wolfson-Reichlin, M., and Lee, L. A. (1994). Concentration of autoantibodies to native 60-kD Ro/SS-A and denatured 52kD Ro/SS-A in eluates from the heart of a child who died with congenital complete heart block. *Arthritis Rheum.* **37**(No. 11), 1698–1703.
44. Horsfall, A. C., Venables, P. J. W., Taylor, P. V., and Maini, R. N. (1991). Ro and La antigens and maternal autoantibody idiotype on the surface of myocardial fibers in congenital heart block. *J. Autoimmun*. **4**, 165–176.
45. Provost, T. T., Talal, N., Harley, J. B., Reichlin, M., and Alexander, E. (1988). The relationship between anti-Ro(SS-A) antibody positive Sjögren's syndrome and anti-Ro(SS-A) antibody positive lupus erythematosus. *Arch. Dermatol*. **124**, 63–71.

46. Provost, T. T., Talal, N., Bias, W., Harley, J. B., Reichlin, M., and Alexander, E. (1988). Ro(SS-A) positive Sjögren's/lupus erythematosus (SC/LE) overlap patients are associated with the HLA-DR3 and/or DRw6 phenotypes. *J. Invest. Dermatol.* **91**, 369–371.
47. O'Brien, C. A., and Wolin, S. L. (1994). A possible role for the 60-kD Ro autoantigen in a discard pathway for defective 5S RNA precursors. *Genes Dev*. **8**, 2891–2903.
48. Rinke, J., and Steitz, J. A. (1982). Precursor molecules of both human 5S ribosomal RNA and transfer RNAs are bound by a cellular protein reactive with anti-La lupus antibodies. *Cell* **29**, 149–159.
49. Lerner, M. R., Andrews, N. C., Miller, G., and Steitz, J. A. (1981). Two small RNAs encoded by Epstein Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **78**, 805–809.
50. Reichlin, M. (1995). Autoantibodies to the RoRNP particles. *Clin. Exp. Immunol*. **99**, 7–9.
51. McCauliffe, D. P., Lux, F. A., and Lieu, T. S. (1990). Molecular cloning expression and chromosome 19 localization of a human Ro/SSA autoantigen. *J. Clin. Invest.* **85**, 1379–1391.
52. Schur, P. H., Moroz, L. E., and Kunkel, H. G. (1967). Precipitating antibodies to ribosomes in the serum of patients with systemic lupus erythematosus. *Immunochemistry* **4**, 447–453.
53. Sturgill, B., and Carpenter, R. R. (1965). Antibody to ribosomes in systemic lupus erythematosus. *Arthritis Rheum.* **19**, 213–218.
54. Sturgill, B. C., and Preble, M. R. (1967). Antibody to ribosomes in systemic lupus erythematosus: Demonstration by immunofluorescence and precipitation in agar. *Arthritis Rheum.* **10**, 538–543.
55. Lamon, E. W., and Bennett, J. C. (1970). Antibodies to ribosomal RNA (rRNA) in patients with systemic lupus erythematosus (SLE). *Immunology* **19**, 439–442.
56. Koffler, D., Faiferman, I., and Gerber, M. A. (1977). Radioimmunoassay for antibodies to cytoplasmic ribosomes in human serum. *Science* **198**, 741–743.
57. Koffler, D., Miller, T. E., and Lahita, R. G. (1979). Studies on the specificity and clinical correlation of antiribosomal antibodies in systemic lupus erythematosus sera. *Arthritis Rheum.* **22**, 463–470.
58. Elkon, K. B., Parnassa, A. P., and Foster, C. L. (1985). Lupus autoantibodies target ribosomal P proteins. *J. Exp. Med*. **162**, 459–471.
59. Elkon, K., Skelly, S., Parnassa, A., Moller, W., Danho, W., Weissbach, H., and Brot, N. (1986). Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **83**, 7419–7423.
60. Bonfa, E., Golombek, S. J., Kaufman, L. D., Skelly, S., Weissbach, H., Brot, N., and Elkon, K. B. (1987). Association between lupus psychosis and anti-ribosomal P protein antibodies. *N. Eng. J. Med*. **317**, 265–271.
61. Schneebaun, A. B., Singleton, J. D., West, S. G., Blodgett, J. K., Allen, L. G., Cheronis, J. C., and Kotzin, B. L. (1991). Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am. J. Med*. **90**, 54–62.
62. Teh, L. S., and Isenberg, D. A. (1994). Antiribosomal P protein antibodies in systemic lupus erythematosus, a reappraisal. *Arthritis Rheum*. **37**, 307–315.
63. Hulsey, M., Goldstein, R., Scully, L., Surbeck, W., and Reichlin, M. (1995). Antiribosomal P antibodies in systemic lupus erythematosus: A case-control study correlating hepatic and renal disease. *Clin. Immunol. Immunopathol*. **74**, 252–256.
64. Arnett, F. C., and Reichlin, M. (1995). Lupus hepatitis: An under recognized disease feature associated with autoantibodies to ribosomal P. *Am. J. Med*. **99**, 465–472.
65. Martin, A. L., and Reichlin, M. (1996). Fluctuation of antibody to ribosomal P proteins correlate with appearance and remission of nephritis in SLE. *Lupus* **5**, 22–29.
66. Chindalore, V., Neas, B. R., and Reichlin, M. (1998). The association between antiribosomal P antibodies and active nephritis in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **87**, 292–296.
67. Koren, E., Koscec, M., Wolfson-Reichlin, M., Fugate, R. D., and Reichlin M. (1997). Autoantibodies to ribosomal P proteins penetrate into live hepatocytes and cause cellular dysfunction in culture. *J. Immunol*. **159**, 2033–2041.
68. Reichlin, M. (1996). Presence of ribosomal P protein on the surface of human umbilical vein endothelial cells. *J. Rheumatol*. **23**, 1123–1125.

12

ANTIHISTONE ANTIBODIES

Robert L. Rubin

HISTORICAL PERSPECTIVE

Histone-reactive antibodies hold a preeminent position in the history of fundamental discoveries in autoimmunity. The first indication that autoimmunity may underlie diseases such as systemic lupus erythematosus is generally attributed to the observations of Hargraves *et al*. [1] on the lupus erythematosus (LE) cell phenomenon in which *in vivo* polymorphonuclear leukocytes (PMN) were deduced to have phagocytosed nuclear material derived from dead cells. This phenomenon was produced *in vitro* by Hamburger [2] and Miescher and Fauconnet [3] by mixing normal PMNs with SLE plasma. Holman and Deicher [4] showed that the responsible factor in SLE plasma was an antinuclear antibody (ANA) but not anti-DNA (as demonstrated by absorption experiments) and was capable of fixing complement when bound to nuclei [5]. Absorption of sera with calf thymus nucleohistone and histone–DNA complexes greatly reduced the LE cell phenomenon [6], suggesting that the fine specificity of this ANA was that of histone-reactive antibodies. LE cells have also been observed in drug-induced lupus (DIL) [7], a syndrome invariably associated with antihistone antibodies [8].

The necessity for the presence of histones in maintaining the antigenicity of nuclei for the LE cell factor was verified by a number of studies published in 1959. Holborow and Weir [9] demonstrated that these antibodies bound to spermatocyte nuclei, but after histones are replaced by protamines during the formation of mature sperm, antibody binding was no longer detected. The LE cell factor was absorbed out and could be eluted from a histone-containing, DNase predigested residue of nucleoprotein [4]; binding of antibody to nuclei was also not diminished after DNase pretreatment [10]. Direct demonstration of the antigenicity of free histones was obtained by Holman *et al*. [11] and Kunkel *et al*. [12] using a complement-depletion assay.

Much of the subsequent, older work on histone-reactive antibodies in SLE was restricted to histone-containing nucleoproteins. Complexes of histones with sonicated DNA were shown to form immunoprecipitins with SLE sera using classic Ouchterlony analysis [13]. Many SLE patients had complement-fixing anti-DNA and anti-(histone-DNA), which tended to increase along with clinical disease [14]. Various immunofluorescence patterns (rim, fibrillar, homogeneous) were attributed to antibodies to nucleoprotein [13, 14]. Over 50% of SLE sera had antibodies to soluble nucleoprotein that were not removed by absorption of sera with DNA [15], but the fine specificity of these autoantibodies was not determined until years later [16]. It was generally inferred from these early studies that antibodies that bind nucleoprotein are antibodies to histone–DNA complexes, and this specificity is equivalent to the LE cell factor. More recent studies by Hannestad *et al*. [17] and Rekvig and Hannestan [18] suggested that a complex of histones in the absence of DNA possessed antigenic activity for the LE factor. Precisely what specificities determine the LE factor activity have still not been completely resolved, although studies have demonstrated that antibodies to histone H1 are a major contributor [19]. Nevertheless, the recognition that LE cells in SLE and DIL are caused by histone-reactive antibodies not only led to numerous studies on the significance of these antibodies, but also

opened up the laboratory investigation of autoimmunity in general.

HISTONE STRUCTURE AND FUNCTION

Histones are normally found in eukaryotic cells associated with DNA, and this nucleoprotein is the major constituent of chromatin. Chromatin is insoluble in physiologic solutions, but histones dissociate from DNA in 2 *M* NaCl or dilute acid. Acid-solubilized histones are monomeric, and five major polypeptides can be distinguished by polyacrylamide gel electrophoresis (Fig. 1). These proteins can be separated by gel permeation chromatography [20], and individual histone classes can be purified readily on a preparative scale. The amino acid sequences of all five histones and numerous variants from many animals and plants have been determined [22, 23].

Primary Structures and Histone Variants

Histones H3 and H4 are two of the most conserved proteins in evolution [24]—bovine H3 and H3 from the pea plant differ by only 4 of 135 amino acids, and bovine and pea H4 are identical, except for two conservative amino acid substitutions. Human and calf H4 are identical, including the presence of three posttranslationally modified residues [25]. The primary structure of human spleen H3 is identical to calf thymus H3 except for serine in the place of cysteine at position 96 and for the presence of two minor variants in the human histone [26]. The primary structure of H2B also displays remarkable similarity in the C-terminal half of the molecule among a wide variety of animals, but considerable variation in the amino acid sequence of the N-terminal half of H2B has been detected within the animal kingdom [23]. However, human spleen H2B is identical with calf thymus H2B except for two minor variants in human H2B, each of which has a single amino acid substitution [27]. The entire molecule of H2A appears to be more highly conserved than H2B. Calf thymus H2A is identical to human spleen H2A except for a minor variant having a histidine deletion at residue 123 or 124 [28]. The vast majority of histone variants are identical among mammalian species, suggesting their importance in the biology of the cell. Because of this near identity between human core histones and the respective histones from calf thymus (the source tissue of choice for its high nuclear/cytoplasmic mass ratio), bovine histones can generally be considered as valid autoantigens for analyzing human autoantibodies.

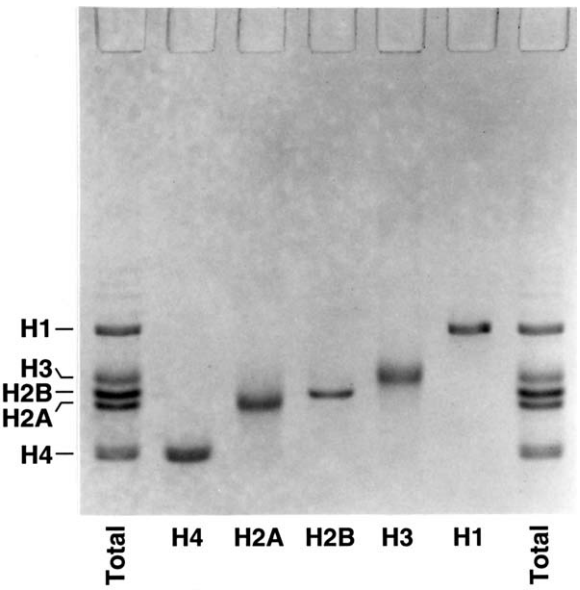


FIGURE 1 The five major histone classes. Total histones derived by the acid extraction of chromatin were separated by gel exclusion chromatography [20] and subjected to polyacrylamide gel electrophoresis [21].

The H1 class of histones is considerably more complex and heterogeneous. An internal segment of approximately 70 amino acids displays extensive homology among the animal H1 histones sequenced, but the N-terminal 40 residues and C-terminal half of the protein show substantial primary structure divergence [23]. H1 includes at least eight variants, which differ in primary structure (see later), and many of these, especially human [29–33], have only recently been sequenced. Mammalian H1° is present mainly in terminally differentiated, quiescent cells and shows extensive homology with histone H5, which is found in phylogenetically lower animals and is generally isolated from avian-nucleated erythrocytes. Human H1° and chicken H5 share eight essentially identical segments of 6–21 amino acids long if only one amino acid deletion, addition, or substitution is allowed per segment [31]. However, these histones show less than 10% homology with the main H1 class [34]. The nomenclature for the H1 family of histones has not been formally standardized, so structural comparisons between species are presently ambiguous. However, all the H1 histones, including H1° and H5, share a nearly identical 16 amino acid long region in the globular domain (human H1.1 94–108, human H1.2 91–105, human H1° 79–94, bovine H1.1 91–106, chicken H5 80–95), as well as a highly conserved octapeptide in the C-terminal domain (human H1.1 151–158, H1.2 153–160, and H1° 152–159, chicken H5 148–155) [33]. Although extensive antigenic cross-reactivity can be expected among H1 variants within an

animal and between species, the complexity of this histone class and the difficulty in physically separating and identifying these variants presently confounds the interpretation of studies involving antibody to the H1 family of histones.

The different histone classes have very limited amino acid sequence similarities [35, 36], but these may account for some cross-reactions among antihistone antibodies. H2A shows homologies with H4, H2B, and H3, each in different parts of the H2A molecule, an octapeptide in H3 is similar to a decapeptide in H4, and H3 and H2B share an identical tetrapeptide (see Rubin [37] for exact sequence). Although all the histones are rich in basic amino acids, especially in their amino-terminal regions, extensive, uninterrupted strings of such residues are generally not observed. A pentapeptide in H2B and H4 consisting of only basic amino acids can be found, but an array of greater than three lysine, arginine, or histidine residues does not occur in any histone, including H1. This may explain why naturally occurring antihistone antibodies display only weak or negligible cross-reactivity with basic homopolypeptides.

Within each class of histones, numerous subtypes or variants have been identified [38]. Variants occur among different species as well as within a species, such as between stages of development, among different tissues, and even within a homogeneous cell type. This heterogeneity in primary structure can be the result of transcription from multiple genes for a histone class with slightly different coding sequences or due to postsynthetic modifications. Posttranslational modifications occur on only a portion of the total histone pool of a cell or tissue, resulting in a variety of subtypes being present simultaneously. Phosphorylation occurs on certain serine residues of all histones, threonine on H3 and H1, histidine on H4, and lysine on H1. H1 is phosphorylated extensively during the S phase of cell division [39]. From two to four lysine residues may be acetylated in histones H3, H4, H2A, and H2B, as is the amino terminus of H2A, H4, and H1 [39]. A portion of all the histones is methylated at certain lysine residues and probably at the guanidine nitrogen of arginine. Poly(ADP-ribose) has been found covalently linked to H1, and ADP-ribose was present on up to 15% of H2B when nuclei were incubated *in vitro* with ^{14}C-NAD^+ [40, 41]. However, definitive evidence for ADP-ribosylation of histones *in vivo* is lacking [38]. Approximately 10% of calf thymus H2A (and 1% of H2B) is covalently associated at lysine 119 with ubiquitin, a protein of 76 amino acids, resulting in a variant of H2A possessing an electrophoretic mobility equivalent to twice the size of the parent molecule [42–44]. As mentioned earlier, there are at least six H1 variants in addition to H1° and H5, some of which have been identified only by deduction from the nucleotide sequence of cloned genes. The significance of the H1 variants has been the subject of intensive study for years and appears to be related to the role of H1 in affecting the higher order structure of chromatin (see the following section) and consequently the overall transcriptional activity of the genome.

Clearly, very subtle variants as well as highly altered forms of histones have been identified among higher organisms. Even single amino acid differences in histone variants could potentially be recognized immunologically [45], and many of the side chain modifications of histones are biochemically profound. Phosphorylation introduces a charge, lysine acetylation neutralizes a charge, and poly(ADP-ribose) or ubiquitin is obviously a very bulky modification. Autoantibodies to poly(ADP-ribose) [46] and to ubiquitin [47] have been reported, but because these modifications are found on many other proteins, their relationship to antihistone antibodies is unclear. The significance of posttranslationally modified histones as well as of the variants with different amino acid sequences in the elicitation of antihistone antibodies or the antigenicity of the targeted histones is unknown.

Higher Order Structure Comprising the Nucleosome

Histones are not randomly associated with DNA in chromatin but are organized into a well-defined subunit called the nucleosome [48–51]. The nucleosome consists of two molecules of each of the "core" histones, H2A, H2B, H3, and H4, along with one H1 molecule, and DNA of approximately 200 bp in length. DNA of 146-bp-long wraps with 1.75 superhelical turns around an octamer of histones H2A, H2B, H3, and H4, and this core particle is connected to the adjacent core particle by the linker segment of DNA. Histones H1 or H5 appear to bind close to the exposed face of one of the two H2A molecules, creating asymmetry in the nucleosome that provides a ramp where DNA exits and is directed to the adjacent nucleosome [52]. The amount of linker DNA shows wide interspecies and even intertissue variation so that nucleosomes may contain from 166 to 231 bp of DNA. The linear array of nucleosomes held together by a continuous strand of approximately 2×10^7 bp of DNA forms the primary chromatin fiber of approximately 100 Å in diameter. This thin filament has a tendency to supercoil into higher order solenoid-like structures of increasing diameter [53], compacting the DNA 5000- to 10,000-fold as depicted in Fig. 2. In a typical chromosome, approximately 7 m of DNA is condensed to 1 μm by the organizing capacity of about 100,000 nucleosomes.

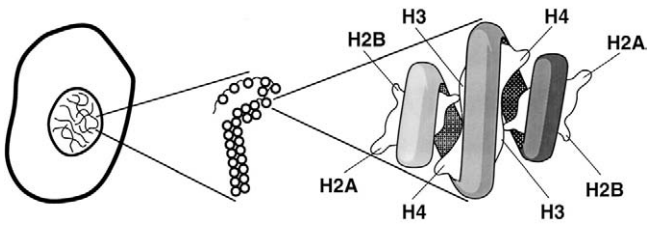


FIGURE 2 Higher ordered structure of histones in chromatin. (Right) A diagrammatic representation of the core particle of the nucleosome in which two turns of DNA wrap around an octamer of histones consisting of two H2A-H2B dimers flanking an H3-H4 tetramer. Separation of dimers from the tetramer is exaggerated for clarity—the native core particle is a compact structure stabilized by noncovalent bonds [54, 55]. Association with H1 completes the nucleosome, and an array of polynucleosomes supercoil into a solenoid-like structure depicted in the middle diagram. Additional supercoiling into even higher ordered structures is presumed to take place until chromatin fibers are eventually visible in the light microscope as mitotic chromosomes.

The natural tendency of histones to interact with each other is of fundamental importance in the generation of the core particle. A tetramer of histones H3 and H4 is stable under physiologic conditions, as is a dimer of H2A and H2B, and the H3–H4 tetramer can be readily separated from the H2A–H2B dimer by ion-exchange chromatography on carboxymethylcellulose [56]. Two H2A–H2B dimers and the H3–H4 tetramer self-assemble to form an octamer, stable at high salt concentration. The octamer stability is due largely to contacts of residues 16–25 and 71–92 of the two H4 molecules in the H3–H4 tetramer with residues 76–97 of H2B in each H2A–H2B complex, but other interactions occur within the octamer, including H3–H2A′, H4–H2A′, H2A–H2A′, and H2B–H2B′ [55]. All the histones participate in limited contacts with DNA at 10-bp intervals whenever the minor grove of the double helix faces the histone core [57–59], resulting in 14 regions on the twisting DNA anchored to arginine side chains of the histones [55]. These multiple interactions account for the stability of the nucleosome and constrain the DNA to curve tightly around the octamer. As discussed later, the discovery of autoantibodies specific to certain histone–histone and histone–DNA complexes suggests that histones in the form of nucleosomes drive these autoimmune responses.

Domain Structure of Histones

Histones in chromatin (but not the individual proteins) show a remarkable resistance to trypsin digestion considering their high content of lysine and arginine, the amino acids immediately N-terminal of the specific cleavage site of trypsin. The trypsin-resistant "limit digest" products can be separated by sodium dodecyl sulfate (SDS) gel electrophoresis, and their compositions are shown in Table 1. Because similar polypeptide products are observed after trypsin digestion of nuclei, chromatin, or core particles but not of histones in low ionic strength environments, resistance is attributed to the protection of potential cleavage sites by the tertiary or quaternary conformation of the histones in the nucleosome. DNA does not afford principal protection of histones from trypsin because the DNA-free octamer in 2 *M* NaCl displays a similar resistance to trypsin digestion. [In fact, the trypsin-sensitive sites are the highly positively charged regions believed previously (incorrectly) to be bound stably to the DNA phosphates: the 11–30 amino acid residues of the N-terminal ends of the four core histones and the C-terminal 11 residues of H2A and six residues of H3.] Biophysical studies on trypsinized core particles indicate that they are largely indistinguishable from their native counterparts [61]. The trypsin-sensitive tails are highly mobile with no stable conformation or binding sites on DNA, although they have been suggested to provide relatively nonspecific electrostatic shielding of DNA phosphates, stabilizing the higher order chromatin structure [62]. Apparently, these domains are accessible to the enzymes involved in histone posttranslational modification because almost all the sites for phosphorylation, acetylation, methylation and ubiquitination are at the trypsin-sensitive regions. It has also been claimed that these domains comprise the predominant epitope for antihistone antibodies in SLE, but as discussed in a later section, this is an oversimplification.

The protruding histone tails may make contact with DNA and/or histone of the adjacent nucleosome, and a candidate site of interaction between a seven acidic residue region on the outward face of the H2A–H2B

TABLE 1 Structural Domains within Histones[a]

Histone	Number of residues in parent molecule	Major trypsin-resistant domain	
		Residue number	Designation
H3	135	27–129	P1
H2A	129	12–118	P2′
H2B	125	21–125	P2
		24–125	P3
H4	102	18–102	P4
		20–102	P5
H1	212–222	36–121	P0 (G-H1)

[a] Compilation is based on Böhm and Crane-Robinson [60] and refers to calf thymus histones. Histones are arranged in order of increasing electrophoretic mobility of the trypsin-resistant domain.

dimer and the amino-terminal tail of H4 from the adjacent nucleosome has been identified [55]. These kinds of interactions may "lock" the DNA into a higher order structure (Fig. 2) that suppresses transcriptional activity. Transcription factors associated with histone acetyltransferase activity can add acetyl groups to the protruding histone tails, disrupting this compact structure, thereby allowing access to transcriptional machinery [63].

All the H1 variants, including H5 and H1°, bear a similar three-domain structure as described previously. The highly conserved central globular domain is involved in the critical function of redirecting the DNA as it exits the core particle, and the flanking regions, which are rich in the basic amino acids arginine and lysine, presumably play a role in internucleosomal interactions involved in chromatin condensation into higher order structures [52]. Histone H5 possesses an unusually large arginine-rich C-terminal domain believed to condense chromatin to the transcriptionally inactive form present in avian erythrocytes. The otherwise closely related H1° retains lysines in the C-terminal domain characteristic of the main H1 family, but is associated with chromatin in terminally differentiated cells, which have only selected genes that are transcriptionally active. The highly conserved 16-mer in the globular domain and octamer in the C-terminal region of all members of the H1 family are presumably involved in critical structural/functional roles. Although autoantibodies to H1 have been frequently reported (see later), the domain structure and the complexity of the H1 family complicate interpretation of these data.

ASSAYS FOR ANTIHISTONE ANTIBODIES

The measurement of antihistone antibody has had a tortuous history. Application of conventional immunoassay techniques such as Ouchterlony immunodiffusion, immunoprecipitation, or Farr ammonium sulfate assay for detection of antihistone antibodies is complicated by the tendency of histones to aggregate and to interact with nonimmune IgG and IgM and other serum proteins such as α_2-macroglobulin [64]. The quality of histones used as antigens is highly variable, and commercial sources are often degraded or contaminated with nonhistone proteins. Inhibition of endogenous proteolysis during histone isolation is necessary to obtain intact histones, as shown in Fig. 1. Complete sets of individual histones or of subnucleosome complexes are not widely available commercially and require a biochemistry laboratory for preparation. It is difficult to remove all traces of DNA, a serious problem when assessing sera that may also contain anti-DNA antibodies. In addition, the propensity for histones to bind DNA in biological fluids can result in artifacts such as false-positive reactions with anti-DNA antibodies (see later).

The first assay for antihistone antibodies was an adaptation of the microcomplement fixation method using lysis of sensitized red blood cells as the indicator [12, 65]. Antihistone antibodies were reported to be infrequent in SLE using this assay [66] and reactive predominantly with histone H1, although a few sera displayed binding to all histones [65]. It was also inferred from these and other studies [13–15, 67] that a complex of histones with DNA was required to generate antigenicity. The stringent requirements of this assay [68], the procomplementary nature of histones [69], and its apparent insensitivity [64] have led to abandoning the complement fixation assay for detecting antihistone antibodies.

An immunofluorescence procedure was modified to render it specific for histone-binding antibodies [70]. Histones can be extracted from mouse kidney tubule nuclei by 0.1 *N* HCl, preventing the binding of antihistone antibodies. The histone content of such nuclei can be partially "reconstituted" by incubation with a histone solution. Sera with antihistone activity display binding to histone-reconstituted nuclei and to untreated mouse kidney but not to acid-extracted sections. This assay is highly specific for antihistone antibodies but cannot be used with sera that contain anti-DNA antibodies, which display background binding to acid-extracted nuclei. Furthermore, sera with antihistone activity predominantly to H3 and H4 displayed negligible reaction in the immunofluorescence assay [71, 72], presumably because of the failure of these histones to bind to DNA with this technique. Thus, the immunofluorescence assay is largely selective for antibodies reactive with H1, H2A, and H2B and may preferentially detect epitopes requiring a histone–DNA complex.

Solid-phase assays have largely replaced conventional assays for antihistone antibodies. Histones, histone complexes, and chromatin adsorb readily to polystyrene, and antibody binding can be quantified with a class-specific enzyme-, fluorescent-, or radiolabeled anti-immunoglobulin. The first solid-phase assays detected antibodies to histones raised in rabbits [73, 74], in human SLE [72, 75, 76], and in murine lupus [77]. Most studies on antihistone antibody in the past decade have relied on purified histones in solid-phase enzyme-linked immunosorbent assays (ELISA) or on immunoblots (Western blots) of histones separated by SDS polyacrylamide gel electrophoresis. Subnucleosome structures have also been adapted to

ELISA formats [78], which allow measurement of autoantibodies requiring these higher ordered structures.

PREVALENCE AND SPECIFICITY OF ANTIHISTONE ANTIBODIES

Overall Disease Association

Reports of antihistone antibodies in various diseases are listed in Table 2. Although antihistone antibodies have been observed in a variety of (generally rheumatologic) diseases, most of the studies have focused on SLE or DIL. Reported prevalences ranged from 17 to 95% in SLE (average = 51%) and 67 to 100% in DIL (average = 92%). Antihistone antibodies have also been consistently observed in rheumatoid arthritis (RA) (average prevalence = 11%) and juvenile rheumatoid arthritis (JRA) (average prevalence = 51%). RA patients with ANA were two to seven times more likely to have antihistone antibodies [110–112] than ANA-negative RA patients. Other than SLE in which other ANA commonly coexist, the bulk of the ANA in sera with antihistone antibodies is probably due to these antibodies, although this relationship has been formally demonstrated only in DIL and drug-induced autoimmunity [133].

Limited studies on antihistone antibodies in various other syndromes have appeared. In some cases, remarkably high prevalences of antihistone antibodies were observed, especially in primary biliary cirrhosis [124, 125], autoimmune hepatitis in all immunoglobulin classes [127], ANA-positive neoplastic diseases [109], RA-related diseases, and patients with undifferentiated connective tissue disease who develop predominately IgM anti-H3 producing a variable large speckled ANA pattern [134, 135]. Antichromatin antibodies were reported in 53% of patients with autoimmune hepatitis type I [136]. In addition to DIL, there have been isolated case reports of unusual antihistone antibodies such as anti-H1° in patients with sensory neuropathy [137, 138] and antibody to chymotrypsin-digested H2B in a patient with vasculitis [139]. However, antihistone antibodies have generally not been observed in signifi-

TABLE 2 Reported Prevalences of Histone-Reactive Antibody in Human Disease

Disease/syndrome	Method[a]	Prevalence[b]
Systemic lupus erythematosus	IF	24% [79], 35% [8], 46% [80]
	RIA	48% [75], 55% [81]
	FIA	83% [82]
	ELISA	8%(M) [83], 17% [84], 21%(G) [85], 27%(G) [83], 28%(M) [86], 36% [87], 42% [88], 43%(G) [89], 43%(M) [90], 44% [76], 45%(G) [91], 50%(G) [92], 52% [93], 52% [94], 55%(G) [90], 57%(G) [95], 57% [96], 60% [97], 61% [98], 68%(M) [16], 74% [99], 77% [100], 80%(A) [86], 84%(G) [86], 8%(G) [16], 95% [101]
	WB	38%(M) [90], 46% [102], 52% [103], 53%(G) [90]
Drug-induced lupus	IF	67% [104], 96% [8]
	RIA	100% [71]
	ELISA	82% [101], 100% [88], 100% [105], 100% [106], 100% [107]
Drug-induced ANA	ELISA	22% [88], 24% [108], 39% [109], 67% [105], 81% [106], 79–95% [107]
Rheumatoid arthritis	IF	8% [109], 14% [110], 24% (ANA+) [110], 36% [80]
(RA)	ELISA	0% [91], 5% [87], 5% [98], 5% [97], 9% [83], 11% [100], 13% [111], 33% (ANA+) [111], 80% (ANA+) [112]
RA-vasculitis	FIA	75% [82]
RA-Felty's	ELISA	79% [111]
Juvenile RA	ELISA	44% [113], 48% [114], 72% [115], 72% [116, 117], 75% [118]
	WB	42% [119], 47%(G) [120]
Inflammatory bowel disease	ELISA	13–16%(G anti-H1) [121]
Neoplastic diseases	ELISA/IF	14% [122], 17–78% (G anti-H2B) [123], 79% (ANA+) [109]
Primary biliary cirrhosis	ELISA	50% [124], 60% [125], 81% [94]
Liver cirrhosis/hepatitis	ELISA	35% [126], 40% [127], 50% [124]
Scleroderma disorders	ELISA	29–44% [128], 42% [129], 47% [130], 62% [131]
Poly/dermatomyositis	ELISA	17% [132]

[a] A, IgA antibodies; ANA, antinuclear antibody; ELISA, enzyme-linked immunosorbent assay; FIA, solid-phase fluorescence immunoassay; G, IgG antibodies; IF, histone reconstituted immunofluorescence assay; M, IgM antibodies; RIA, solid-phase radioimmunoassay; WB, Western (immuno-)blot.

[b] Elevated IgG and/or IgM antibody to total histone or to at least one histone class.

cant prevalences in other rheumatic diseases, including Sjögren's syndrome, progressive systemic sclerosis, and mixed connective tissue disease; a low prevalence of predominately anti-H1 antibodies has been reported in patients with dermatomyositis/polymyositis [132]. However, reports have found antihistone antibodies in a substantial proportion of patients with scleroderma-related disorders [128, 130, 131, 140], especially localized scleroderma with generalized morphea [129, 130]. In addition, anti-H2B antibodies were reported in over half of HIV+ patients with persistent lymphadenopathy [141] or patients with squamous cell carcinoma [123], but these results have not been reproduced. Although approximately 11% of patients with RA have antihistone antibodies, these tend to be low level (low titer). Therefore, most studies have concentrated on antibodies in DIL, drug-induced ANA, and SLE, diseases in which antihistone antibody levels are generally high.

The basis for the discrepancies in the reported prevalences of antihistone antibodies shown in Table 2 is probably multifactorial. Certainly, methodology can affect both sensitivity and specificity of the immunoassay. For example, the immunofluorescence histone reconstitution assay tends to produce false negatives because of its insensitivity to antibodies reacting with histones H3 and/or H4 or with epitopes that are blocked by DNA as discussed previously. Thus, this assay cannot detect some antihistone antibodies in hydralazine-induced lupus, whereas these antibodies can be detected readily in solid-phase and Western blot assays using DNA-free histones [71, 72, 142–144]. Methodological differences among the various studies include the nature of the detecting reagent (polyvalent vs immunoglobulin class-specific vs protein A from *S. aureus*, which selects predominantly IgG1, IgG2, and IgG4), serum dilution, and quality and quantity of the antigen on the solid phase. A collection of normal human sera generally establishes the cut-off value used to assign an experimental sample to a positive category, but borderline values are always difficult to interpret in quantitative immunoassays and these low-level reactivities may sometimes be due to increased nonspecific binding as a result of elevated γ globulins commonly observed in sera from patients with rheumatic diseases. Antibody binding to histones appears to be more prevalent in elderly, healthy people [145], making the need for aged-matched controls important. Finally, ethnic or regional genetic differences in the patient samples, diagnostic criteria for patient selection, or the therapeutic regimen (especially the use of corticosteroids, which may suppress immune responses) can account for discrepancies. Considering these potential variables and excluding the extreme reports, there is fairly good agreement on the prevalence of antihistone antibodies in various human diseases.

TABLE 3 Prevalence of IgG Anti-[(H2A-H2B)–DNA] in Idiopathic and Drug-Induced Lupus

Disease	Prevalence of anti-[(H2A-H2B)–DNA]
SLE, Oriental sample, untreated	70% [16]
SLE, American samples, treated	59% [16], 31% [131]
Procainamide-induced lupus	96% [107, 146]
Lupus induced by penicillamine, isoniazid, acebutalol, methyldopa, timolol, sulfasalazine	Very common[a] [146–151]
Quinidine-induced lupus	53% [107, 146]
Hydralazine-induced lupus	43% [107]

[a] All patients with lupus induced by these drugs had anti-[(H2A-H2B)–DNA] reactivity, but few patients have been tested because lupus induced by these drugs is rare.

In addition to the occurrence of antibodies reactive with isolated histones, patients with lupus-like disorders commonly have autoantibodies to histone–DNA complexes, and the (H2A-H2B)–DNA complex largely accounts for reactivity to these higher order structures in chromatin. Table 3 summarizes this information. Anti-[(H2A-H2B)–DNA] antibodies were found in over two-thirds of untreated (Oriental) patients; a somewhat lower prevalence and titer were observed in (American) SLE patients under medication [16]. Anti-nucleosome (and anti-nDNA) appear to be particularly sensitive to corticosteroid therapy [152]. Most patients with lupus induced by procainamide, penicillamine, isoniazid, acebutolol, methyldopa, timolol, and sulfasalazine also have strong reactivity with the (H2A-H2B)–DNA complex, although a substantial number of patients have only been examined with lupus induced by procainamide. Drug-induced lupus related to quinidine and hydralazine was associated with anti-[(H2A-H2B)–DNA] in 53 and 43%, respectively.

The overall relationships among these histone-dependent antibodies are depicted in Fig. 3 and are detailed further elsewhere [153]. In SLE, antibodies requiring native forms of nucleohistone dominate the autoantibody serology, although antibodies to DNA-free histones (really anti-*denatured* histone antibodies) are commonly present at lower titer. Antichromatin (which is synonymous with antinucleosome) antibodies were found in 72 to 88% of SLE patients from three separate sources [16, 155, 156], although the prevalence of this type of serological abnormality in SLE ranged from 31 to 56% in three other studies [131, 157, 158]. Anti-[(H2A-H2B)–DNA] antibodies are a major component of antichromatin, and antinative (n) DNA antibodies can be considered a subset of the antichromatin response. Drug-induced lupus is more restricted to

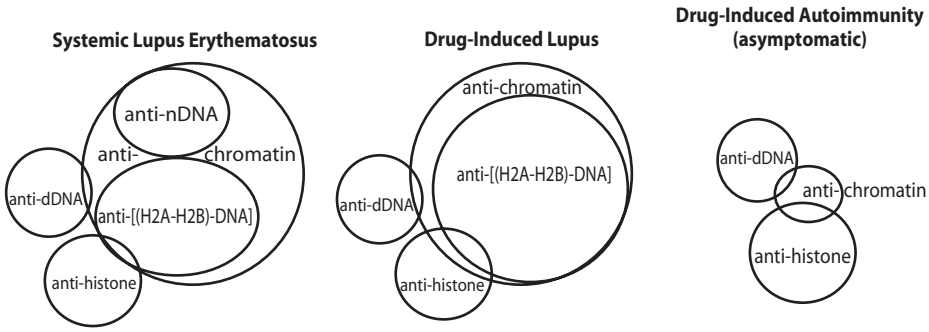


FIGURE 3 Venn diagrams depicting relationships among the major autoantibodies to chromatin components in patients with SLE, DIL, and DIA. Not included are antibodies to high-mobility group proteins, which are present in the three patient groups [91, 154], as well as other important autoantibodies in SLE that react with antigens not stably bound to chromatin. In contrast to epitopes for antinative DNA antibodies (anti-nDNA), epitopes on denatured DNA (dDNA) and some individual histones are probably not expressed on truly native chromatin. However, some of these antibodies may bind chromatin because of localized denatured regions or partial degradation of isolated chromatin.

antichromatin, as anti-nDNA (and other types of autoantibodies) are generally absent. Anti-[(H2A-H2B)–DNA] largely accounts for the antichromatin activity, and, as in SLE, anti-(denatured) histone antibodies are often present at lower titer. In patients who remain asymptomatic during treatment with lupus-inducing drugs, chromatin-reactive antibodies rarely develop and antihistone antibodies account for the bulk of the autoimmune serology. Antibodies to denatured (d) DNA are common in all forms of lupus (and in asymptomatic, drug-treated patients), and these antibodies generally do not bind chromatin unless it contains regions of single-stranded DNA.

Associations with Clinical Disease Activity and Symptoms

Systemic Lupus Erythematosus

Studies on the association of antihistone antibodies with disease activity or severity, with the predominant organ system affected, or with specific clinical features have been inconsistent. Some reports showed no association between the presence or the amount of antihistone antibodies and any measure of disease activity [80, 82, 85, 88, 152, 159] with the exception of a history of photosensitivity in one report [82]. However, in one of the earliest reports on histone-reactive antibodies, Rothfield and Stollar [14] observed that 14 out of 15 patients with active lupus contained antibodies to histone–DNA complexes, whereas only 4 of 26 patients in remission had these antibodies. Subsequently, associations of antihistone antibodies with active disease were observed with solid-phase [93] and histone-reconstituted immunofluorescence assays [109]. The presence of anti-[(H2A-H2B)–DNA] antibodies was significantly correlated with glomerulonephritis [16, 160] and the antihistone antibody level with the renal activity score [95], supporting the older study on association with renal manifestations [14]. Population correlations of the presence of antihistone antibodies with neuropsychiatric involvement [79], skin and joint symptoms [92], or overall disease severity have been reported [86]. However, no quantitative association has been observed between the level of antihistone antibodies and any specific array of clinical symptoms or the overall disease activity. Perhaps variants of SLE characterized by increased disease severity commonly display antihistone antibody because of the general linkage between symptoms and signs in SLE. This view is supported by the association of antihistone or antinucleohistone antibodies with antibodies to native DNA [85, 95, 97, 161], a well-established correlate of disease activity. Taken together, these studies indicate that antihistone antibodies are generally not a useful independent parameter for the clinical form or the activity of disease in SLE, but high levels of antihistone antibodies tend to be linked with active disease.

Drug-Induced Lupus

Rheumatologic symptoms associated with DIL are strongly correlated with the presence of IgG anti-[(H2A-H2B)–DNA] antibodies (Table 3 and Rubin [162]). In contrast, patients remaining asymptomatic for lupus-like disease during treatment with procainamide or chlorpromazine [107] or isoniazid [108] develop antihistone antibodies that are generally inhibited by DNA.

Thus, although antibodies to total histones and to non-native preparations of H2A-H2B do not show a strong correlation with symptomatic drug-induced lupus [106, 163, 164], IgG antibodies to the native H2A-H2B complex [106], especially when bound to DNA [107], are highly correlated with symptomatic disease. In fact, anti-(H2A-H2B) has been observed to precede overt clinical symptoms and therefore may have predictive as well as diagnostic value [165, 166]. Anti-(H2A-H2B) has a sensitivity of 100% and a specificity of >90% for symptomatic procainamide-induced lupus compared to asymptomatic procainamide-treated patients [106] and an even higher specificity when an (H2A-H2B)–DNA complex is used as the screening antigen [107].

Rheumatoid Arthritis

RA patients with antihistone antibodies did not display more active disease than antihistone antibody-negative RA patients [112]. However, in Felty's syndrome, which is considered to be a severe form of RA, antihistone levels were considerably higher than in non-Felty's RA, especially of the IgG isotype [111].

Juvenile Rheumatoid Arthritis

Antihistone antibodies were reported in 93% [114] and 67% [120] of JRA patients with uveitis compared to 33% of patients without uveitis [114]. However, associations with uveitis were not observed in other studies [113, 119, 167], although patients with uveitis (active or inactive) tended to have higher levels of antihistone antibody compared to those JRA patients with no history of uveitis [118]. Antihistone antibodies tended to be more common in pauci- or poly-articular types of JRA compared to systemic onset JRA [119, 120]. These antibodies react preferentially with DNA-free histones [115, 167], similar to those in asymptomatic drug-treated patients, so may not contribute substantially to the ANA commonly occurring in these children.

Isotypes of Histone-Reactive Antibodies

Antihistone antibodies can be found in all the major immunoglobulin classes in patients with SLE, but as discussed in detail [37], there is little agreement as to the predominant isotype. There appears to be a significant correlation between the amount or at least presence of IgG (but not IgM) antihistone antibody and overall clinical disease activity [83, 86], although there was no correlation between isotype and predominant organ system involvement [86]. The isotype profile tended to remain constant over time [81] and for IgG antibodies consisted mainly of IgG1 and IgG3 with notably negligible amounts of the IgG2 antihistone antibody [81, 168]. IgG1 and IgG3 are strong complement-fixing subclasses, implicating these antihistone antibodies in pathogenic processes. A broader distribution of the IgG subclasses was observed in drug-induced autoimmunity in which antihistone activity commonly occurred in all four IgG subclasses [168]. These patients, as well as patients with frank drug-induced lupus, are more likely than SLE patients to have IgG, IgA, and IgM antihistone and anti-[(H2A-H2B)–DNA] antibodies. These autoantibody isotypes often appear to arise simultaneously during procainamide treatment, although patients who remain asymptomatic fail to develop *IgG* anti-[(H2A-H2B)–DNA] [166]. Interestingly, as treatment with the lupus-inducing drug continued, a gradual shift to predominantly IgG1 antihistone antibodies occurred [168], similar to the isotype profile in idiopathic SLE. The substantial IgA autoantibody levels in procainamide [166]- and isoniazid [108]-treated patients is remarkable, suggesting induction of autoantibody synthesis within the gastrointestinal mucosal immune system where drug concentration is presumably highest. The rather slow development of autoantibodies, the apparent concordance of IgG, IgA, and IgM isotypes, and the perpetuation of IgM autoantibodies for many years in asymptomatic, procainamide-treated patients suggest that the mechanism underlying drug induction of autoantibodies is unlike a classical immune phenomenon.

Fine Specificities of Autoantibodies to Individual Histones

Histone heterogeneity and chromatin structure (see the previous section on *histone structure and function*) raise the possibility that useful information may be derived from examining antibody activity to individual histones, domain regions within individual histones, or to subnucleosome particles. Most studies have focused on the five major histone classes, although antibody activity to histone variants such as H1° [137], H5 [120], and ubiquitinated H2A [169] has been reported. The methods employed have been either analytical separation of total histones by gel electrophoresis followed by Western blot or ELISA using biochemically purified histones or histone complexes.

Data on antibody activity to a panel of histones have been reported in various ways. In many studies, antibody binding to each histone is averaged over the entire sample of patients. More informative is the profile of reactivity of individual patients with a panel of histones. The former analysis results in information on the average prevalence or level of antibody activity to the tested histones in a patient sample. In general, SLE

TABLE 4 Antibodies to Histone Classes in Individual Patients[a]

Disease	Method	Immunoglobulin class	Histone class reativity (ranking order)	Ref.
SLE	ELISA	G, M	Variable	75, 90, 99, 170
	ELISA	G	H2B ≥ H2A > H3 = H4	91
	WB	Undefined	H1 = H2B > H4 > H3 > H2A	102, 103
	FIA	Undefined	Variable	82
	WB	G, M	H1 = H2B = H3 > H2A = H4	90, 142
	ELISA and WB	G	Variable H1 = H2B > H2A > H3 > H4	92
DIL-PA	RIA/ELISA	G	H2A-H2B	71, 105, 106
	WB	Undefined	Complex > > H2A = H2B	143
	WB & ELISA	Undefined	H2B > H3 = H1 > H4 = H2A H2A-H2B Complex > H2A = H2B > H4	142
DIL-HY	FIA	Undefined	Variable	144
	WB	Undefined	H3 > H2B = H2A = H1 > H4	143
	WB and ELISA	Undefined	H3 > H4 > > H2B > H2A	142
DIA-PA	ELISA	M	Relatively uniform	105, 170

[a] Abbreviations as in Table 2; DIL-HY, hydralazine-induced lupus; DIL-PA, procainamide-induced lupus; DIA-PA, procainamide-induced ANA in asymptomatic patients.

patients with antihistone antibodies tended to have antibody to all histones, especially H1 and/or H2B [82, 89, 91, 92, 97, 101, 102, 144]. However, there has been only modest agreement within these studies in that anti-H1 [89, 144] or anti-H2B [159] was sometimes observed to be a minor antibody or that other histones, especially H3 [82, 83, 89], were major antigenic targets for antihistone antibodies in SLE sera. Biases in assays, differences in patient population, and small sample size contribute to these discrepancies. Furthermore, patient-to-patient variability is obscured by this type of analysis.

Table 4 summarizes the reported average profiles in individual patients of antibody activities to a panel of histones. The aforementioned predominance of anti-H1 and anti-H2B in SLE can be observed in the patient profiles of only half the reports; the other studies showed no particular profile because of substantial patient-to-patient variability. Many of these studies used a nonclass-specific immunoglobulin-detecting reagent, adding additional ambiguity because the specificity of antihistone antibodies may depend on the immunoglobulin class being examined. Considerable disagreement on the characteristic antihistone antibody profile in DIL is also apparent (discussed in Rubin [37]). With procainamide-induced lupus, a discrete profile of reactivity was only discernible when the dimer of histones H2A and H2B was included as a test antigen, although reactivity with the H2A-H2B complex may also be due to antibody binding to the individual, constituent histones [163, 164].

H2A and H2B

There is good agreement that antibody to H2A and/or H2B in most SLE sera does not bind in Western blot to the large trypsin-resistant domain of these histones (Table 1). Thus, only 15% [142], 8% [101], and 0% [171] of SLE sera with anti-H2B retained activity on the C-terminal H2B polypeptide [21–125), i.e., when the N-terminal 20 amino acids were removed. Direct evidence that the N-terminal region of H2B contained a predominant epitope was obtained by Hardin and Thomas [102], who showed that 63% of sera with anti-H2B reacted with the N-terminal cyanogen bromide polypeptide (1–59), whereas there was no reactivity with the C-terminal (63–125) polypeptide. Studies on monoclonal anti-H2B antibodies from the lupus-prone MRL/*lpr* mouse also demonstrated predominant reactivity with the amino terminus of H2B [172]. Similarly, removal of the N-terminal 11 and C-terminal 11 amino acids from H2A reduced its antigenicity dramatically so only 20% [101] 15% [142], and 11% [171] of anti-H2A in SLE retained activity on the H2A polypeptide (12–118). These studies would suggest that the major epitope on H2B contains peptide (1–20) and on H2A peptide (1–11) and/or (119–129). In fact, in ELISA, H2B (1–25) did have antigenicity for 38% of SLE sera [96]. Gohill *et al.* [101] reported that the H2A 38-mer (91–129) retained good antigenicity for SLE sera having anti-H2A activity, but Muller *et al.* [96] found that the smaller C-terminal H2A peptide (116–129) and N-terminal H2A peptide (1–15) displayed only weak

antibody binding for 13 and 29%, respectively, of SLE sera. There are considerable discrepancies in DIL as to the predominant epitopes within the trypsin-resistant domains, as discussed previously [37]. However, the trypsin-resistant domains of H2A and H2B are clearly important because these regions contain the amino acid residues responsible for H2A complex formation with H2B [60, 173] creating the highly antigenic H2A-H2B dimer [71, 105, 106, 142].

H3 and H4

The effect of trypsin digestion on the antigenicity of both H3 and H4 is similar. In Western blot, the H3-derived P1 domain ("A" band [101, 143, 171]) and the H4-derived P4/P5 domain ("C" band [101, 143, 171]) retained antigenicity for fewer than 10% of SLE and procainamide-induced lupus sera having anti-H3 and/or H4 activity [101, 142, 143, 171]. This would suggest that the major epitope for SLE anti-H3 and anti-H4 antibodies resides in the N-terminal 26 amino acid and/or C-terminal six amino acids of H3 and the N-terminal 17 amino acids of H4, assuming no tertiary interaction between these and other regions of the molecules, as would be expected [60, 173]. In fact, in ELISA, H3 peptide (1–21) and (130–135) bound 76 and 39% of SLE sera, respectively, and 54% of SLE sera bound H4 (1–29) [96]. However, the H3 peptide (40–45) was also antigenic [96], and absorption experiments showing that these activities were related to antibody to the parent molecule were not reported.

In contrast to SLE and procainamide-induced lupus, 80% [143] or 100% [142] of hydralazine-induced lupus sera reacted with the trypsin-resistant domains of H3 and H4 to an extent similar to that of the parent histones. The dichotomy between the reactivity of antibodies in hydralazine-induced lupus with those in procainamide-induced lupus and SLE is also manifested on subnucleosome particles (see the preceding section on *overall disease association*) and would suggest a difference in the immunogenic stimulus driving the autoimmune responses in these disease groups.

H1

Chymotrypsin cleaves accessible peptide bonds on the C-terminal side of aromatic amino acids, and brief digestion splits H1 approximately in the middle of the central, globular domain at phenylalanine 106. In Western blot the C-terminal polypeptide (107–220) retained full antigenicity for 100% [92, 98, 174] or 86% [102] of SLE and 100% of procainamide-induced lupus sera [174] having anti-H1 antibodies (both IgM [174], and IgG [98, 143, 174]). In contrast, the N-terminal half (1–106) displayed lower antigenicity in most (but not all [92]) studies in showing reaction with only 28% [98], 16% [174], and 14% [102] of SLE sera with anti-H1 activity. When Gohill and Fritzler [174] tested the antigenicity of the globular domain (36–121) (generated by trypsin digestion of H1) using ELISA, no SLE or procainamide-induced lupus serum reacted. These results suggested that the bulk of H1 antigenicity lies in the C-terminal hydrophilic domain. In a study using synthetic 15-mer peptides, Stemmer *et al*. [175] observed definite skewing of reactivity of SLE sera with C-terminal peptides of human H1b, although N-terminal and globular domain peptides were occasionally antigenic as well. Nevertheless, these data suggest that the apparent lower antigenicity of the N-terminal domain reported using bovine [92, 98, 174] or chicken [102] H1 fragments applies to human H1 as well, presumably the bona fide immunogen driving the autoimmune response. The highly conserved octapeptide in the C-terminal region (see earlier section on *primary structure and histone variants*) might be a candidate epitopic region that would cross species barriers and is included in the second most antigenic peptide in H1b [175]. It is unlikely, however, that there is an immunodominate epitope in H1, consistent with the heterogeneity of anti-H1 antibodies in murine lupus [176]. In addition, SLE sera appear to have substantial antibody heterogeneity within the H1 variants; of nine SLE sera examined, H1.5 was recognized by all nine patients, but from none to all of the five other H1 variants showed antigenicity by Western blot [177].

Antibodies to H1 from JRA sera have also received considerable attention. Of particular interest was the report by Pauls *et al*. [120] that a small proportion (5 of 51) of JRA patients had antibody activity to chicken histone H5 without concomitant reactivity with the major H1 class. A similar preferential reactivity with H5 compared to H1 with SLE sera was observed by Stemmer *et al*. [175]. Because H5 is not present in human tissues, it was suggested that the anti-H5 immune response was driven by an unknown non-histone cross-reacting antigen. However, as discussed in the section on *primary structure and histone variants*, H5 has extensive homology with H1°, the variant found in terminally differentiated cells. In fact, Monestier *et al*. [118] showed that JRA sera with anti-H5 had even higher reactivity with human brain H1°. Antibody binding to H1° from human brain without binding to the major H1 fraction was also detected in a patient with sensory neuropathy [137]. There are seven sequences in human H1° not present in H1 that are nearly identical to sequences in chicken H5, which are distributed mainly in the globular and N-terminal region [31], possibly accounting for cross-reaction between H1° and

H5. In fact, epitope mapping demonstrated that peptides within the globular domain of H5 had the highest antigenicity in SLE and procainamide-induced lupus [178]. Antibodies in JRA displayed poor binding to the central globular domain of H1 whether or not the amino terminal segment (1–32) was also present, whereas the highest reactivity was observed on the C-terminal segment containing the globular domain (33–220) [118]. This reactivity appears to be similar to that of SLE sera, but coblocking studies between IgM antibodies in JRA and IgG antibodies in SLE have not been performed.

Significance of Antihistone Antibody Fine Specificity

The specificity of histone-reactive antibodies in SLE and in lupus induced by most drugs is consistent with some form of chromatin being the predominant *in vivo* immunogen driving these B-cell responses. Antibodies in SLE and DIL that react with intact histones but not with histones from trypsinized chromatin can be explained most readily as having been elicited by chromatin rather than by free histones because the trypsin-resistant domain structure of histones is preserved in the form of chromatin and nucleosomes but not in individual histones. However, subnucleosome particles and histones in the form of the DNA-free H2A-H2B dimer and the H3-H4 tetramer are also resistant to trypsin digestion. Furthermore, the argument that chromatin drives the autoantibody response is weakened by observations that antibodies reacting with the trypsin-resistant cores of H2A and/or H2B also occur in some SLE sera and were the predominant activity in procainamide-induced lupus [107, 142]. This discrepancy is especially pronounced with antibodies in hydralazine-induced lupus, which retain reactivity with histones after trypsinization of chromatin [142, 143]. IgM antibodies from patients taking hydralazine or chlorpromazine [107], as well as IgG antihistone antibodies from patients with JRA [115], bound strongest to DNA-free histones, less to subnucleosome structures, and very little or not at all with chromatin. Thus, immune tolerance to more native forms of nucleohistone is largely preserved in JRA and with antibodies induced by chlorpromazine, isoniazid, and IgM antihistone antibodies in hydralazine-induced lupus. This is the same pattern of antibody binding found when normal mice were immunized with histones [179] and is more consistent with some unknown form of DNA-free histone driving the immune response to histones in these patients.

A more convincing argument that chromatin drives the bulk of the histone-reactive antibody response in SLE and most DIL can be made from data that compare the antigenicity of various forms of histones. As discussed earlier, antibodies from patients with SLE and lupus induced by 10 drugs (**Table 3**), as well as antibodies from murine lupus, bound prominently to a structural epitope in the (H2A-H2B)–DNA complex. Some patients with SLE also bound native DNA and the $(H3\text{-}H4)_2$–DNA subnucleosome, but reactivity with individual histones was much lower [16, 152, 157]. In murine lupus, antibodies to (H2A-H2B)–DNA were found early in disease, before antibodies to native DNA and $(H3\text{-}H4)_2$–DNA arose [180]. Absorption with chromatin removed most of the antibody reactivity to subnucleosome structures, indicating that regions buried in chromatin were not antigenic in SLE, DIL, or murine lupus. Thus, histone-reactive antibodies in SLE can be explained most readily by a autoimmunization with chromatin accompanied by a sequential loss of tolerance first to the (H2A-H2B)–DNA region and then to $(H3\text{-}H4)_2$–DNA and native DNA. Loss of immune tolerance to epitopes on DNA-free histones, which can be considered "denatured histones," and to denatured DNA (and to other nuclear antigens) may accompany the immune disregulation associated with lupus-related disorders, but because of the complexity of these epitopes and the heterogeneity of this immune response, only with nucleosome-reactive antibodies can a strong case be made for the putative *in vivo* existence of a chromatin-like immunogen.

Artifactual Antihistone Antibodies

Special problems arise when measuring antihistone antibodies in complex biological fluids. Three features of this antigen–antibody system contribute to ambiguous results: (1) histones have a net positive charge and readily bind soluble polyanions such as DNA in physiological medium or sulfated macromolecules on membranes, (2) DNA and/or histones are not uncommon contaminants of antibody-containing fluids or of other components of the assay, and (3) anti-DNA and antihistone activities commonly coexist in SLE sera. Soluble DNA in human serum can be responsible for false-positive antihistone antibody signals by forming a macromolecular bridge between anti-DNA antibodies and histone bound to the solid phase. These interactions result in anti-DNA antibody binding to histones, indistinguishable from bona fide antihistone antibody activity (see the following section on *antilymphocyte antibodies*). This phenomenon can be clearly seen when harvesting tissue culture supernatants from a hybridoma secreting anti-DNA antibodies [181]. DNase treatment of the antibody preparation removes the antihistone activity, consistent with the involvement of DNA in the generation of artifactual antihistone activity.

Similar artifacts can occur in serum. Subiza *et al*. [182] demonstrated that DNase pretreatment of SLE sera resulted in a significant decrease in antihistone antibody activity in 7/11 sera. Affected sera invariably had anti-DNA antibodies (whose activity increased after DNase digestion) and the histone-binding activity could be regenerated by the addition of DNA. These results would suggest that a significant portion of the antihistone activity in SLE might actually be due to DNA–anti-DNA immune complexes. However, Suzuki *et al*. [90] observed a DNase effect on the antihistone activity in only 27% of SLE sera; this decrease was relatively small and uniform across all histone classes. Therefore, it appears that measurement of antihistone activity in well-prepared samples of SLE sera gives largely valid results even in the presence of anti-DNA antibodies.

Nucleohistone in bovine milk (a common blocking medium) or serum can bind to histone bands transferred to nitrocellulose, introducing artifacts [183]. Histones were isolated from bovine serum and milk by affinity chromatography on DNA-cellulose [184], and this material mediated the binding of antihistone antibodies to solid-phase DNA [181]. Indirect evidence strongly suggests that DNA exists in serum in the form of mono- and oligonucleosomes [185, 186], which can be immunoprecipitated with antihistone antibodies [186]. Therefore, blocking media from these natural biological fluids should be avoided when measuring antihistone antibodies. This type of phenomenon may also have pathologic significance, as suggested by the report of Schmiedke *et al*. [187] that circulating nucleohistone binding to the negatively charged residues on heparin sulfate in the glomerular basement membrane (GBM) may mediate the binding of DNA and anti-DNA antibodies to the glomerulus.

Cross-Reactions of Antihistone Antibodies

Rheumatoid Factors (RF)

Polyclonal IgG RF with ANA activity were first clearly demonstrated by Hannestad and Johanessen [188], and an IgM RF with the capacity to bind nucleosomes containing core histones was isolated from another RA patient [189]. Several subsequent studies verified and extended these findings [110, 190–194], suggesting that RF with histone-binding activity was quite common. Trivial explanations such as contaminating immune complexes were ruled out by the discovery of monoclonal RF produced in tissue culture with similar properties [195]. Although the structural basis for these cross-reactions is unclear, it is possible that the combining site of an antibody is considerably larger than that needed to accommodate a single epitope, permitting distinct regions within the Fab part of an antibody molecule to react with unrelated epitopes.

Antilymphocyte Antibodies

Of 27 LE factor-positive sera, 26 contained ANA that bound to and could be eluted from viable leukocytes [196]. These "X-ANA" reacted with nucleosomes [197], and absorption experiments indicated that ANA, LE factor, and antilymphocyte activities were properties of the same antibody population [18]. In addition to human leukocytes, LE factor also bound to mouse and rabbit splenocytes, rat hepatocytes, and human endothelial cells but not human or chicken erythrocytes [198, 199]. X-ANA behaved as a subset of the cold reactive antilymphocyte antibody repertoire [198]. The related capacity of anti-DNA antibodies to bind a "lupus-associated membrane protein" (LAMP) has been shown to be mediated by DNA or nucleohistone debris contaminating the anti-DNA preparations [200, 201], although apparently DNA-independent anti-DNA antibody binding to cell membranes has been described [202]. The capacity of mononuclear cells or granulocytes to bind X-ANA was not mediated by the Fc region of IgG or by the Fc or C3 receptors on leukocytes, and, in contrast to anti-LAMP antibodies, nuclei derived from dead cells appeared not to be responsible for the capacity of leukocytes to immunoadsorb X-ANA [197, 203, 204]. The cell membrane antigen is similar to an epitope in the core histone octamer (in the presence or absence of DNA) but not in nucleosomes depleted of H2A and H2B [196], and solid-phase H2B or H2B peptide (6–18) reacted with antibody affinity purified on leukocytes [205]. A peptide derived from H2B also appears to be present on murine B cells [206]. However, studies with a panel of histones, as well as antihistone and anti-DNA antibodies, failed to detect other chromatin-related antigens on cell membranes [207]. In addition, Holers and Kotzin [208] detected antihistone antibody binding to mononuclear cells only after overnight culture at 37°C. Apparently activation of monocytes by endotoxin in the medium induces expression of a nucleohistone receptor that binds chromatin debris released into the medium [209]. This receptor may be a 94-kDa protein in a cell membrane extract, which bound DNA and nucleohistone [200], a 30-kDa protein, which is a component of a DNA receptor on mononuclear cells [210, 211], or a 50-kDa protein that acts as a nucleosome receptor on a fibroblast cell line [212]. However, there is no evidence that SLE patients, in which anti-H2B and/or anti-DNA antibodies are commonly observed, display membrane-associated antibody on circulating mononuclear cells *in vivo*.

MURINE EXPERIMENTAL MODELS WITH ANTIHISTONE ANTIBODIES

Strains of mice in which serum antihistone antibodies appear spontaneously during their natural life history include (NZB × NZW)F1 [77, 176, 213, 213–215], MRL-*lpr/lpr* [87, 176, 180, 214–217], MRL-+/+ [215, 216, 218], BXSB [180], (SWR × NZB)F1 [219, 220] Palmerston North [87], Swan [87], NZB [176, 216, 217], ddY [221], and (C57Bl/6 × DBA/2)F1 mice undergoing chronic graft-vs-host (GVH) disease after injection of DBA/2 T cells [179, 214, 222–226]. As with most human SLE and some DIL, predominant reactivity with linear epitopes in the trypsin-sensitive region of the core histones was also observed with spontaneously arising antibodies in murine lupus [213, 224]. Many monoclonal histone-reactive antibodies have been derived by the hybridoma technique from autoimmune mice [181, 218, 223, 227, 228] and subjected to comparative sequence analyses [217, 218, 220, 229, 230]. Monoclonal antibodies to (H2A-H2B)-DNA obtained from MRL-+/+ mice were clonally related and possessed numerous charged residues in the heavy chain as a result of somatic mutations and various $V_H DJ_H$ rearrangement processes [218, 229], indicating antigen selection rather than polyclonal activation. However, the replacement/silent mutation ratios even in the complementarity-determining regions were much lower than the ratio expected for random mutations [229], suggesting that once B cells with sufficient avidity for chromatin arise, something else, presumably T-cell help, causes their expansion. This view is supported by the detailed sequence comparisons of anti-(H2A-H2B) antibodies derived from an (SWR × NZB)F1 [220] and an (NZB × NZW)F1 [231] mouse; these antibodies showed very few somatic mutations, suggesting that chromatin-reactive T cells selected a single precursor of these B cells for expansion at an early stage in their development and limited further V region mutations to an extent corresponding to only 1 week of antigen-driven autoimmunization.

When the kinetics of autoantibody appearance in the lupus-prone MRL-*lpr/lpr* and BXSB mouse were examined over short time intervals, the earliest autoantibodies detected reacted with native chromatin rather than its constituents such as histones and DNA [180, 232]. Anti-[(H2A-H2B)–DNA] accounted for the bulk of the early antichromatin activity [180]. This serology was similar to that observed in 14 out of 40 newly diagnosed SLE patients, and the mouse and human sera efficiently coblocked, indicating a similar target epitope [16]. Another 14 SLE sera had elevated reactivity to multiple antigens in chromatin, including (H2A-H2B)-DNA, native DNA, H3-H4 tetramer, and various individual histones [16]. This serology, in turn, was very much like that in older lupus mice, which had rapidly developed antibodies to multiple epitopes on chromatin [180]. These data suggests that, as in DIL, the autoimmune response in human and murine SLE is initially directed to the (H2A-H2B)–DNA component of chromatin, but unlike DIL spreads to other regions in chromatin (and to other nuclear antigens).

HISTONE-REACTIVE T CELLS

Lymphocytes from 54% of SLE patients showed a proliferative response to nucleosomes *in vitro* [158]. Chromatin-specific T cells were isolated from mouse [219] and human [233] SLE, and histone-reactive T-cell lines were obtained from SLE and a normal individual [234]. T-cell clones derived from lupus-prone (SWR × NZB)F1 mice displayed proliferative and cytokine responses to nucleosomes but not their component macromolecules. Of the clones that could be mapped, five reacted with peptides corresponding to H4 (13–39) and/or H4 (73–90) and one with H2B (10–33) [235], and their specificity seemed to be conferred primarily by the α chain of the T-cell receptor [236]. Interestingly, these T-cell epitopes were similar to those commonly recognized by T cells from SLE patients; human lupus T cells also recognized H3 (91–105 or 100–114), H2A (34–48), and H4 (49–63) [237]. Nucleosome-reactive clones provided helper activity to primary B cells for secretion of IgG anti-"histone/DNA" *in vitro* [235]. A controlling role for such chromatin-specific T cells in the humoral response of BXSB mice was the demonstration that injection of chromatin *in vivo* directly into thymic lobes delayed the appearance of antichromatin and anti-DNA antibodies, probably due to the enhanced deletion (negative selection) of autoreactive T cells [238]. Histone-reactive T-cell lines have been developed from normal individuals after stimulation with nucleosome–polyomavirus T antigen complexes [239, 240], demonstrating that quiescent (anergic) histone-reactive T cells may be part of the normal T-cell repertoire.

The notion that the antichromatin response is limited by the availability of T_H cells is supported by immunization studies. Normal mouse strains generally fail to produce autoantibodies reactive with native self-antigens using various immunization protocols [241]. Thus, host B cells from (C57BL/6 × DBA/2)F1 mice produce anti-[(H2A-H2B)–DNA] antibodies after *in vivo* transfer of DBA/2 T cells due to the GVH reaction [222, 242], but only antibodies to nonative regions in chromatin can be detected after immunization of this strain with various chromatin-related antigens [179]. These results are consistent with the findings that immunoglobulin receptors for (H2A-H2B)–DNA are

derived by limited somatic mutations of the normal B-cell repertoire [218, 220, 229], but these cells or their precursors remain quiescent until appropriate T-cell help becomes available. Apparently, DBA/2 T cells provide this help in the GVH model, but direct immunization does not break T-cell tolerance to chromatin. However, when a reactive metabolite of the lupus-inducing drug procainamide was injected into the thymus of (C57BL/6 × DBA/2)F1 mice, anti-[(H2A-H2B)–DNA] antibodies arose [243], consistent with the view that chromatin-reactive T cells, created by disruption of central T-cell tolerance, have sufficient helper capacity to drive precursors of [(H2A-H2B)–DNA]-specific B cells to somatically mutate, expand, and secrete autoantibodies.

PATHOGENIC POTENTIAL OF ANTIHISTONE ANTIBODIES

The pathogenic mechanisms in SLE and DIL have not been clearly elucidated, but there is a general consensus that complexes between autoantibody and its cognate antigen play at least a contributing role. Anti-nDNA antibodies have a long history of being implicated in glomerulonephritis in murine and human SLE [244], but serum anti-nDNA is not closely correlated with renal disease (reviewed in [153, 245]), and anti-[(H2A-H2B)-DNA] activity was a better statistical correlate of lupus nephritis than anti-nDNA [16]. The presence of antichromatin was also strongly linked to early death in the progeny of (NZB × NZW)F2 intercross mice [246], and IgG antichromatin antibodies showed a strong statistical correlation with the SLEDAI score of disease activity in SLE [161]. Although one study found that anti-DNA antibodies correlated better with lupus nephritis than anti-[(H2A-H2B)-DNA] activity [156], the predominance of antinucleosome antibodies and their correlation with active lupus nephritis were demonstrated in large samples of SLE patients from France and Brazil [157]. IgG3 antinucleosome antibodies were found to be an overrepresented subclass in SLE patients with renal disease, further implicating immune complex-mediated pathology involving this strong complement-fixing isotype [155].

A pathogenic role for chromatin-reactive antibodies in lupus nephritis, but not antibodies to (denatured) histones [247], is suggested by studies in experimental animal models of nephritis and *in vitro* assays for glomerular-binding immunoglobulin. Antinucleosome–nucleosome immune complexes bound to the glomerulus *in vivo* and to the GBM *in vitro* [248], and immunoglobulin eluted from glomeruli of MRL/*lpr* mice reacted most strongly with histones in the nucleosome core particle [249]. However, in SLE, circulating, preformed immune complexes appear to be of minor importance [250]. Although serum from 81% of SLE patients with nephritis showed elevated IgG binding to a preparation of basement membranes isolated from human glomeruli in an ELISA format [250], GBM-binding activity was decreased substantially by DNase treatment of the GBM (but not the sera), and nucleosome preparations were especially potent in restoring antibody binding to GBM. These and other data [187, 248, 251–255] demonstrate that chromatin or nucleosome core particles readily adhere to type IV collagen and/or heparin sulfate in the GBM (but less so to other types of basement membranes), providing an *in situ* surface for binding chromatin-reactive antibodies. Approximately 20% of SLE patients have increased levels of circulating nucleosomal material, although plasma chromatin did not correlate with disease activity or SLEDAI score [161].

Of SLE patients with active nephritis, those with the highest GBM-associated immunoglobulin showed more severe clinical disease, especially hypocomplementemia and poorer prognosis than SLE patients with low GBM-binding activity [256]. Although there are some data to the contrary [80, 257], complement proteins C3, C4, and properdin deposited on nuclei of HEp-2 cells preincubated with histone-reactive antibodies [258], consistent with the finding that IgG1 and IgG3, potent complement-fixing immunoglobulins, are the predominant isotypes of these antibodies in SLE and DIL [155, 168]. In fact, depressed complement levels have been detected in patients with lupus induced by procainamide [259] and hydralazine [260], and a prospective study of a patient with procainamide-induced lupus demonstrated elevated C4d/C4 ratios during symptomatic disease [165], indicating activation of the classical pathway *in vivo*. It is highly likely, therefore, that complement activation and its accompanying inflammatory mediators are a consequence of the appearance of histone-reactive antibodies in SLE and DIL.

It could be envisioned that anti-nDNA, anti-[(H2A-H2B)–DNA], and other chromatin-reactive antibodies cause nephritis by binding to nucleosomal material that deposited in the glomerulus from the circulation [186]. However, sera from a substantial proportion of SLE patients without nephritis also showed a capacity to bind glomerular structures [256] or GBM [250] *in vitro*. Furthermore, sera from patients with DIL displayed a range of *in vitro* GBM-binding capacity similar to that of SLE sera [250]. As SLE but not DIL patients develop glomerulonephritis, anti-[(H2A-H2B)–DNA] antibodies cannot be sufficient to cause this pathology.

Collectively, these observations suggest that kidney damage in SLE requires not only chromatin-reactive

antibodies (including anti-DNA) but also at least another abnormality, such as a compromised capacity to clear immune complexes after chromatin followed by antichromatin antibodies are deposited in the kidney. Patients with drug-induced anti-[(H2A-H2B)–DNA] antibodies presumably have adequate capacity to remove immune complexes involving these antibodies from the kidney, but the systemic symptoms of DIL [162] may be a consequence of the operation of the inflammatory machinery involved in clearance of these immune complexes. The strong correlation between anti-[(H2A-H2B)–DNA] antibodies and both nephritis in SLE and rheumatologic symptoms in DIL suggests that there is a general intolerance of these antibodies, contributing to symptomatic disease.

References

1. Hargraves, M. M., Richmond, H., and Morton, R. (1948). Presentation of two bone marrow elements: The "Tart" cell and the "L.E." cell. *Proc. Staff Mtg. Mayo Clinic* **23**, 25–28.
2. Hamburger, R. N. (1950). Induction of the lupus erythematosus ("L.E.") cell *in vitro* in peripheral blood. *Yale J. Biol. Med.* **22**, 407–410.
3. Miescher, P., and Fauconnet, M. (1954). L'absorbtion du facteur "L.E." par des noyaux cellulaires isolés. *Experientia* **10**, 252.
4. Holman, H., and Deicher, H. R. (1959). The reaction of the lupus erythematosus (L.E.) cell factor with deoxyribonucleoprotein of the cell nucleus. *J. Clin. Invest.* **38**, 2059–2072.
5. Robbins, W. C., Holman, H. R., Deicher, H., and Kunkel, H. G. (1957). Complement fixation with cell nuclei and DNA in lupus erythematosus. *Proc. Soc. Exp. Biol. Med.* **96**, 575.
6. Friou, G. J. (1958). Identification of the nuclear component of the interaction of lupus erythematosus globulin and nuclei. *J. Immunol.* **80**, 476–481.
7. Vivino, F. B., and Schumacher, H. R. J. (1989). Synovial fluid characteristics and the lupus erythematosus cell phenomenon in drug-induced lupus. *Arthritis Rheum.* **32**, 560–568.
8. Fritzler, M. J., and Tan, E. M. (1978). Antibodies to histones in drug-induced and idiopathic lupus erythematosus. *J. Clin. Invest.* **62**, 560–567.
9. Holborow, E. J., and Weir, D. M. (1959). Histone: An essential component for the lupus erythematosus antinuclear reaction. *Lancet* **1**, 809.
10. Aisenberg, A. C. (1959). Studies on the mechanism of the lupus erythematosus (L.E.) phenomenon. *J. Clin. Invest.* **38**, 325.
11. Holman, H. R., Deicher, H. R. G., and Kunkel, H. G. (1959). The L.E. cell and the L.E. serum factors. *Bull. N. Y. Acad. Med.* **35**, 409–418.
12. Kunkel, H. G., Holman, H. R., and Deicher, H. R. G. (1960). Multiple "autoantibodies" to cell constituents in systemic lupus erythematosus. *In* "Ciba Foundation Symposium on Cellular Aspects of Immunity," pp. 429–437. Ciba, Basel.
13. Tan, E. M. (1967). Relationship of nuclear staining patterns with precipitating antibodies in systemic lupus erythematosus. *J. Lab. Clin. Med.* **70**, 800.
14. Rothfield, N. F., and Stollar, B. D. (1967). The relation of immunoglobulin class, pattern of antinuclear antibody, and complement-fixing antibodies to DNA in sera from patients with systemic lupus erythematosus. *J. Clin. Invest.* **46**, 1785.
15. Robitaille, P., and Tan, E. M. (1973). Relationship between deoxyribonucleoprotein and deoxyribonucleic acid antibodies in systemic lupus erythematosus. *J. Clin. Invest.* **52**, 316–323.
16. Burlingame, R. W., Boey, M. L., Starkebaum, G., and Rubin, R. L. (1994). The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J. Clin. Invest.* **94**, 184–192.
17. Hannestad, K., Rekvig, O. P., and Husebekk, A. (1981). Cross-reacting rheumatoid factors and lupus erythematosus (LE)-factors. *Springer Semin. Immunopathol.* **4**, 133.
18. Rekvig, O. P., and Hannestad, K. (1981). Lupus erythematosus (LE) factors recognize both nucleosomes and viable human leukocytes. *Scand. J. Immunol.* **13**, 597.
19. Schett, G., Rubin, R. L., Steiner, G., Hiesberger, H., Muller, S., and Smolen, J. (2000). The lupus erythematosus cell phenomenon: Comparative analysis of antichromatin antibody specificity in lupus erythematosus cell-positive and -negative sera. *Arthritis Rheum.* **43**, 420–428.
20. Bohm, E. L., Strickland, W. N., Strickland, M., Theraits, B. H., Von der Westhuizen, D. R., and Von Holt, C. (1973). Purification of the five main calf thymus histone fractions by gel exclusion chromatography. *FEBS Lett.* **34**, 217–221.
21. Panyim, S., and Chalkley, R. (1969). High resolution acrylamide gel electrophoresis of histones. *Arch. Biochem. Biophys.* **130**, 337–346.
22. Isenberg, I. (1979). Histones. *Annu. Rev. Biochem.* **48**, 159.
23. Von Holt, C., Strickland, W. N., Brandt, W. F., and Strickland, M. S. (1979). More histone structures. *FEBS Lett.* **100**, 201.
24. Isenberg, I. (1978). Protein-protein interactions of histones. *In* "The Cell Nucleus" (H. Busch, ed.), p. 135. Academic Press, New York.
25. Hayashi, T., Ohe, Y., Hayashi, H., and Iwai, K. (1982). Human spleen histone H4. Isolation and amino acid sequence. *J. Biochem.* **92(6)**, 1995–2000.
26. Ohe, Y., and Iwai, K. (1981). Human spleen histone H3: Isolation and amino acid sequence. *J. Biochem.* **90**, 1205.
27. Ohe, Y., Hayashi, H., and Iwai, K. (1979). Human spleen histone H2B. *J. Biochem.* **85**, 615.
28. Hayashi, T., Ohe, Y., Hayashi, H., and Iwai, K. (1980). Human spleen histone H2A. *J. Biochem.* **88**, 27.
29. Carozzi, N., Marashi, F., Plumb, M., Zimmerman, S., and Zimmerman, A. (1984). Clustering of human H1 and core histone genes. *Science* **224**, 1115–1117.

30. Ohe, Y., Hayashi, H., and Iwai, K. (1986). Human spleen histone H1: Isolation and amino acid sequence of a main variant, H1b. *J. Biochem.* **100(2)**, 359–368.
31. Doenecke, D., and Tönjes, R. (1986). Differential distribution of lysine and arginine residues in the closely related histones H1 and H5. *J. Mol. Biol.* **187**, 461–464.
32. Ohe, Y., Hayashi, H., and Iwai, K. (1989). Human spleen histone H1: Isolation and amino acid sequences of three minor variants, H1a, H1c, and H1d. *J. Biochem.* **106(5)**, 844–857.
33. Eick, S., Nicolai, M., Mumberg, D., and Doenecke, D. (1989). Human H1 histones: Conserved and varied sequence elements in two H1 subtype genes. *Eur. J. Cell Biol.* **49**, 110–115.
34. Coles, L. S., Robins, A. J., Madley, L. K., and Wells, J. R. E. (1987). Characterization of the chicken histone H1 gene complement. *J. Biol. Chem.* **262**, 9656–9663.
35. Delange, R. J., and Smith, E. L. (1975). Histone function and evolution as viewed by sequence studies. *In* "The Structure and Function of Chromatin: Ciba Foundation Symposium 28" (E. M. Bradbury, ed.), pp. 59–76. American Elsevier, New York.
36. Wuilmart, C., and Wyns, L. (1977). An evolutionary scheme for the histones as derived from a study of internal repetitions and homologies among the different classes. *J. Theor. Biol.* **65**, 231–252.
37. Rubin, R. L. (1992). Antihistone antibodies. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), pp. 247–271. Churchill Livingstone, NY.
38. Wu, R. S., Panusz, H. T., Hatch, C. L., and Bonner, W. M. (1986). Histones and their modifications. *Crit. Rev. Biochem.* **20**, 201–263.
39. Dixon, G. H., Candido, E. P. M., and Honda, B. M. (1975). The biological roles of post-synthetic modifications of basic nuclear proteins. *In* "The Structure and Function of Chromatin: Ciba Foundation Symposium 28" (E. M. Bradbury, ed.), pp. 229–258. Elsevier, New York.
40. Hayashi, O., and Veda, K. (1977). Poly (ADP-Ribose) and ADP-ribosylation of proteins. *Annu. Rev. Biochem.* **46**, 95.
41. Burzio, L. O., Riquelme, P. T., and Koide, S. S. (1979). ADP ribosylation of rat liver nucleosomal core histones. *J. Biol. Chem.* **254**, 3029.
42. Goldknopf, I. L., and Busch, H. (1977). Isopeptide linkage between nonhistone and histone 2A polypeptides of chromosomal conjugate-protein A24. *Proc. Natl. Acad. Sci. USA* **74**, 864.
43. Hunt, L. T., and Dayhoff, M. O. (1977). Amino terminal sequence identity of ubiquitin and the nonhistone component of A24. *Biochem. Biophys. Res. Commun.* **74**, 650.
44. Busch, H., and Goldknopf, I. L. (1981). Ubiquitin—protein conjugates. *Mol. Cell. Biochem.* **40**, 173–187.
45. Benezra, R., Blankstein, L. A., Stollar, B. D., and Levy, S. B. (1981). Immunological and organizational heterogeneity of histone H2a variants within chromatin of cells at different stages of Friend leukemia. *J. Biol. Chem.* **256**, 6837.
46. Okolie, E. E., and Shall, S. (1979). The significance of antibodies to poly(adenosine diphosphate-ribose) in systemic lupus erythematosus. *Clin. Exp. Immunol.* **36**, 151–164.
47. Muller, S., Briand, J.-P., and Van Regenmortel, M. H. V. (1988). Presence of antibodies to ubiquitin during autoimmune response associated with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **85**, 8176–8180.
48. Kornberg, R. D. (1977). Structure of chromatin. *Annu. Rev. Biochem.* **46**, 931.
49. Igo-Kemenes, T., Horz, W., and Zachau, H. G. (1982). Chromatin. *Annu. Rev. Biochem.* **51**, 89–121.
50. Allan, J., Hartman, P. G., Crane-Robinson, C., and Aviles, F. X. (1980). The structure of histone H1 and its location in chromatin. *Nature* **288**, 675–679.
51. Pederson, D. S., Thoma, F., and Simpson, R. T. (1986). Core particle, fiber, and transcriptionally active chromatin structure. *Annu. Rev. Cell Biol.* **2**, 117–147.
52. Pruss, D., Bartholomew, B., Persinger, J., Hayes, J., Arents, G., Moudrianakis, E. N., and Wolffe, A. P. (1996). An asymmetric model for the nucleosome: A binding site for linker histones inside the DNA gyres. *Science* **274**, 614–617.
53. McGhee, J. D., Rau, D. C., Charney, E., and Felsenfeld, G. (1980). Orientation of the nucleosome within the higher order structure of chromatin. *Cell* **22**, 87.
54. Arents, G., Burlingame, R. W., Wang, B.-C., Love, W. E., and Moudrianakis, E. N. (1991). The nucleosomal core histone octamer at 3.1 Å resolution: A tripartite protein assembly and a left-handed superhelix. *Proc. Natl. Acad. Sci. USA* **88**, 10148–10152.
55. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251–260.
56. Godfrey, J. E., Baxevanis, A. D., and Moudrianakis, E. N. (1990). Spectropolarimetric analysis of the core histone octamer and its subunits. *Biochemistry* **29**, 965–972.
57. Shick, V. V., Belyavsky, A. V., Bavykin, S. G., and Mirzabekov, A. D. (1980). Primary organization of the nucleosome core particles: Sequential arrangement of histones along DNA. *J. Mol. Biol.* **139**, 491.
58. Nelson, D. A., Mencke, A. J., Chambers, S. A., Oosterhof, D. K., and Rill, R. L. (1982). Subnucleosomes and their relationships to the arrangement of histone binding sites along nucleosome deoxyribonucleic acid. *Biochemistry* **21**, 4350–4362.
59. Arents, G., and Moudrianakis, E. N. (1993). Topography of the histone octamer surface: Repeating structural motifs utilized in the docking of nucleosomal DNA. *Proc. Natl. Acad. Sci. USA* **90**, 10489–10493.
60. Böhm, L., and Crane-Robinson, C. (1984). Proteases as structural probes for chromatin: The domain structure of histones. *Biosci. Rep.* **4**, 365–386.
61. Whitlock, J. P., Jr., and Stein, A. (1978). Folding of DNA by histones which lack their NH_2-terminal regions. *J. Biol. Chem.* **253**, 3857–3861.
62. Allan, J., Harborne, N., Rau, D. C., and Gould, H. (1982). Participation of core histone "tails" in the stabilization of the chromatin solenoid. *J. Cell Biol.* **93**, 285–297.

63. Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. *Nature* **389**, 349–352.
64. Stollar, B. D., and Rezuke, W. (1978). Separation of antihistone antibodies from nonimmune histone precipitating serum proteins, predominantly alpha 2-macroglobulin. *Arch. Biochem. Biophys.* **190**, 398.
65. Stollar, B. D. (1971). Reactions of systemic lupus erythematosus sera with histone fractions and histone-DNA complexes. *Arthritis Rheum.* **4**, 485–492.
66. Stollar, B. D. (1969). Varying specificity of systemic lupus erythematosus sera for histone fractions and a periodate-sensitive antigen associated with histones. *J. Immunol.* **103**, 804–808.
67. Stollar, B. D. (1967). Studies on nucleoprotein determinants for systemic lupus erythematosus serum. *J. Immunol.* **99**, 959–965.
68. Levine, L. (1973). Micro-complement fixation. *In* "Immunochemistry" (D. M. Weir, ed.), pp. 22.1 Blackwell Scientific, Oxford.
69. Hekman, A., and Sluyser, M. (1973). Antigenic determinations on lysine-rich histones. *Biochim. Biophys. Acta* **295**, 613.
70. Tan, E. M., Robinson, J., and Robitaille, P. (1976). Studies on antibodies to histones by immunofluorescence. *Scand. J. Immunol.* **5**, 811–818.
71. Portanova, J. P., Rubin, R. L., Joslin, F. G., Agnello, V. D., and Tan, E. M. (1982). Reactivity of anti-histone antibodies induced by procainamide and hydralazine. *Clin. Immunol. Immunopathol.* **25**, 67–79.
72. Rubin, R. L., Joslin, F. J., and Tan, E. M. (1982). A solid-phase radioimmunoassay for antihistone antibodies in human sera: Comparison with an immunofluorescence assay. *Scand. J. Immunol.* **15**, 63–70.
73. Blankstein, L. A., Stollar, B. D., and Levy, S. B. (1980). Immunochemical distinctions among histones and their variants in a solid-phase radioimmunoassay. *Anal. Biochem.* **104**, 168–172.
74. Romac, J., Bouley, J. P., and Van Regenmortel, M. H. V. (1981). Enzyme-linked immunosorbent assay in the study of histone antigens and nucleosome structure. *Anal. Biochem.* **113**, 366–371.
75. Rubin, R. L., Joslin, F. G., and Tan, E. M. (1982). Specificity of antihistone antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **25**, 779–782.
76. Aitkaci, A., Monier, J. C., and Mamelle, N. (1981). Enzyme-linked immunosorbent assay for antihistone antibodies and their presence in systemic lupus erythematosus sera. *J. Immunol. Methods* **44**, 311–322.
77. Gioud, M., Kotzin, B. L., Rubin, R. L., Joslin, F. G., and Tan, E. M. (1983). *In vivo* and *in vitro* production of antihistone antibodies in NZB/NZW mice. *J. Immunol.* **131**, 269–279.
78. Burlingame, R. W., and Rubin, R. L. (1990). Subnucleosome structures as substrates in enzyme-linked immunosorbent assays. *J. Immunol. Methods* **134**, 187–199.
79. Fishbein, E., Alarcón-Segovia, D., and Vega, J. M. (1979). Antibodies to histones in systemic lupus erythematosus. *Clin. Exp. Immunol.* **36**, 145–150.
80. Fritzler, M., Ryan, P., and Kinsella, T. D. (1982). Clinical features of systemic lupus erythematosus patients with antihistone antibodies. *J. Rheumatol.* **9(1)**, 46–51.
81. Fellows, G., Gittoes, N., Scott, D. G. I., Coppock, J. S., Wainwright, A., Goodall, M., and Turner, B. M. (1988). Individual variation in the isotype profile of antihistone autoantibodies in systemic lupus erythematosus. *Clin. Exp. Immunol.* **72**, 440–445.
82. Bernstein, R. M., Hobbs, R. N., Lea, D. J., Ward, D. J., and Hughes, G. R. V. (1985). Patterns of antihistone antibody specificity in systemic rheumatic disease. *Arthritis Rheum.* **28**, 285–293.
83. Shoenfeld, Y., and Segol, O. (1989). Antihistone antibodies in SLE and other autoimmune diseases. *Clin. Exp. Rheumatol.* **7**, 265–271.
84. Gripenberg, M., Helve, T., and Kurki, P. (1985). Profiles of antibodies to histones, DNA and IgG in patients with systemic rheumatic diseases determined by ELISA. *J. Rheumatol.* **12(5)**, 934–939.
85. Krippner, H., Springer, B., Merle, S., and Pirlet, K. (1984). Antibodies to histones of the IgG and IgM class in systemic lupus erythematosus. *Clin. Exp. Immunol.* **58**, 49–56.
86. Gompertz, N. R., Isenberg, D. A., and Turner, B. M. (1990). Correlation between clinical features of systemic lupus erythematosus and levels of antihistone antibodies of the IgG, IgA, and IgM isotypes. *Ann. Rheum. Dis.* **49**, 524–527.
87. Costa, O., and Monier, J. C. (1983). Detection of antibodies to histones in human systemic lupus erythematosus and in murine lupus-like syndromes using micro-ELISA. *Ann. Immunol. (Inst. Pasteur)* **134c**, 365–376.
88. Epstein, A., Greenberg, M., Halpert, S., Kramer, L., and Barland, P. (1986). The clinical application of an ELISA technique for the detection of antihistone antibodies. *J. Rheumatol.* **13**, 304–307.
89. Shoenfeld, Y., Segol, G., Segol, O., Neary, B., Klajman, A., Stollar, B. D., and Isenberg, D. A. (1987). Detection of antibodies to total histones and their subfractions in systemic lupus erythematosus patients and their asymptomatic relatives. *Arthritis Rheum.* **30(2)**, 169–175.
90. Suzuki, T., Burlingame, R. W., Casiano, C. A., Boey, M. L., and Rubin, R. L. (1994). Antihistone antibodies in systemic lupus erythematosus: Assay dependency and effects of ubiquitination and serum DNA. *J. Rheumatol.* **21**, 1081–1091.
91. Bustin, M., Reisch, J., Einck, L., and Klippel, J. H. (1982). Autoantibodies to nucleosomal proteins: Antibodies to HMG-17 in autoimmune diseases. *Science* **215(5)**, 1245–1247.
92. Konstantinov, K., Russanova, V., and Russeva, V. (1986). Antibodies to histones and disease activity in systemic lupus erythematosus: A comparative study with an enzyme-linked immunosorbent assay and immunoblotting. *Arch. Dermatol. Res.* **278**, 410–415.
93. Gioud, M., Aitkaci, A., and Monier, J. C. (1982). Histone antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **25**, 407–413.

94. Chou, M. J., Lee, S. L., Chen, T. Y., and Tsay, G. J. (1995). Specificity of antinuclear antibodies in primary biliary cirrhosis. *Ann. Rheum. Dis.* **54**, 148–151.
95. Kohda, S., Kanayama, Y., Okamura, M., Amatsu, K., Negoro, N., Takeda, T., and Inoue, T. (1989). Clinical significance of antibodies to histones in systemic lupus erythematosus. *J. Rheumatol.* **16**, 24–28.
96. Muller, S., Bonnier, D., Thiry, M., and Van Regenmortel, M. H. V. (1989). Reactivity of autoantibodies in systemic lupus erythematosus with synthetic core histone peptides. *Int. Arch. Allergy Appl. Immunol.* **89**, 288–296.
97. Costa, O., and Monier, J.-C. (1986). Antihistone antibodies detected by ELISA and immunoblotting in systemic lupus erythematosus and rheumatoid arthritis. *J. Rheumatol.* **13(4)**, 722–725.
98. Costa, O., Tchouatcha-Tchouassom, J. C., Roux, B., and Monier, J. C. (1986). Anti-H1 histone antibodies in systemic lupus erythematosus: epitope localization after immunoblotting of chymotrypsin-digested H1. *Clin. Exp. Immunol.* **63**, 608–613.
99. Cohen, M. G., Pollard, K. M., and Webb, J. (1992). Antibodies to histones in systemic lupus erythematosus: Prevalence, specificity, and relationship to clinical and laboratory features. *Ann. Rheum. Dis.* **51**, 61–66.
100. Rubin, R. L. (1997). Enzyme-linked immunosorbent assay for antibodies to native DNA, histones and (H2A-H2B)-DNA. *In* "Manual of Clinical Laboratory Immunology" (N. R. Rose, E. C. de Macario, J. D. Folds, H. C. Lane, and R. M. Nakamura, eds.), pp. 935–941. American Society for Microbiology, Washington, DC.
101. Gohill, J., Cary, P. D., Couppez, M., and Fritzler, M. J. (1985). Antibodies from patients with drug-induced and idiopathic lupus erythematosus react with epitopes restricted to the amino and carboxyl termini of histone. *J. Immunol.* **135**, 3116–3121.
102. Hardin, J. A., and Thomas, J. O. (1983). Antibodies to histones in systemic lupus erythematosus: Localization of prominent autoantigens on histones H1 and H2B. *Proc. Natl. Acad. Sci. USA* **80**, 7410–7414.
103. Raska, I., Petrasovicova, V., Jarnik, M., Cebecauser, L., Lukacovicova, L., Lejnar, J., Viklicky, V., Macha, J., Jira, M., and Trnavsky, K. (1991). Autoantibodies against histones and actin in patients with rheumatic diseases assessed by the Western blot method. *Czech. Med.* **14**, 135–145.
104. Grossman, L., and Barland, P. (1981). Histone reactivity of drug-induced anti-nuclear antibodies. *Arthritis Rheum.* **24**, 927–930.
105. Rubin, R. L., McNally, E. M., Nusinow, S. R., Robinson, C. A., and Tan, E. M. (1985). IgG antibodies to the histone complex H2A-H2B characterize procainamide-induced lupus. *Clin. Immunol. Immunopathol.* **36**, 49–59.
106. Totoritis, M. C., Tan, E. M., McNally, E. M., and Rubin, R. L. (1988). Association of antibody to histone complex H2A-H2B with symptomatic procainamide-induced lupus. *N. Engl. J. Med.* **318**, 1431–1436.
107. Burlingame, R. W., and Rubin, R. L. (1991). Drug-induced anti-histone autoantibodies display two patterns of reactivity with substructures of chromatin. *J. Clin. Invest.* **88**, 680–690.
108. Vázquez-Del Mercado, M., Casiano, C. A., and Rubin, R. L. (1995). IgA antihistone antibodies in isoniazid-treated tuberculosis patients. *Autoimmunity* **20**, 105–111.
109. Klajman, A., Kafri, B., Shohat, T., Drucker, I., Moalem, T., and Jaretzky, A. (1983). The prevalence of antibodies to histones induced by procainamide in old people, in cancer patients, and in rheumatoid-like diseases. *Clin. Immunol. Immunopathol.* **27**, 1–8.
110. Aitcheson, C. T., Peebles, C., Joslin, F., and Tan, E. M. (1980). Characteristics of antinuclear antibodies in rheumatoid arthritis. *Arthritis Rheum.* **23**, 528.
111. Cohen, M. G., and Webb, J. (1989). Antihistone antibodies in rheumatoid arthritis and Felty's syndrome. *Arthritis Rheum.* **32(10)**, 1319–1324.
112. Muzellec, Y., Le Goff, P., Jouquan, J., Fauquert, P., Muller, S., and Youinou, P. (1988). Antibodies to histones in rheumatoid arthritis. *Diagn. Clin. Immunol.* **5**, 326–331.
113. Tuaillon, N., Muller, S., Pasquali, J.-L., Bordigon, P., and Van Regenmortel, M. H. V. (1990). Antibodies from patients with rheumatoid arthritis and juvenile chronic arthritis analyzed with core histone synthetic peptides. *Arch. Allergy Appl. Immunol.* **91**, 297–305.
114. Ostensen, M., Fredriksen, K., Kåss, E., and Rekvig, O.-P. (1989). Identification of antihistone antibodies in subsets of juvenile chronic arthritis. *Ann. Rheum. Dis.* **48**, 114–117.
115. Burlingame, R. W., Rubin, R. L., and Rosenberg, A. M. (1993). Autoantibodies to chromatin components in juvenile rheumatoid arthritis. *Arthritis Rheum.* **36**, 836–841.
116. Stemmer, C., Tuaillon, N., Prieur, A. M., and Muller, S. (1995). Mapping of B-cell epitopes recognized by antibodies to histones in subsets of juvenile chronic arthritis. *Clin. Immunol. Immunopathol.* **76**, 82–89.
117. Leak, A. M., Tuaillon, N., Muller, S., and Woo, P. (1993). Study of antibodies to histones and histone synthetic peptides in pauciarticular juvenile chronic arthritis. *Br. J. Rheumatol.* **32**, 426–431.
118. Monestier, M., Losman, J. A., Fasy, T. M., Debbas, M. E., Massa, M., Albani, S., Bohm, L., and Martini, A. (1990). Antihistone antibodies in antinuclear antibody-positive juvenile arthritis. *Arthritis Rheum.* **33(12)**, 1836–1841.
119. Malleson, P., Petty, R. E., Fung, M., and Candido, P. M. (1989). Reactivity of antinuclear antibodies with histones and other antigens in juvenile rheumatoid arrthritis. *Arthritis Rheum.* **32(7)**, 919–923.
120. Pauls, J. D., Silverman, E., Laxer, R. M., and Fritzler, M. J. (1989). Antibodies to histones H1 and H5 in sera of patients with juvenile rheumatoid arthritis. *Arthritis Rheum.* **32(7)**, 877–883.
121. Reumaux, D., Mézère, C., Colombel, J.-F., Duthilleul, P., and Muller, S. (1995). Distinct production of autoantibodies to nuclear components in ulcerative colitis and in Crohn's disease. *Clin. Immunol. Immunopathol.* **77**, 349–357.
122. Shoenfeld, Y., El-Roeiy, A., Ben-Yehuda, O., and Pick, A. I. (1987). Detection of anti-histone activity in sera of

patients with monoclonal gammopathies. *Clin. Immunol. Immunopathol.* **42**, 250–258.
123. Kamei, M., Kato, M., Mochizuki, K., Kuroda, K., Sato, S., Hashizume, S., Yasumoto, K., Murakami, H., and Nomoto, K. (1992). Serodiagnosis of cancers by ELISA of anti-histone H2B antibody. *Biotherapy* **4**, 17–22.
124. Konikoff, F., Swissa, M., and Shoenfeld, Y. (1989). Autoantibodies to histones and their subfractions in chronic liver diseases. *Clin. Immunol. Immunopathol.* **51**, 77–82.
125. Penner, E., Muller, S., Zimmermann, D., and Van Regenmortel, M. H. V. (1987). High prevalence of antibodies to histones among patients with primary biliary cirrhosis. *Clin. Exp. Immunol.* **70**, 47–52.
126. Czaja, A. J., Ming, C., Shirai, M., and Nishioka, M. (1995). Frequency and significance of antibodies to histones in autoimmune hepatitis. *J. Hepatol.* **23**, 32–38.
127. Chen, M., Shirai, M., Czaja, A. J., Kurokohchi, K., Arichi, T., Arima, K., Kodama, T., and Nishioka, M. (1998). Characterization of anti-histone antibodies in patients with type 1 autoimmune hepatitis. *J. Gastroenterol. Hepatol.* **13**, 483–489.
128. Sato, S., Ihn, H., Kikuchi, K., and Takehara, K. (1994). Antihistone antibodies in systemic sclerosis. Association with pulmonary fibrosis. *Arthritis Rheum.* **37**, 391–394.
129. Parodi, A., Drosera, M., Barbieri, L., and Rebora, A. (1995). Antihistone antibodies in scleroderma. *Dermatology* **191**, 16–18.
130. Sato, S., Ihn, H., Soma, Y., Igarashi, A., Tamaki, T., Kikuchi, K., Ishibashi, Y., and Takehara, K. (1993). Antihistone antibodies in patients with localized scleroderma. *Arthritis Rheum.* **36**, 1137–1141.
131. Wallace, D. J., Lin, H.-C., Shen, G. Q., and Peter, J. B. (1994). Antibodies to histone (H2A-H2B)-DNA complexes in the absence of antibodies to double-stranded DNA or to (H2A-H2B) complexes are more sensitive and specific for scleroderma-related disorders than for lupus. *Arthritis Rheum.* **37**, 1795–1797.
132. Kubo, M., Ihn, H., Yazawa, N., Sato, S., Kikuchi, K., and Tamaki, K. (1999). Prevalence and antigen specificity of anti-histone antibodies in patients with polymyositis/dermatomyositis. *J. Invest Dermatol.* **112**, 711–715.
133. Rubin, R. L., Reimer, G., McNally, E. M., Nusinow, S. R., Searles, R. P., and Tan, E. M. (1986). Procainamide elicits a selective autoantibody immune response. *Clin. Exp. Immunol.* **63**, 58–67.
134. Molden, D. P., Klipple, G. L., Peebles, C. L., Rubin, R. L., Nakamura, R. M., and Tan, E. M. (1986). IgM anti-histone H-3 antibody associated with undifferentiated rheumatic disease syndromes. *Arthritis Rheum.* **29**, 39–43.
135. Burlingame, R. W., and Rubin, R. L. (1992). Anti-histone autoantibodies recognize centromeric heterochromatin in metaphase chromosomes and hidden epitopes in interphase cells. *Hum. Antibodies Hybridomas* **3**, 40–47.
136. Li, L., Chen, M., Huang, D. Y., and Nishioka, M. (2000). Frequency and significance of antibodies to chromatin in autoimmune hepatitis type I. *J. Gastroenterol. Hepatol.* **15**, 1176–1182.
137. Vila, J. L., Juarez, C., Illa, I., Agusti, M., Gelpi, C., Amengual, M. J., and Rodriguez, J. L. (1987). Autoantibodies against the $H1^0$ subtype of histone H1. *Clin. Immunol. Immunopathol.* **45**, 499–503.
138. Monestier, M., Fasy, T. M., Bohm, L., and Lieberman, F. S. (1991). Anti-histone antibodies in subacute sensory neuropathy. *J. Neurooncol.* **11**, 71–75.
139. Caturla, A., Colome, J. A., Bustos, A., Chamorro, M. J., Figueredo, M. A., Subiza, J. L., and de la Concha, E. G. (1991). Occurrence of antibodies to protease treated histones in a patient with vasculitis. *Clin. Immunol. Immunopathol.* **60**, 65–71.
140. Martin, L., Pauls, J. D., Ryan, J. P., and Fritzler, M. J. (1993). Identification of a subset of patients with scleroderma with severe pulmonary and vascular disease by the presence of autoantibodies to centromere and histone. *Ann. Rheum. Dis.* **52**, 780–784.
141. Williams, W. M., Whalley, A. S., Comacchio, R. M., Rosenberg, J., Watts, R. A., Isenberg, D. A., McCutchan, J. A., and Morrow, W. J. W. (1996). Correlation between expression of antibodies to histone H2B and clinical activity in HIV-infected individuals. *Clin. Exp. Immunol.* **104**, 18–24.
142. Portanova, J. P., Arndt, R. E., Tan, E. M., and Kotzin, B. L. (1987). Antihistone antibodies in idiopathic and drug-induced lupus recognize distinct intrahistone regions. *J. Immunol.* **138**, 446–451.
143. Craft, J. E., Radding, J. A., Harding, M. W., Bernstein, R. M., and Hardin, J. A. (1987). Autoantigenic histone epitopes: A comparison between procainamide- and hydralazine-induced lupus. *Arthritis Rheum.* **30**, 689–694.
144. Hobbs, R. N., Clayton, A.-L., and Bernstein, R. M. (1987). Antibodies to the five histones and poly(adenosine diphosphate-ribose) in drug induced lupus: Implications for pathogenesis. *Ann. Rheum. Dis.* **46**, 408–416.
145. Xavier, R. M., Yamauchi, Y., Nakamura, M., Tanigawa, Y., Ishikura, H., Tsunematsu, T., and Kobayashi, S. (1995). Antinuclear antibodies in healthy aging people: A prospective study. *Mech. Ageing Dev.* **78**, 145–154.
146. Rubin, R. L., Bell, S. A., and Burlingame, R. W. (1992). Autoantibodies associated with lupus induced by diverse drugs target a similar epitope in the (H2A-H2B)-DNA complex. *J. Clin. Invest.* **90**, 165–173.
147. Enzenauer, R. J., West, S. G., and Rubin, R. L. (1990). D-penicillamine-induced systemic lupus erythematosus. *Arthritis Rheum.* **33**, 1582–1585.
148. Salazar-Paramo, M., Rubin, R. L., and Garcia-de la Torre, I. (1992). Isoniazid-induced systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 1085–1087.
149. Nordstrom, D. M., West, S. G., and Rubin, R. L. (1989). Methyldopa-induced systemic lupus erythematosus. *Arthritis Rheum.* **32**, 205–208.
150. Zamber, R., Martens, H., Rubin, R. L., and Starkebaum, G. (1992). Drug-induced lupus due to ophthalmic timolol. *J. Rheumatol.* **19**, 977–979.
151. Bray, V. J., West, S. G., Schultz, K. T., Boumpas, D. T., and Rubin, R. L. (1994). Antihistone antibody profile in sulfasalazine induced lupus. *J. Rheumatol.* **21**, 2157–2158.

152. Massa, M., De Benedetti, F., Pignatti, P., Albani, S., Di Fuccia, G., Monestier, M., and Martini, A. (1994). Anti-double stranded DNA, anti-histone, and anti-nucleosome IgG reactivities in children with systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **12**, 219–225.
153. Burlingame, R. W., and Rubin, R. L. (1996). Autoantibody to the nucleosome subunit (H2A-H2B)-DNA is an early and ubiquitous feature of lupus-like conditions. *Mol. Biol. Rep.* **23**, 159–166.
154. Ayer, L. M., Rubin, R. L., Dixon, G. H., and Fritzler, M. J. (1994). Antibodies from patients with drug-induced autoimmunity react with high mobility group (HMG) proteins. *Arthritis Rheum.* **37**, 98–103.
155. Amoura, Z., Koutouzov, S., Chabre, H., Cacoub, P., Amoura, I., Musset, L., Bach, J. F., and Piette, J. C. (2000). Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases: Antinucleosome antibodies of the IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 76–84.
156. Mohan, C., Liu, F., Xie, C., and Williams, R. C., Jr. (2001). Anti-subnucleosome reactivities in systemic lupus erythematosus (SLE) patients and their first-degree relatives. *Clin. Exp. Immunol.* **123**, 119–126.
157. Chabre, H., Amoura, Z., Piette, J.-C., Godeau, P., Bach, J.-F., and Koutouzov, S. (1995). Presence of nucleosome-restricted antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **38**, 1485–1491.
158. Bruns, A., Blass, S., Hausdorf, G., Burmester, G. R., and Hiepe, F. (2000). Nucleosomes are major T and B cell autoantigens in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 2307–2315.
159. Muller, S., Barakat, S., Watts, R., Joubaud, P., and Isenberg, D. (1990). Longitudinal analysis of antibodies to histones, Sm-D peptides and ubiquitin in the serum of patients with systemic lupus erythematosus, rheumatoid arthritis and tuberculosis. *Clin. Exp. Rheumatol.* **8**, 445–453.
160. Mizushima, N., Kubota, T., Nanki, T., Koike, R., Kohsaka, H., and Miyasaka, N. (1996). Two cases of lupus nephritis having a high titer of anti-(histone-DNA) complex antibody without IgG anti-dsDNA antibody. *Rinsho Byori* **44**, 585–589.
161. Amoura, Z., Piette, J.-C., Chabre, H., Cacoub, P., Papo, T., Wechsler, B., Bach, J.-F., and Koutouzov, S. (1997). Circulating plasma levels of nucleosomes in patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 2217–2225.
162. Rubin, R. L. (1997). Drug-induced lupus. *In* "Dubois' Lupus Erythematosus" (D. J. Wallace and B. H. Hahn, eds.), pp. 871–901. Williams and Wilkens, Baltimore, MD.
163. Mongey, A.-B., Donovan-Brand, R., Thomas, T. J., Adams, L. E., and Hess, E. V. (1992). Serologic evaluation of patients receiving procainamide. *Arthritis Rheum.* **35**, 219–223.
164. Rubin, R. L. (1992). A complex epitope: Comment on the article by Mongey *et al. Arthritis Rheum.* **35**, 1108.
165. Rubin, R. L., Nusinow, S. R., Johnson, A. D., Rubenson, D. S., Curd, J. G., and Tan, E. M. (1986). Serological changes during induction of lupus-like disease by procainamide. *Am. J. Med.* **80**, 999–1002.
166. Rubin, R. L., Burlingame, R. W., Arnott, J. E., Totoritis, M. C., McNally, E. M., and Johnson, A. D. (1995). IgG but not other classes of anti-[(H2A-H2B)-DNA] is an early sign of procainamide-induced lupus. *J. Immunol.* **154**, 2483–2493.
167. Massa, M., De Benedetti, F., Pignatti, P., Albani, S., Monestier, M., and Martini, A. (1995). Lack of temporal association of iridocyclitis with IgG reactivities to core histones and nucleosome subparticles in pauciarticular juvenile chronic arthritis. *Br. J. Rheumatol.* **34**, 507–511.
168. Rubin, R. L., Tang, F.-L., Chan, E. K. L., Pollard, K. M., Tsay, G., and Tan, E. M. (1986). IgG subclasses of autoantibodies in systemic lupus erythematosus, Sjogren's syndrome, and drug-induced autoimmunity. *J. Immunol.* **137**, 2528–2534.
169. Plaué, S., Muller, S., and Van Regenmortel, M. H. V. (1989). A branched, synthetic octapeptide of ubiquitinated histone H2A as target of autoantibodies. *J. Exp. Med.* **169**, 1607–1616.
170. Rubin, R. L., and Waga, S. (1987). Anti-histone antibodies in systemic lupus erythematosus. *J. Rheumatol.* **14**, 118–126.
171. Thomas, J. O., Wilson, C. M., and Hardin, J. A. (1984). The major core histone antigenic determinants in systemic lupus erythematosus are in the trypsin-sensitive regions. *FEBS Lett.* **169**, 90–96.
172. Monestier, M., Decker, P., Briand, J. P., Gabriel, J. L., and Muller, S. (2000). Molecular and structural properties of three autoimmune IgG monoclonal antibodies to histone H2B. *J. Biol. Chem.* **275**, 13558–13563.
173. McGhee, J. D., and Felsenfeld, G. (1980). Nucleosome structure. *Annu. Rev. Biochem.* **49**, 1115–1156.
174. Gohill, J., and Fritzler, M. J. (1987). Antibodies in procainamide-induced and systemic lupus erythematosus bind the C-terminus of histone 1 (H1). *Mol. Immunol.* **24**, 275–285.
175. Stemmer, C., Briand, J.-P., and Muller, S. (1994). Mapping of linear epitopes of human histone H1 recognized by rabbit anti-H1/H5 antisera and antibodies from autoimmune patients. *Mol. Immunol.* **31**, 1037–1046.
176. Monestier, M., Fasy, T. M., Debbas, M. E., and Bohm, L. (1990). Specificities of IgM and IgG anti-histone H1 autoantibodies in autoimmune mice. *Clin. Exp. Immunol.* **81**, 39–44.
177. Wesierska-Gadek, J., Penner, E., Lindner, H., Hitchman, E., and Sauermann, G. (1990). Autoantibodies against different histone H1 subtypes in systemic lupus erythematosus sera. *Arthritis Rheum.* **33**, 1273–1278.
178. Pauls, J. D., Edworthy, S. M., and Fritzler, M. J. (1993). Epitope mapping of histone 5 (H5) with systemic lupus erythematosus, procainamide-induced lupus and hydralazine-induced lupus sera. *Mol. Immunol.* **30**, 709–719.
179. Rubin, R. L., Tang, F.-L., Tsay, G., and Pollard, K. M. (1990). Pseudoautoimmunity in normal mice: Anti-histone antibodies elicited by immunization versus

induction during graft-versus-host reaction. *Clin. Immunol. Immunopathol.* **54**, 320–332.
180. Burlingame, R. W., Rubin, R. L., Balderas, R. S., and Theofilopoulos, A. N. (1993). Genesis and evolution of antichromatin autoantibodies in murine lupus implicates T-dependent immunization with self antigen. *J. Clin. Invest.* **91**, 1687–1696.
181. Rubin, R. L., and Theofilopoulos, A. N. (1987). Monoclonal autoantibodies reacting with multiple structurally related and unrelated macromolecules. *Intern. Rev. Immunol.* **3**, 71–95.
182. Subiza, J. L., Caturla, A., Pascual-salcedo, D., Chamorro, M. J., Gazapo, E., Figueredo, M. A., and de la Concha, E. G. (1989). DNA-anti-DNA complexes account for part of the antihistone activity found in patients with systemic lupus erythematosus. *Arthritis Rheum.* **32(4)**, 406–412.
183. Waga, S., Tan, E. M., and Rubin, R. L. (1986): Histones in biological fluids: Effect on anti-histone antibody specificities. *Arthritis Rheum.* **29**, S72. [Abstract]
184. Waga, S., Tan, E. M., and Rubin, R. L. (1987). Identification and isolation of soluble histones from bovine milk and serum. *Biochem. J.* **244**, 675–682.
185. Morimoto, C., Sano, H., Abe, T., Homma, M., and Steinberg, A. D. (1982). Correlation between clinical activity of systemic lupus erythematosus and the amounts of DNA in DNA/anti-DNA antibody immune complexes. *J. Immunol.* **139(5)**, 1960–1965.
186. Rumore, P. M., and Steinman, C. R. (1990). Endogenous circulating DNA in systemic lupus erythematosus. *J. Clin. Invest.* **86**, 69–74.
187. Schmiedeke, T. M. J., Stöckl, F. W., Weber, R., Sugisaki, Y., Batsford, S. R., and Vogt, A. (1989). Histones have high affinity for the glomerular basement membrane. *J. Exp. Med.* **169**, 1879–1894.
188. Hannestad, K., and Johannessen, A. (1976). Polyclonal human antibodies to IgG (rheumatoid factors) which cross-react with cell nuclei. *Scand. J. Immunol.* **5**, 541–547.
189. Hannestad, K., and Stollar, B. D. (1978). Certain rheumatoid factors react with nucleosomes. *Nature* **275**, 671–673.
190. Johnson, P. M. (1979). IgM-rheumatoid factors cross-reactive with IgG and a cell nuclear antigen: apparent "masking" in original serum. *Scand. J. Immunol.* **9**, 461–466.
191. Agnello, V., Arbetter, A., Ibanez de Kasep, G., Powell, R., Tan, E. M., and Joslin, F. (1980). Evidence for a subset of rheumatoid factors that cross-react with DNA-histone and have a distinct cross-idiotype. *J. Exp. Med.* **151**, 1514–1527.
192. Hobbs, R. N., Lea, D. J., Phua, K. K., and Johnson, P. M. (1983). Binding of isolated rheumatoid factors to histone proteins and basic polycations. *Ann. Rheum. Dis.* **42**, 435–438.
193. Quismorio, F. P., Beardmore, T., Kaurman, R. L., and Mongan, E. S. (1983). IgG rheumatoid factors and antinuclear antibodies in rheumatoid vasculitis. *Clin. Exp. Immunol.* **52**, 333–340.
194. Tuaillon, N., Martin, T., Knapp, A. M., Pasquali, J. L., and Muller, S. (1992). Double reactivity of monoclonal and polyclonal rheumatoid factors for IgG and histones: Mapping of binding sites by means of histone synthetic peptides and anti-Id antibodies. *J. Autoimmun.* **5**, 1–14.
195. Rubin, R. L., Balderas, R. S., Tan, E. M., Dixon, F. J., and Theofilopoulos, A. N. (1984). Multiple autoantigen binding capabilities of mouse monoclonal antibodies selected for rheumatoid factor activity. *J. Exp. Med.* **159**, 1429–1440.
196. Rekvig, O. P., and Hannestad, K. (1980). Human autoantibodies that react with both cell nuclei and plasma membranes display specificity for the octamer of histones H2A, H2B, H3, and H4 in high salt. *J. Exp. Med.* **152**, 1720–1733.
197. Rekvig, O. P., and Hannestad, K. (1979). The specificity of human autoantibodies that react with both cell nuclei and plasma membranes: The nuclear antigen is present on core mononucleosomes. *J. Immunol.* **123**, 2673–2681.
198. Searles, R. P., Messner, R. P., and Bankhurst, A. D. (1979). Cross-reactivity of antilymphocyte and antinuclear antibodies in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **14**, 292–299.
199. Horneland, M., Rekvig, O. P., Jorgensen, L., and Hannestad, K. (1983). Cultured human endothelial cells display an antigen that is recognized by certain human antichromatin autoantibodies. *Clin. Exp. Immunol.* **54**, 373–377.
200. Jacob, L., Viard, J.-P., Allenet, B., Anin, M.-F., Slama, F. B. H., Vandekerckhove, J., Primo, J., Markovits, J., Jacob, F., Bach, J.-F., Le Pecq, J.-B., and Louvard, D. (1989). A monoclonal anti-double-stranded DNA autoantibody binds to a 94-kDa cell-surface protein on various cell types via nucleosomes or a DNA-histone complex. *Proc. Natl. Acad. Sci. USA* **86**, 4669–4673.
201. Kubota, T., Kanai, Y., and Miyasaka, N. (1990). Interpretation of the cross-reactivity of anti-DNA antibodies with cell surface proteins: The role of cell surface histones. *Immunol. Lett.* **23**, 187–194.
202. Raz, E., Ben-Bassat, H., Davidi, T., Shlomai, Z., and Eilat, D. (1993). Cross-reactions of anti-DNA autoantibodies with cell surface proteins. *Eur. J. Immunol.* **23**, 383–390.
203. Rekvig, O. P., and Hannestad, K. (1979). Properties of antinuclear antibodies that crossreact with plasma membranes. *Scand. J. Immunol.* **9**, 325–332.
204. Rekvig, O. P., and Hannestad, K. (1977). Certain polyclonal antinuclear antibodies crossreact with the surface membrane of human lymphocytes and granulocytes. *Scand. J. Immunol.* **6**, 1041–1054.
205. Rekvig, O. P., Muller, S., Briand, J. P., Skogen, B., and Van Regenmortel, M. H. V. (1987). Human antinuclear autoantibodies crossreacting with the plasma membrane and the N-terminal region of histone H2B. *Immunol. Invest.* **16(7)**, 535–547.
206. Mecheri, S., Dannecker, G., Dennig, D., Poncet, P., and Hoffmann, M. K. (1993). Anti-histone autoantibodies react specifically with the B cell surface. *Mol. Immunol.* **30**, 549–557.
207. Rekvig, O. P. (1989). Intrinsic cell membrane antigens recognized by antichromatin autoantibodies. *Scand. J. Immunol.* **29**, 7–13.

208. Holers, V. M., and Kotzin, B. L. (1985). Human peripheral blood monocytes display surface antigens recognized by monoclonal antinuclear antibodies. *J. Clin. Invest.* **76**, 991–998.
209. Emlen, W., Holers, V. M., Arend, W. P., and Kotzin, B. (1992). Regulation of nuclear antigen expression on the cell surface of human monocytes. *J. Immunol.* **148**, 3042–3048.
210. Bennett, R. M., Gabor, G. T., and Merritt, M. M. (1985). DNA binding to human leukocytes: Evidence for a receptor-mediated association, internalization, and degradation of DNA. *J. Clin. Invest.* **76**, 2182–2190.
211. Bennett, R. M., Kotzin, B. L., and Merritt, M. J. (1987). DNA receptor dysfunction in systemic lupus erythematosus and kindred disorders. *J. Exp. Med.* **166**, 850–863.
212. Koutouzov, S., Cabrespines, A., Amoura, Z., Chabre, H., Lotton, C., and Bach, J. F. (1996). Binding of nucleosomes to a cell surface receptor: Redistribution and endocytosis in the presence of lupus antibodies. *Eur. J. Immunol.* **26**, 472–486.
213. Portanova, J. P., Cheronis, J. C., Blodgett, J. K., and Kotzin, B. L. (1990). Histone autoantigens in murine lupus. *J. Immunol.* **144**, 4633–4640.
214. Portonova, J. P., Arndt, R. E., and Kotzin, B. L. (1988). Selective production of autoantibodies in graft-vs-host-induced and spontaneous murine lupus. *J. Immunol.* **140**, 755–760.
215. Brick, J. E., Ong, S.-H., Bathon, J. M., Walker, S. E., O'Sullivan, F. X., and DiBartolomeo, A. (1990). Antihistone antibodies in the serum of autoimmune MRL and NZB/NZW F_1 mice. *Clin. Immunol. Immunopathol.* **54**, 372–381.
216. Costa, O., and Monier, J. C. (1986). Antihistone antibodies detected by micro-ELISA and immunoblotting in mice with lupus-like syndrome (MRL/1, MRL/n, PN, and NZB strains). *Clin. Immunol. Immunopathol.* **40**, 276–282.
217. Monestier, M., and Novick, K. E. (1996). Specificities and genetic characteristics of nucleosome-reactive antibodies from autoimmune mice. *Mol. Immunol.* **33**, 89–99.
218. Losman, M. J., Fasy, T. M., Novick, K. E., and Monestier, M. (1992). Monoclonal autoantibodies to subnucleosomes from a MRL/Mp-+/+ mouse. *J. Immunol.* **148**, 1561–1569.
219. Mohan, C., Adams, S., Stanik, V., and Datta, S. K. (1993). Nucleosome: A major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J. Exp. Med.* **177**, 1367–1381.
220. Portanova, J. P., Creadon, G., Zhang, X., Smith, D. S., Kotzin, B. L., and Wysocki, L. J. (1995). An early post-mutational selection event directs expansion of autoreative B cells in murine lupus. *Mol. Immunol.* **32**, 117–135.
221. Wakui, H., Imai, H., Nakamoto, Y., Kobayashi, R., Itoh, H., and Miura, A. B. (1989). Anti-histone autoantibodies in ddY mice, an animal model for spontaneous IgA nephritis. *Clin. Immunol. Immunopathol.* **52**, 248–256.
222. Portanova, J. P., Claman, H. N., and Kotzin, B. L. (1985). Autoimmunization in murine graft-vs-host disease. I. Selective production of antibodies to histones and DNA. *J. Immunol.* **135**, 3850–3856.
223. Pollard, K. M., Chan, E. K. L., Rubin, R. L., and Tan, E. M. (1987). Monoclonal autoantibodies to nuclear antigens from murine graft-versus-host disease. *Clin. Immunol. Immunopathol.* **44**, 31–40.
224. Portanova, J. P., Arndt, R. E., and Kotzin, B. L. (1988). Selective production of autoantibodies in graft-vs.-host-induced and spontaneous murine lupus: Predominant reactivity with histone regions accessible in chromatin. *J. Immunol.* **140**, 755–760.
225. Meziere, C., Stockl, F., Batsford, S., Vogt, A., and Muller, S. (1994). Antibodies to DNA, chromatin core particles and histones in mice with graft-versus-host disease and their involvement in glomerular injury. *Clin. Exp. Immunol.* **98**, 287–294.
226. Van Dam, A. P., Meilof, J. F., Van Den Brink, H. G., and Smeenk, R. J. T. (1990). Fine specificities of anti-nuclear antibodies in murine models of graft-versus-host disease. *Clin. Exp. Immunol.* **81**, 31–38.
227. Kotzin, B. L., Lafferty, J. A., Portanova, J. P., Rubin, R. L., and Tan, E. M. (1984). Monoclonal anti-histone autoantibodies derived from murine models of lupus. *J. Immunol.* **133**, 2554–2559.
228. Kramers, K., Stemmer, C., Monestier, M., van Bruggen, M. C. J., Rijke-Schilder, T. P. M., Hylkema, M. N., Smeenk, R. J. T., Muller, S., and Berden, J. H. M. (1996). Specificity of monoclonal anti-nucleosome auto-antibodies derived from lupus mice. *J. Autoimmun.* **9**, 723–729.
229. Losman, M. J., Fasy, T. M., Novick, K. E., and Monestier, M. (1993). Relationship among antinuclear antibodies from autoimmune MRL mice reacting with histone H2A-H2B dimers and DNA. *Int. Immunol.* **5**, 513–523.
230. Tuaillon, N., Watts, R. A., Isenberg, D. A., and Muller, S. (1994). Sequence analysis and fine specificity of two human monoclonal antibodies to histone H1. *Mol. Immunol.* **31**, 269–277.
231. Brard, F., Jovelin, F., Petit, S., Tron, F., and Gilbert, D. (1996). Structural properties and mutation patterns of anti-nucleosome monoclonal antibodies are similar to those of anti-DNA antibodies. *Eur. J. Immunol.* **26**, 1587–1594.
232. Amoura, Z., Chabre, H., Koutouzov, S., Lotton, C., Cabrespines, A., Bach, J.-F., and Jacob, L. (1994). Nucleosome-restricted antibodies are detected before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp *lpr/lpr* and +/+ mice, and are present in kidney eluates of lupus mice with proteinuria. *Arthritis Rheum.* **37(11)**, 1684–1688.
233. Desai-Mehta, A., Mao, C., Rajagopalan, S., Robinson, T., and Datta, S. K. (1995). Structure and specificity of T cell receptors expressed by potentially pathogenic anti-DNA autoantibody-inducing T cells in human lupus. *J. Clin. Invest.* **95**, 531–541.
234. Voll, R. E., Roth, E. A., Girkontaite, I., Fehr, H., Herrmann, M., Lorenz, H.-M., and Kalden, J. R. (1997). Histone-specific Th0 and Th1 clones derived from systemic lupus erythematosus patients induce double-

stranded DNA antibody production. *Arthritis Rheum.* **40**, 2162–2171.
235. Kaliyaperumal, A., Mohan, C., Wu, W., and Datta, S. K. (1996). Nucleosomal peptide epitopes for nephritis-inducing T helper cells of murine lupus. *J. Exp. Med.* **183**, 2459–2469.
236. Shi, Y., Kaliyaperumal, A., Lu, L., Southwood, S., Sette, A., Michaels, M. A., and Datta, S. K. (1998). Promiscuous presentation and recognition of nucleosomal autoepitopes in lupus: role of autoimmune T cell receptor alpha chain. *J. Exp. Med.* **187**, 367–378.
237. Lu, L., Kaliyaperumal, A., Boumpas, D. T., and Datta, S. K. (1999). Major peptide autoepitopes for nucleosome-specific T cells of human lupus. *J. Clin. Invest* **104**, 345–355.
238. Duncan, S. R., Rubin, R. L., Burlingame, R. W., Sinclair, S. B., Pekny, K. W., and Theofilopoulos, A. N. (1996). Intrathymic injection of polynucleosomes delays autoantibody production in BXSB mice. *Clin. Immunol. Immunopathol.* **79**, 171–181.
239. Andreassen, K., Moens, U., Nossent, H., Marion, T. N., and Rekvig, O. P. (1999). Termination of human T cell tolerance to histones by presentation of histones and polyomavirus T antigen provided that T antigen is complexed with nucleosomes. *Arthritis Rheum.* **42**, 2449–2460.
240. Andreassen, K., Bendiksen, S., Kjeldsen, E., Van Ghelue, M., Moens, U., Arnesen, E., and Rekvig, O. P. (2002). T cell autoimmunity to histones and nucleosomes is a latent property of the normal immune system. *Arthritis Rheum.* **46**, 1270–1281.
241. Rubin, R. L., and Tan, E. M. (1992). B cell epitopes in natural and induced autoimmunity. *In* "The Autoimmune Disease II" (N. R. Rose and I. R. Mackay, eds.), pp. 173–193. Academic Press, San Diego.
242. Gleichmann, E., Pals, S. T., Rolink, A. G., Radaskiewicz, T., and Gleichmann, H. (1984). Graft-versus-host reactions: Clues to the etiopathology of a spectrum of immunological diseases. *Immunol. Today* **5**, 324–332.
243. Kretz-Rommel, A., Duncan, S. R., and Rubin, R. L. (1997). Autoimmunity caused by disruption of central T cell tolerance: A murine model of drug-induced lupus. *J. Clin. Invest.* **99**, 1888–1896.
244. Tan, E. M., Schur, P. H., Carr, R. I., and Kunkel, H. G. (1966). Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J. Clin. Invest.* **45**, 1732–1740.
245. Amoura, Z., Koutouzov, S., and Piette, J. C. (2000). The role of nucleosomes in lupus. *Curr. Opin. Rheumatol.* **12**, 369–373.
246. Kono, D. H., Burlingame, R. W., Owens, D. G., Kuramochi, A., Balderas, R. S., Balomenos, D., and Theofilopoulos, A. N. (1994). Lupus susceptibility loci in New Zealand mice. *Proc. Natl. Acad. Sci. USA* **91**, 10160–10172.
247. van Bruggen, M. C., Walgreen, B., Rijke, T. P., Tamboer, W., Kramers, K., Smeenk, R. J., Monestier, M., Fournie, G. J., and Berden, J. H. (1997). Antigen specificity of antinuclear antibodies complexed to nucleosomes determines glomerular basement membrane binding *in vivo*. *Eur. J. Immunol.* **27**, 1564–1569.
248. Kramers, C., Hylkema, M. N., van Bruggen, M. C. J., van de Lagemaat, R., Dijkman, H. B. P., Assmann, K. J. M., Smeenk, R. J. T., and Berden, J. H. M. (1994). Antinucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane *in vivo*. *J. Clin. Invest.* **94**, 568–577.
249. Minota, S., Yoshio, T., Iwamoto, M., Takeda, A., Masuyama, J., Mimori, A., Yamada, A., and Kano, S. (1996). Selective accumulation of anti-histone antibodies in glomeruli of lupus-prone lpr mice. *Clin. Immunol. Immunopathol.* **80**, 82–87.
250. Lefkowith, J. B., Kiehl, M., Rubenstein, J., Di Valerio, R., Bernstein, K., Kahl, L., Rubin, R. L., and Gourley, M. (1996). Heterogeneity and clinical significance of glomerular-binding antibodies in systemic lupus erythematosus. *J. Clin. Invest.* **98**, 1373–1380.
251. Di Valerio, R., Bernstein, K. A., Varghese, E., and Lefkowith, J. B. (1995). Murine lupus glomerulotropic monoclonal antibodies exhibit differing specificities but bind via a common mechanism. *J. Immunol.* **155**, 2258–2268.
252. Bernstein, K. A., Di Valerio, R., and Lefkowith, J. B. (1995). Glomerular binding activity in MRL *lpr* serum consists of antibodies that bind to a DNA/histone/type IV collagen complex. *J. Immunol.* **154**, 2424–2433.
253. Minota, S., Yoshio, T., Iwamoto, M., Takeda, A., Masuyama, J., Mimori, A., Yamada, A., and Kano, S. (1996). Selective accumulation of anti-histone antibodies in glomeruli of lupus-prone 1pr mice. *Clin. Immunol. Immunopathol.* **80**, 82–87.
254. van Bruggen, M. C., Walgreen, B., Rijke, T. P., Corsius, M. J., Assmann, K. J., Smeenk, R. J., van Dedem, G. W., Kramers, K., and Berden, J. H. (1996). Heparin and heparinoids prevent the binding of immune complexes containing nucleosomal antigens to the GBM and delay nephritis in MRL/1pr mice. *Kidney Int.* **50**, 1555–1564.
255. Woitas, R., Fujigaki, Y., Batsford, S. R., and Vogt, A. (1994). Histone mediates glomerular deposition of small size DNA anti-DNA complex. *Kidney Int.* **45**, 991–997.
256. Budhai, L., Oh, K., and Davidson, A. (1996). An *in vitro* assay for detection of glomerular binding IgG autoantibodies in patients with systemic lupus erythematosus. *J. Clin. Invest.* **98**, 1585–1593.
257. Klajman, A., Farkas, R., and Ben-Efraim, S. (1973). Complement-fixing activity of antinuclear antibodies induced by procainamide treatment. *Isr. J. Med. Sci.* **9**, 627–630.
258. Kanayama, Y., Peebles, C., Tan, E. M., and Curd, J. G. (1986). Complement activating abilities of defined antinuclear antibodies. *Arthritis Rheum.* **29**, 748–754.
259. Utsinger, P. D., Zvaifler, N. J., and Bluestein, H. G. (1976). Hypocomplementemia in procainamide-associated systemic lupus erythematosus. *Ann. Intern. Med.* **84**, 293.
260. Weinstein, J. (1978). Hypocomplementemia in hydralazine-associated systemic lupus erythematosus. *Am. J. Med.* **65**, 553.

13

ANTIBODIES TO NONHISTONE ANTIGENS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Marvin J. Fritzler
Edward K. L. Chan

INTRODUCTION

Systemic lupus erythematosus (SLE) is a disease in which an unbalanced immune response is invoked on the background of susceptibility genes and environmental stimuli. One manifestation of this response is the presence of serum autoantibodies directed against intracellular and extracellular antigens. Autoantibodies directed against nuclear (ANA) and other intracellular components predominate in SLE, and for that reason they have achieved clinical importance. The past three decades have witnessed a tremendous increase in our knowledge of the clinical relevance and complexity of ANA in SLE. This has made it necessary to know that antinuclear antibodies are present in serum and to determine the immunologic specificity of the autoantibody responses.

There are several reasons why the determination of autoantibody specificity is important. First, a positive ANA is no longer considered a specific test for any systemic rheumatic disease because ANA occur in high frequency in several of them as well as in nonrheumatic conditions. Second, important diagnostic and prognostic information can be gained from determining autoantibody specificities because certain autoantibodies are specific for SLE while others are specific for other diseases [1–4]. Moreover, some ANA specificities are closely related to certain clinical manifestations of SLE. Therefore, autoantibodies to a variety of nuclear antigens, including nonhistone macromolecules, have served as extremely useful aids in differential diagnosis and in the detection of early forms of several diseases, including SLE.

The importance of ANA was recognized in the 1982 revised criteria for the classification of SLE [5] where they are identified as an independent criterion. Anti-double-stranded DNA and anti-Sm were included with lupus erythematosus (LE) cell test and biological false-positive test for syphilis as subitems in a second immunological criterion. This has underscored the diagnostic significance of autoantibodies directed against several autoantigens.

An understanding of the clinical relevance of ANA has been the result of intensive study over the past three decades. From these studies it has been appreciated that ANA specificities can be divided into three general categories: antibodies directed against DNA (see Chapter 10), those directed against a family of basic nuclear proteins or histones (see Chapter 12), and those directed against nonhistone proteins. Although most autoantibodies in SLE react with intranuclear antigens (ANA), many sera also react with cytoplasmic antigens [2, 6] (see Chapter 11). Some antibodies that stain both the cytoplasm and the nucleus are directed against ribosomal proteins, various RNA species, and other cytoplasmic antigens [6, 7]. The discussion in this chapter is limited to autoantibodies directed against nonhistone proteins (Table 1).

TABLE 1 Autoantibodies in SLE: Clinical Association and Biochemical Identity[a]

Autoantigen	Prevalence in SLE (%)	Molecular characteristic
DNA		
dsDNA	40–60	Double-stranded DNA
Histones	60–80	H1,H2A,H2B,H3,H4,H5
Nonhistone nuclear antigens		
nRNP		
Sm	10–30	Proteins 28,29,16, and 13 kDa complexed U1,U2,U4,U5,U6 RNAs
U1 RNP	30–40	Proteins 33 and 22 kDa complexed U1 RNA Protein 68 kDa
hnRNA	15–25	Heterogeneous nuclear RNA
hnRNP A2/RA33	20–25	33-kDa protein of hnRNP complex
Ribonuclease P (RNase P)	25	400 bases RNA; 38-kDa protein
Nuclear and cytoplasmic RNPs		
SS-A/Ro	30–40	Proteins 60 and 52 kDa complexed to Y1–Y5 RNA
SS-B/La	15–25	Phosphoprotein 48 kDa complexed to RNA pol III transcripts—ATPase/dATPase
LSm4	<10	15-kDa protein complexed to U4/U6 snRNP
DNA/chromatin-binding antigens		
PCNA	<5	Protein 36 kDa—auxiliary protein of DNA polymerase δ
Ku p70/p80	10–20	70 and 80 kDa
Poly(ADP-ribose) polymerase (PARP)	35–45	116 kDa
Poly(ADP-ribose)	40–70	Branched homopolymer
DEK oncoprotein	10–25	45 kDa
Transcription activator Sp1	<10	95-kDa component of terminal repeat-binding protein
HMG-17	30–70	9-kDa high-mobility group protein
SSRP1	28	HMG protein involved transcription and repair of DNA and RNA
Ubiquitin	80	Ubiquitin-H2A branched peptide
Nucleolar and ribosomal proteins		
Ribosomal P proteins	10–20	P0, P1, P2 phosphoproteins
L7 eukaryotic protein	30–75	29-kDa large ribosomal subunit
L12/S10 protein subunits	2–40	L12 = 20 kDa (60S); S10 = 20 kDa (40S)
Nucleolar organizer NOR-90	<5	90-kDa upstream binding factor
RNA helicase (Gu)	<20	100 kDa
ASE-1	20	90-kDa protein antisense to ERCC-1
Mitotic spindle-associated proteins		
NuMA-1	<5	235-kDa protein
HsEg5 (NuMA-2)	<10	130-kDa protein
Other nuclear antigens		
p53	25–30	53 kDa regulates cell proliferation and apoptosis
Ki/SL	10–30	~31-kDa protein
Ki-67	3–30	Two isoforms: 315 and 350 kDa
Lamins	<10	Lamins A,B1/B2,C
p80-coilin Cajal/coiled bodies	<10	80-kDa protein in nuclear
Cytoplasmic antigens		
pp75	16[b]	75-kDa protein complexed to SS-A/Ro
20S proteasome	50	Nonlysosomal protein degradation
p57	?	57-kDa protein
Golgi complex	<10	67-, 97-, 160-, 245-, 376-kDa proteins
Endosome	<10	160-kDa early endosome antigen 1
Mitochondria	<10	DNA, cardiolipin, pyruvate dehydrogenase complex
Carbonic anhydrase I, II	20–45	34-kDa protein in erythrocytes

[a] ADP, adenosine diphosphate; HMG, high mobility group; hnRNP, heterogeneous nuclear ribonucleoprotein; NuMA, nuclear mitotic apparatus; PCNA, proliferating cell nuclear antigen; RNP, ribonucleoprotein; scRNP, small cytoplasmic ribonucleoproteins; snRNP, small nuclear ribonucleoproteins; SSRP 1, structure-specific recognition protein 1.

[b] Refers to frequency in neonatal lupus syndrome.

NOMENCLATURE AND MOLECULAR BIOLOGY OF NONHISTONE AUTOANTIGENS

The significance of antibodies directed against nonhistone protein antigens has increased with the ability to characterize their respective antigens at the molecular level. The pioneers in this work, Steitz and colleagues, introduced the techniques of immunoprecipitation of radioisotope-labeled cellular macromolecules and immunoblotting of cell extracts for the identification of intracellular autoantigens [3]. These techniques are now widely used for the characterization of autoantigens and for the recognition of nucleic acids, particularly RNAs. These RNAs are complexed to a number of protein antigens in a highly specific and characteristic association. The technology being applied to the study of nonhistone antigens also includes cloning of these antigens, the isolation of cDNA probes to study their role in cell biology, and the use of recombinant proteins in antigen arrays and other assays to detect autoantibodies [8–10].

Studies of RNA and protein antigens by immunoprecipitation and immunoblotting techniques have suggested that autoantigens reacting with SLE autoantibodies are frequently coupled as supramolecular complexes such as RNA–protein (RNP, spliceosomes), DNA–protein (DNP, nucleosome), and protein–protein (70/80 Ku subunits, ribosomal P proteins) complexes [11–14]. This discussion focuses on RNPs because they are the most prevalent nonhistone autoantigens described in SLE.

A review of Tables 1 and 2 reveals that an understanding of molecular biology and biochemistry is required to understand the nomenclature used in the identification of nonhistone antigens. Therefore, a brief overview of molecular and cell biology is presented to help the reader understand the nomenclature of autoantibodies seen in SLE and to appreciate basic concepts of cell biology.

TABLE 2 Clinical Features Associated with SS-A/Ro Antibodies

	Ref.
Neonatal lupus syndrome	134–136
Photosensitive skin rash/subacute cutaneous lupus	137–140
Interstitial pneumonitis	141, 142
Homozygous C2 deficiency	143, 144
Thrombocytopenia purpura	145–147
Vasculitis	132, 148
Lymphopenia	149
With anti-SS-B/La = decreased frequency of nephritis	149–151
ANA-negative lupus	169, 170

RNA

The RNA molecules that make up RNP complexes in eukaryotic cells can be categorized as messenger RNAs (mRNA), heterogeneous nuclear RNAs (hnRNA), large ribosomal RNAs, and several classes of low molecular weight RNA [13, 15]. Low molecular weight RNAs can be subclassified into uncapped small cytoplasmic RNAs (scRNA) and capped small nuclear RNAs (snRNA). snRNAs typically have a high content of uridine and are therefore designated UsnRNA. At least 12 UsnRNAs have been identified. UsnRNAs are, for the most part, transcribed by RNA polymerase II, whereas scRNAs are transcribed by RNA polymerase III. Transfer RNA (tRNA), ribosomal 5S RNA, 7SK RNA, the 7SL RNA of the signal recognition particle, translational control RNA, and 4.5S RNA are all examples of scRNA. A common feature of scRNAs is the addition of a few uridine residues to the 3′ terminus by RNA polymerase III at the termination of transcription. scRNAs include a class of low molecular weight molecules of 83–112 bases referred to as yRNA. The y designates their primary cytoplasmic localization. When yRNA are extracted from human tissues, they are given the additional designation of hyRNA. There are four classes of hyRNA: hy1, hy3, hy4, and hy5. hy2 is a breakdown product of hy1.

Some snRNAs are independently associated with their own RNP particle so that each snRNP particle contains only one snRNA. An exception is U4/U6 snRNAs, which are present in the same complex. A schematic representation of the RNA molecules precipitated and bound by RNP and Sm sera is shown in Fig. 1. Figure 1 shows that anti-Sm sera coprecipitate snRNAs U1, U2, U4, U5, and U6. However, the antibody to nuclear RNP only coprecipitates U1 RNA. Although autoimmune sera target predominantly the protein component of RNPs, autoantibodies directed against the RNA molecules themselves have been detected in association with autoantibodies to snRNP and scRNP [16–22]. For example, up to 60% of sera reactive with U1-RNP have autoantibodies directed against U1-RNA, and this reactivity is correlated with the HLA marker DR2/DR4, Raynaud's phenomenon, and synovitis [22].

Proteins

Identification and characterization of the proteins associated with RNPs have been aided greatly by

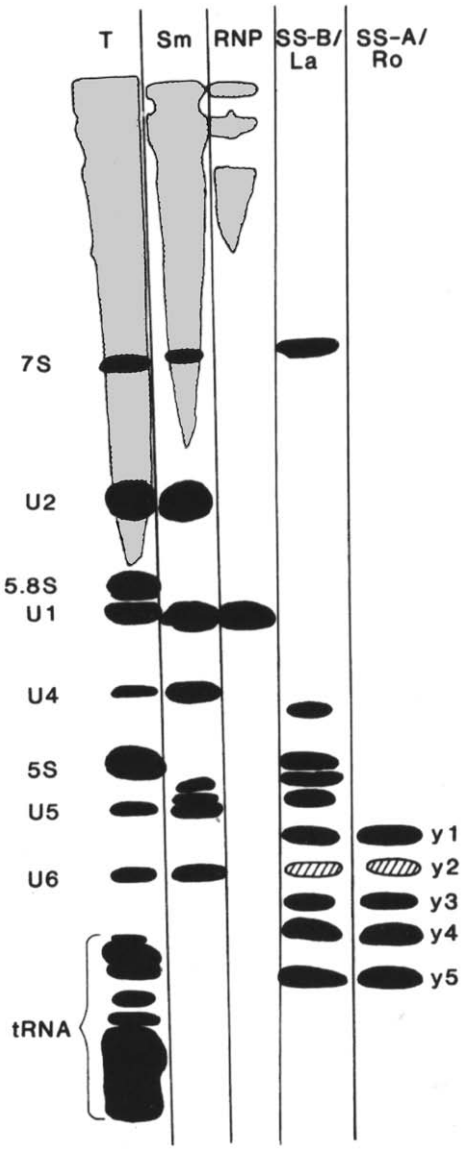


FIGURE 1 Schematic representation of small nuclear RNAs (snRNAs) immunoprecipitated by anti-Sm and anti-nRNP sera and small cytoplasmic RNAs (scRNAs) immunoprecipitated by anti-SS-B/La and anti-SS-Ro sera. HeLa cells are labeled with ^{32}P, and the soluble extract of these cells immunoprecipitated with anti-Sm (lane 2), anti-U1-RNP (lane 3), anti-SS-B/La (lane 4), and anti-SS-A/Ro (lane 5). Total RNA of the cell extracts is displayed in lane 1 (T). Anti-Sm precipitates five species of uridine-rich snRNA (U1, U2, U4, U5, and U6), whereas anti-U1-RNP precipitates only U1 RNA. Anti-SS-B/La sera precipitates 7S, 5S, 4.5S, y1, y3, y4, and y5 scRNAs, whereas anti-60-kDa SS-A/Ro precipitates only y1–y5 scRNAs. Stippled areas represent other high molecular weight RNA coprecipitated by the sera. The shading of the y2 RNA band is used to indicate that it is a breakdown product of y1.

autoantibodies from SLE patients. Immunoaffinity, immunoprecipitation, immunoblotting, other biochemical techniques, and molecular cloning have been used to identify polypeptides that are the constituents of the major human snRNPs U1, U2, U4, U5, and U6 [1, 15, 23]. At least seven of these core proteins are present on all the snRNPs. These have been designated by the letters B/B′ (27/28 kDa), D1/D2/D3 (14 kDa), E (12 kDa), F (11 kDa), and G (9 kDa). Analysis of individual snRNPs has disclosed that in addition to shared peptides, there are some proteins specific for certain snRNAs. U1 snRNPs contain three such proteins designated 70k (68/70 kDa), A (32 kDa), and C (22 kDa). The 70k protein is bound to the nuclear matrix and is associated with newly transcribed messenger RNA [24, 25]. U2 snRNPs have at least two unique proteins: A′ (29 kDa) and B″ (27 kDa). Two-dimensional electrophoretic gel and biophysical analyses of these proteins have shown that, except for the F protein, they are all basic (p*I* 8–10). The 70k protein, for one, has additional heterogeneity that is likely based on varying degrees of post-translational modifications such as phosphorylation. Several proteins have been shown to be associated with hyRNAs. A 60-kDa protein recognized by anti-SS-A/Ro sera interacts directly with one of the hyRNAs, and the 52-kDa SS-A/Ro polypeptide has been reported to be associated to this protein [26]. Finally, the 48-kDa phosphoprotein SS-B/La has been shown to be associated with RNA polymerase III transcripts, including hyRNAs [27]. A profile of some proteins that react with SLE sera is shown schematically in Fig. 2.

BIOCHEMICAL AND CLINICAL ASPECTS OF NONHISTONE ANTIGEN/ANTIBODY SYSTEMS

Sm

The Sm antigen is a complex system consisting of different proteins (Fig. 2) associated with the small nuclear RNAs (snRNA) U1, U2, U4/U6, and U5 (Fig. 1). These RNA polymerase II transcripts are complexed with proteins to form small nuclear ribonucleoproteins (snRNP). With the aid of immunoblotting and immunoprecipitation techniques, most investigators have come to the conclusion that five or six proteins in these complexes are the primary target antigens of human anti-Sm antibodies [15, 23, 28–31]. These are the Sm core proteins B/B′, D1, D2, D3, E, F, and G proteins described earlier [15, 23, 30, 32]. These proteins share a common sequence motif in two short polypeptide segments, Sm1 and Sm2, separated by a short variable linker. Crystal structures of two Sm protein complexes,

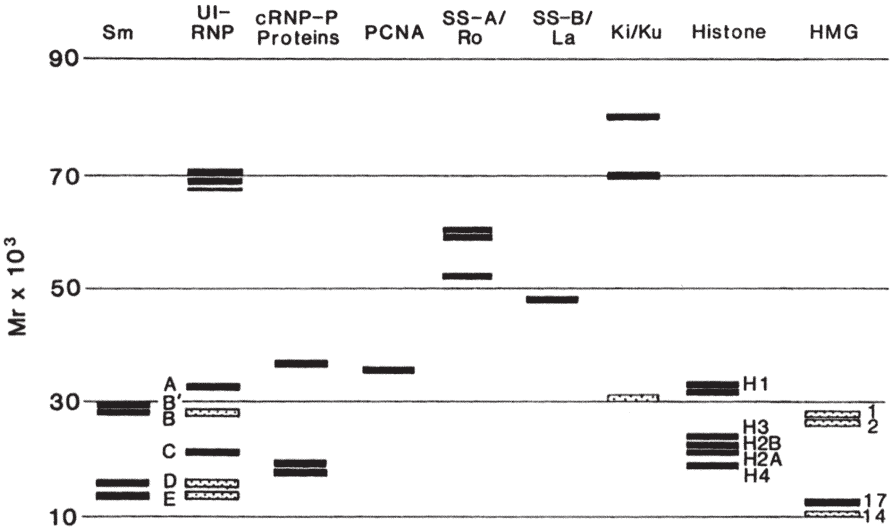


FIGURE 2 Schematic representation of [^{35}S]methionine-labeled HeLa cell proteins immunoprecipitated with various sera and analyzed on SDS polyacrylamide gels. The first two lanes show the typical pattern of polypeptides 70 k and A through E immunoprecipitated by anti-Sm and anti-U1RNP sera. The 70 k antigen is represented by a triplet of 68–70 kDa. Ribosomal RNP P proteins are identified as 38- 19- and 17-kDa polypeptides. Anti-PCNA antibodies precipitate a single 35-kDa protein. When extracts of nucleated mammalian cells are used, antibodies to SS-A/Ro precipitate 60- and 52-kDa proteins. Monospecific anti-SS-B/La sera precipitate a 45- to 48-kDa phosphoprotein. It should be understood that virtually all SS-B/La sera are not monospecific and usually precipitate some of the proteins in the adjacent SS-A/Ro lane. Ku antibodies precipitate proteins of ~80 and 70 kDa. Ki antibodies bind to a ~31-kDa protein (shaded). Histone proteins H1, H2A, H2B, H3, and H4 are bound by some SLE sera. SLE sera with HMG antibodies immunoprecipitate HMG-17 (~17 kDa), HMG-1 (~28 kDa), HMG-2 (~26 kDa), and HMG-14. Mr, relative mobility.

D3/B and D1/D2, suggest that the seven Sm core proteins could form a closed ring and the snRNAs may be bound in the positively charged central hole [33]. These core proteins are common to all major snRNPs, and for that reason a wider range of snRNAs are immunoprecipitated by Sm antibodies than are precipitated by U1RNP antibodies.

Anti-Sm produces a speckled staining pattern on HEp-2 cells by standard indirect immunofluorescence (IIF) assays but it cannot be detected with certainty by this technique [1]. Immunodiffusion, immunoblotting, analysis of precipitated RNAs (see Figs. 1 and 2), or enzyme-linked immunoassays are preferred to definitively identify this autoantibody. Advances in cloning and epitope mapping of Sm-related peptides have led to the use of synthetic peptides in clinical studies [34–37]. The techniques required to demonstrate antibody to Sm necessitate the use of standard reference sera. These prototype sera are available through the Centers for Disease Control [38].

The antibody to Sm was the first nonhistone antibody reported in what has become an expanding list of autoantigens (Table 1). Over the years, it has been confirmed repeatedly that this antibody is present almost exclusively in SLE and is considered a diagnostic marker for the disease to the extent that it is included in criteria for the classification of the disease [5]. Although anti-Sm is present in only 10–30% of patients with SLE, it is a highly specific disease marker. It has not been detected in normal sera or in patients with other systemic rheumatic diseases such as Sjögren's syndrome (SS), mixed connective tissue disease (MCTD), scleroderma, polymyositis, or drug-induced LE [39, 40]. Rising titers of anti-Sm have been correlated with more active disease [41] and disease flares [42]. This tends to support other studies that showed that anti-Sm was associated with milder renal disease and a less central nervous system disease [43–45] despite a high prevalence of late-onset proteinuria and a poorer prognosis than patients without Sm autoantibodies [44]. Because SLE sera tend to have multiple autoantibodies and more sensitive techniques using recombinant and highly purified proteins are under development, it could be concluded that the ability of anti-Sm antibodies to predict the clinical course of SLE is not clearly defined [46]. For example, van Venrooij and associates have reported that SLE sera have a prevalent and unique reactivity to a complex of RNP E-F-G proteins [47]. In

this study, individual snRNP proteins E, F, and G were synthesized and then allowed to form complexes. Although the individual proteins were not recognized in immunoblots, the E-F-G complex was immunoprecipitated preferentially by all anti-Sm sera but not sera from other diseases. These observations suggest that many anti-Sm sera recognize unique conformational epitopes in the E-F-G complex.

As noted earlier, anti-Sm reactivity is characterized by reactivity to several proteins (see Fig. 2), including the A, C, and 70k proteins of U1 RNP [1, 12]. Because U1RNP antibodies are considered to be a serological feature of MCTD, some investigators have questioned if unique serological profiles could distinguish MCTD from SLE. Analyses of SLE sera that contained both anti-Sm and anti-U1RNP antibodies have shown that some SLE patients have antibodies that bind to U1-U6 RNA-associated proteins, but they lack antibodies to the 70k protein [24, 48]. The importance of these observations is discussed in more detail in the next section.

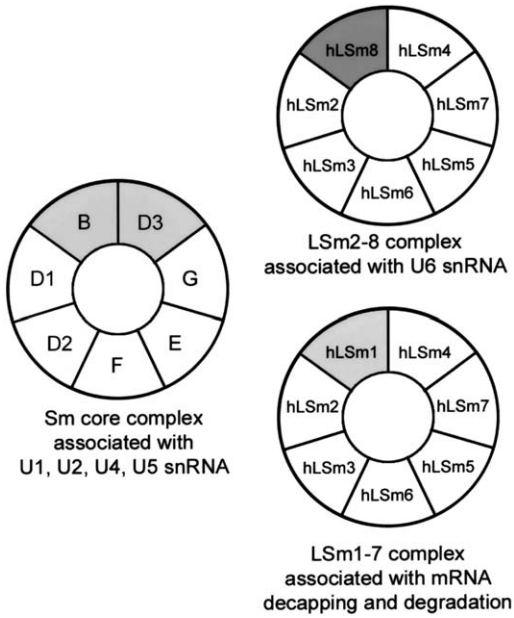


FIGURE 3 Diagrammatic representation of Sm core and hLSm protein complexes illustrating the proposed structural similarity among these seven protein doughnut-shaped structures. For both Sm core and LSm complexes, it has been proposed that the center space of the doughnut shape is involved in RNA binding.

LSm4 and LSm Complex

Studies have identified complexes composed of Sm-like proteins that form complexes distinct from Sm core snRNP complexes. They have been named like-Sm proteins, or LSm proteins (LSm1 to LSm8), as they each bear sequence homology with one of the seven Sm core proteins [49, 50]. For example, LSm4 was found to have the highest sequence similarity to SmD3, whereas hLSm1 and LSm8 have the highest similarity to the core domain of B/B′ [51]. Likewise, D1, D2, E, F, and G are most similar to LSm2, LSm3, LSm5, LSm6, and LSm7, respectively. The complexes they form with each other are postulated to have the same seven member ring doughnut configuration as the Sm protein complexes (Fig. 3) [33, 50]. The LSm complex, therefore, appears to exist in at least two configurations: the LSm2–8 complex associated with U6 snRNA and pre-mRNA splicing and the LSm1–7 complex that is involved in mRNA decapping and degradation [50, 52].

In one report [53], autoantibodies to LSm4 were detected using immunoprecipitation and *in vitro*-translated LSm4 in ~80% of all anti-Sm sera analyzed. A small fraction (7.2%) (28/391) of the same group of anti-Sm sera immunoprecipitated LSm4 together with the LSm complex from HeLa cell extracts. These findings document IgG autoantibodies to LSm4 are detected in a large overlapping subset of anti-Sm positive SLE. Interestingly, a small subset of anti-Sm sera LSm complex can be recognized independently of the Sm core protein antigens. Data suggest that the hLSm complex can be a separate, independent autoantigenic complex distinct from snRNP core complexes. LSm4 could be immunoprecipitated from a few lupus sera that contain little or no coprecipitating anti-Sm core proteins. Thus the presence of autoantibodies to hLSm4 or the hLSm complex may not be readily explained by epitope spreading via the association of LSm with the snRNP complex. The clinical significance of anti-LSm antibodies remains to be determined.

U1RNP

The target proteins of autoantibodies to nuclear U1RNP are predominantly the 70k (68/70kDa), A (32kDa), and C (22kDa) proteins (Fig. 2) [28–32, 48, 54]. Some sera containing antibody to nuclear RNP have been shown to have antibodies directed against the U2 snRNA-associated A′ (29kDa) and B″ (27kDa) proteins [55–57].

The antibody to U1 RNP was first reported by Sharp *et al.* [58, 59] in mixed connective tissue disease, a syndrome characterized by symptoms and signs mimicking SLE, scleroderma, and polymyositis. The main clinical features of MCTD are a high prevalence of Raynaud's phenomenon, edema of the fingers, arthritis/arthralgias, myositis, serositis, and a relative absence of renal disease [58, 59]. The characteristic feature of MCTD is the presence of high titers of U1 RNP antibodies and, with

the possible exception of anti-A2/RA33 (discussed later), no other types of ANA. This is unlike SLE where multiple other autoantibodies are characteristically observed.

In contrast to anti-Sm, anti-U1-RNP antibodies were also found in patients with a variety of systemic rheumatic diseases, including SLE, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome, polymyositis, discoid LE, and following procainamide therapy [1, 39, 40]. From these data one may conclude that the antibody to U1 RNP is not an exclusive serological marker for MCTD. The observation that anti-U1 RNP occurs with high frequency in patients with anti-Sm may be a reflection of autoimmune responses directed to macromolecular complexes [20, 60] through a process called "epitope spreading" [61–64]. It is clear that genetic influences are involved in regulating these autoantibody responses since they are correlated with HLA-DR4 [65] and are found in higher frequency in Black SLE patients than in patients of European extraction [66].

It is important to appreciate that some SLE and MCTD sera bind most of the snRNP polypeptides (as shown in Fig. 2), whereas others bind to little, if any, 70k or C polypeptides. In one study, only 8% SLE sera containing U1 RNP antibodies bound to the 70k antigen, but 76% of MCTD sera bound this protein [54]. The higher frequency of anti-70k antibodies in MCTD has been reported in several studies to be as high as 95% in MCTD while the range of reactivity in SLE is 20–50% [48]. The reported frequency of autoantibody frequency is highly dependent on the assay employed because when recombinant 70k protein was used in an ELISA, up to 85% of SLE patients showed elevated antibody levels [37]. It has been suggested that the presence of anti-70k provides a positive indicator for the presence of Raynaud's phenomenon, esophageal dysmotility, and myositis and as a negative indicator for the presence of renal disease. Taken together, data suggest that antibodies to the 70k protein may distinguish SLE from MCTD. These may be important observations for the clinician who attempts to identify patients at high risk of developing end organ disease when they present with only a few features (e.g., Raynaud's phenomenon, myositis) of other systemic rheumatic diseases.

Although antibodies to the 70k protein vary during the disease course [67], there is little evidence that they correlate with disease activity or that they are involved in disease pathogenesis [37, 45, 54, 68]. Evidence has renewed interest in the possibility that anti-U1 RNP antibodies have a unique property of being able to "penetrate" living cells [69, 70]. This property of U1 RNP antibodies may account for other occasional observations that anti-U1 RNP antibodies appear in nuclei of patients' tissue specimens [71].

Antibodies to the A and C proteins are found in approximately 25% of unselected SLE cohorts and in 75% of SLE patients preselected for antibodies to U1 RNP [30, 37, 45, 54, 72–75]. Like antibodies to the 70k protein, titers may vary during the course of the disease but there is no clear evidence that they predict disease activity or that they are involved in the pathogenesis [37, 45].

Heterogeneous RNP

As described earlier, heterogeneous nuclear RNA and heterogeneous nuclear ribonucleoproteins are intermediate components between gene transcription and translation [76]. Like snRNPs, hnRNP are associated with unique protein components that have a role in the processing, alternative splicing, and transport of larger hnRNA (pre-mRNA) transcripts [77]. Autoantibodies from patients with mixed connective tissue disease and SLE have been shown to bind hnRNA [25] and protein components [78–81] of hnRNP. One of these studies has demonstrated that approximately 20% of SLE, 35% of rheumatoid arthritis, and 50% of mixed connective tissue disease patients have antibodies to the A2/RA33 protein [79]. Unlike rheumatoid arthritis patients where antibodies to snRNPs are relatively rare, antibodies directed against the A2/RA33 protein in SLE and MCTD are often accompanied by anti-U1 RNP. This has led to the conclusion that when antibodies to A2/RA33 occur in the absence of antibodies to U1-RNP, there is 96% specificity for the diagnosis of rheumatoid arthritis [82]. By comparison, one study showed that anti-RA33 antibodies distinguish erosive arthritis in patients with SLE and RA [83]. Both SLE and RA sera bind to a conformational epitope in the RNA-binding amino terminus of the A2/RA33 protein, whereas MCTD sera tend to recognize another epitope [79]. The recognition of RNA-binding domains as major epitopes has been observed in other autoantigens, including the SS-B/La and the 70k and A U1-RNP proteins [84].

U2 RNP, RNase P, and Other nRNP

U2 RNP

Autoantibodies directed against U2 RNP react with the A′ and B″ proteins of the U2 particle [56, 57, 85]. Because U2 RNP antibodies were often accompanied by U1RNP antibodies, reactivity to the 70k and C proteins was also observed. It has been reported that up to 15% of SLE sera have autoantibodies directed against

U2 RNP [31]. In one study, antibodies to U2 RNP were not seen in 141 SLE patients but were identified in 8 patients with an overlap syndrome that most commonly included features of myositis [85]. Two of these 8 patients had features of SLE. One of the SLE patients also had features of scleroderma and an erosive arthropathy.

Ribonuclease P and tRNA

Ribonuclease P (RNase P) is an enzyme that cleaves precursor tRNA molecules to yield the correct 5′ terminal sequences of the mature tRNAs [86, 87]. Because this enzyme is complexed to a 400 nucleotide RNA molecule known as *H1RNA*, it is classified as a RNP particle [87]. Although some sera containing RNase P antibodies bind a 38-kDa protein [88], the identity of RNase P in immunoblots has not been clearly established. An interesting feature of this molecule is that the catalytic activity resides in the RNA subunit of the enzyme [87].

RNase P antibodies were first identified in approximately 25% of selected SLE sera [89]. Antibodies to the Th RNP/RNase MRP antigen are a common accompaniment of RNase P antibodies, suggesting that these two antigens are associated *in vivo* [90]. More recently, two antigens that copurify with RNase P have been shown to react with systemic sclerosis antibodies [91]. Studies have shown that some SLE sera bind tRNA itself [92, 93].

Other nRNP

Serum autoantibodies from a variety of systemic rheumatic diseases bind proteins that are components of U4/U6 [31, 94, 95], U5 [96], U7 [97], and U11 [98] snRNPs. These autoantibodies appear to be rare in SLE and tend to immunoprecipitate specific snRNPs because of their unique reactivity with snRNP particles. By comparison, nucleolar snRNPs (U3, U8, U13) do not contain the common Sm proteins but they do have the 35-kDa polypeptide fibrillarin in common. Hence, U3, U8, and U13 RNPs can be immunoprecipitated by antibodies directed against fibrillarin [99].

SS-A/Ro

There are two main classes of ANA in patients with SLE that are related to ANA seen in patients with Sjögren's syndrome. These two antigens were termed SS-A and SS-B by Alspaugh and Tan [100] because they were precipitated with the sera of patients with Sjögren's syndrome. Another group of investigators described a soluble nuclear antigen, termed Ha, that was precipitated with the sera of patients with SLE—Sjögren's overlap syndrome [101]. It was shown that SS-B and Ha were identical to La, another antibody system demonstrated by the same individuals. Through interlaboratory exchange of sera and antigen extracts, it was determined that SS-A was identical to Ro, an antibody system described in 1974 by Mattioli and Reichlin [102] and by Alspaugh and Madison [103]. Anti-SS-A/Ro antibodies are rarely found in normal individuals [104] or in hospitalized patients without systemic rheumatic diseases [105].

Studies of the molecular characteristics of the SS-A/Ro antigen have generated much interest. The reactive antigen has been described as various molecular species ranging from 50 to 150 kDa [1, 27, 106, 107]. An RNP particle composed of a 60-kDa protein and human cytoplasmic RNAs (hyRNA) was the first antigenic moiety described [108, 109]. The 60-kDa protein was thought to be the site of antibody binding, as SS-A/Ro sera did not bind to hyRNA alone. Some sera bind to unique determinants expressed when hy5RNA is complexed to the 60-kDa protein [110, 111]. Besides the 60-kDa protein, greater than 80% of SS-A/Ro sera identified in conventional double immunodiffusion assays bound a 52-kDa protein [106]. The reason this protein was not detected in earlier studies was likely due to the coexistence of SS-B/La antibodies and the incomplete separation of the 52-kDa SS-A/Ro and 47-kDa SS-B/La polypeptides by gel electrophoresis [112]. Expression cloning of cDNAs for the 52-kDa [113–115] and 60-kDa proteins [115, 116] and gene structure analysis showed that these two antigens are encoded by distinct genes on human chromosomes 1 and 11, respectively [117, 118]. The predominant cellular 60 kDa has a RNA recognition motif and has been shown to bind hy RNAs directly in an *in vitro* reconstitution assay [116]. The RNA-binding domain is conserved among human, mouse [119], and *Xenopus* 60-kDa protein [120]. A zinc finger was originally postulated in the center domain of the 60-kDa protein [115, 116], this putative zinc finger is not conserved in the mouse homologue [119]. The 60-kDa protein has been proposed to function as part of a novel quality control or discard pathway for 5S rRNA precursors in *Xenopus* oocytes [121]. Because 5S RNA is not known to be associated with the 60-kDa protein in mammalian cells, the function of the 60-kDa protein in mammalian cells remains to be determined.

Based on the N-terminal zinc finger domains and two coiled-coil regions, including a leucine zipper identified in the 52-kDa protein, the function for the 52-kDa protein has been implicated in transcription regulation because both of these structural motifs are found in transcription factors [113]. Furthermore, the overall structure of the 52-kDa protein is highly homologous to

the rpt-1 protein, a protein that downregulates the expression of the α chain of the IL-2 receptor and the human immunodeficiency virus type 1 proteins [113]. It has been shown that the leucine zipper of the 52-kDa protein is responsible for self-dimer formation [122]. Two forms of the human 52-kDa SS-A/Ro protein autoantigen, 52α and 52β, are products of alternative mRNA splicing [123]. The 52α form is expressed ubiquitously, whereas 52β, lacking the central leucine zipper domain, has been detected at higher levels than 52α during certain stages of fetal development [124]. In cell transfection transcription reporter assays, a six fold increase in transcription activity of the reporter was detected with the 52β construct compared to 52α or the empty vector control [122]. It is speculated that the ratio of cellular 52α and 52β may play an important role in regulating gene expression as a potential repressor and activator, respectively [122].

The consensus is that SS-A/Ro antibodies recognize both the 60- and the 52-kDa protein independently with at least two or more autoepitopes for each protein [125, 126]. Cross-reactivity of these two antibodies has not been observed consistently [127]. It has been shown that the immune response to 52- and 60-kDa proteins is linked in experimental mice when they are immunized with one or the other antigens [128, 129]. These data provide a confirmation that the linked response in human disease can be reproduced *in vitro*. The question whether the 60-kDa protein is directly complexed to the 52-kDa protein has been disputed. Boire *et al*. [130] have not been able to observe the association of the 52-kDa protein with biochemically purified hy RNPs. Work suggests that, in K562 cells, RoRNPs, especially hy3 RNPs, were immunoprecipitated effectively by certain anti-52-kDa SS-A/Ro peptide antibodies, indicating that the 52-kDa SS-A/Ro protein is associated with at least a subset of RoRNP particles [131].

Antibodies to SS-A/Ro occur in 60–70% of patients with Sjögren's syndrome, 5–10% of other connective tissue diseases, and 40% of patients with SLE [27, 132, 133]. SS-A/Ro antibodies are closely associated with disease subsets (Table 2) such as neonatal lupus erythematosus syndrome (NLS) [134–136], a photosensitive skin rash and subacute cutaneous lupus erythematosus [137–140], interstitial pneumonitis [141, 142], homozygous deficiency of complement proteins C2 and C4 [143, 144], idiopathic thrombocytopenic purpura [145–147], and the purpuric vasculitis typical of Sjögren's syndrome [132, 148]. Titers of SS-A/Ro antibodies have been correlated with lymphopenia [149], and when SS-B/La accompanies SS-A/Ro, the patients have an apparent decreased frequency of nephritis [149–151]. The impression that anti-SS-A/Ro antibodies fluctuate with disease activity [152, 153] is still open to debate and may depend on the assay being used. A new 75-kDa phosphoprotein (pp75) that binds to the carboxyl domain of the 60 kDa SS-A/Ro has also been shown to be an autoantigenic target in 17% of mothers of children with NLS and 6% of Sjögren's syndrome [154].

A study of 60 primary Sjögren's syndrome patients and 90 SLE patients showed that there is a dissociation of the immune responses to the 52- and 60-kDa antigens [155]. In this study, SS-A/Ro immunoprecipitins were identified in 78% of SS patients and in 57% of SLE sera. Of note, 13% of Sjögren's syndrome patients and 35% of SLE patients did not react with either protein in immunoblots. One explanation for this finding is that the immunoblotting techniques abolish epitopes that are expressed in the native protein, a finding in keeping with those of Boire *et al.* [60, 110, 111]. In addition, some patients react primarily with the deproteinized hy5 RNA [18, 60].

Studies of SS-A/Ro have also received much interest in the context of the NLS, a condition that occurs in approximately 1 of every 20,000 births and is characterized by a typical rash and/or congenital heart block (CHB) [156]. This interest was generated when it was noted that in three infant–mother pairs, both infants and their mothers had anti-SS-A/Ro in their serum [135]. In follow-up studies it was noted that anti-SS-A/Ro disappeared from the sera of the children, but continued to be present in the mothers. This suggests transplacental passage of antibody from mother to infant. The high association of isolated congenital complete heart block and anti-SS-A/Ro has been verified in a study of sera banked in the European Registry of CHB [136] (Table 3) and in a large study of CHB cases. There are several cases in which an alert physician aware of the association between anti-SS-A/Ro and NLS identified asymptomatic SLE in a mother who had circulating anti-SS-A/Ro antibodies. It has been suggested that NLS is characterized by antibodies directed against the 52-kDa protein [157, 158] and the concomitant occurrence of SS-A/Ro and SS-B/La [159]. Of interest, both SS-A/Ro and SS-B/La antigens have been detected on

TABLE 3 Serologic Findings in 21 Children with Isolated Congenital Complete Heart Block[a]

Age at time of serum collection	Number of children		No. of mothers with anti-SS-A/Ro
	Total	With anti-SS-A/Ro	
<3 months	8	7	7
>6 months	13	0	11

[a] Adapted from Scott *et al*. [136], with permission.

the surface of myocardial fibers in CHB [160], and the 52β isoform of SS-A/Ro is highly expressed in heart tissue during embryogenesis [124]. In other studies, anti-SS-A/Ro has been shown to alter the transmembrane action potential of isolated cardiac cells [161] and Langendorff preparations of adult rabbit hearts [162]. These studies have added evidence that anti-SS-A/Ro autoantibodies play a pathogenic role in congenital heart block.

The availability of cDNAs encoding SS-A/Ro antigens and their respective recombinant proteins has permitted studies that defined the fine specificity of SS-A/Ro autoantibody reactivity. The expression of recombinant SS-A/Ro fragments has facilitated the definition of epitopes on the 60-kDa [125, 163, 164] and 52-kDa [113] antigens. Curiously, some antigenic peptides of the 60-kDa antigen tend to show short sequence homology with vesicular stomatitis virus nucleocapsid protein [165, 166]. Humans infected with vesicular stomatitis virus have low titers of anti-SS-A/Ro [167], and animals immunized with vesicular stomatitis nucleocapsid produce antibodies that react with 60-kDa SS-A/Ro [168]. These interesting studies will need to be reproduced and the association of stomatitis virus and SLE should be examined in the context of the pathogenesis and antibody production in SLE.

There has been some controversy concerning the question of ANA-negative lupus and the observation that many ANA-negative lupus patients have anti-SS-A/Ro [169, 170]. Several studies have clarified some of this controversy. One explanation is that ANA detected by IIF vary in sensitivity from one laboratory to the next [171]. A second explanation is that the concentration of SS-A/Ro antigen varies remarkably from one animal species to the next [172]. In species such as human, monkey, dog, and guinea pig, the SS-A/Ro antigen is present in higher concentrations than in species such as mouse, rat, and hamster. Therefore, when tissues from species such as rat or mouse are used as substrates in indirect immunofluorescence, a serum containing anti-SS-A/Ro antibody may not show reactivity. However, when human or guinea pig tissues are used, the serum would be positive. These observations are supported by studies showing that SS-A/Ro proteins extracted from human tissues demonstrate preferential reactivity with human SS-A/Ro antibodies [107, 173]. Thus ANA-negative lupus may be related to the high solubility of the antigen that can be washed away during tissue processing rather than the absence of ANA in the serum [6]. Therefore, it is important to use a known positive anti-SS-B/La control with each assay to be certain the antigen has not been solubilized.

Some dilemmas of using the ANA test to screen for SS-A/Ro antibodies in human serum are being resolved by using the transcribed recombinant 60-kDa DNA transfected into mammalian cells as an substrate for indirect immunofluorescence [174–180]. Alternatives are to use immunoblotting as a technique [112] and ELISA techniques that are able to identify reactivity that is undetected by conventional serological assays [173, 181, 182]. At present, the commercial kits designed to detect SS-A/Ro are known to have high sensitivity but may suffer from lack of specificity. In addition, there appears to be considerable variation in results obtained from kits supplied by different commercial vendors [182, 183]. It is clear that these techniques require considerable expertise and use of carefully controlled protocols [112, 182].

SS-B/La

The SS-B/La protein, a 46/48 kDa phosphoprotein [27, 184, 185], is highly conserved in mammals and is mainly associated with precursor forms of RNA polymerase III transcripts such as tRNAs, 7S RNA, and 5S RNA (Fig. 1). SS-B/La is increased in transformed cells [186], and virus-encoded RNAs, such as adenovirus-encoded VAI and VAII RNAs [187, 188], Epstein–Barr virus-encoded EBER RNAs [189, 190], and leader RNAs of some negative stranded viruses, are associated with the SS-B/La antigen [191, 192].

The SS-B/La antigen has RNA recognition motifs that bind to uridine-rich residues on the 3′ terminus of scRNAs [27] and may act as a shuttle to carry RNA transcripts from the nucleus to the cytoplasm [193]. The sequence of the antigen predicts a nuclear localization sequence and a putative ATP-binding site. One study has shown that SS-B/La is a nucleic acid-dependent ATPase/dATPase [194]. This enzyme can "melt" or separate the DNA/RNA hybrids that form during transcription and, along with SS-B/La, serves as a transcription initiation [195] and termination factor for RNA polymerase III [196]. All of these features may account for the observation that SS-B/La also increases the efficiency and fidelity of mRNA translation [197–199].

Epitopes of the SS-B/La antigen have been mapped to the RNA-binding domain [84] and a number of other regions [34, 200–204]. Of interest, peptides in the 88–101 segment bear sequence similarity with a retroviral *gag* protein [201], but the significance of this remains to be determined.

The antibody to SS-B-La is detected in 15–20% of SLE and in 45–60% of SS patients [1, 205]. Except for the overlapping presence of Sjögren's syndrome in patients with SLE, there does not appear to be any other distinguishing clinical features associated with the presence of anti-SS-B/La.

As with anti-SS-A/Ro, anti-SS-B/La is frequently detected by precipitin assays in sera yielding a false-negative ANA by IIF techniques [6]. Here it was felt that because of the high solubility of the SS-B/La antigen, it may be solubilized from tissue sections during the processing of the slides. Thus, it is important to use a known positive anti-SS-B/La control with each assay to be certain that the antigen has not been solubilized from the tissue and inadvertently washed from the slides. Such a positive control serum is available from the CDC reference bank in Atlanta, Georgia [38]. As with other autoantigen systems, the use of recombinant proteins in ELISA techniques may provide a more sensitive method to detect SS-B/La autoantibodies [206].

TABLE 4 Functions of Nuclear Autoantigens Inhibited by Autoantibodies[a]

Autoantigen	Function inhibited
Sm/nRNP (U1,2,4–6 snRNP)	Pre-mRNA splicing
PCNA (DNA pol δ auxiliary protein)	DNA replication and repair
Scl-70 (topoisomerase I)	DNA replication and transcription
RNA polymerase I	Transcription of rRNA
Centromere proteins CENP-A,B,C	Chromosome movement in mitosis
DNA polymerase II	DNA replication
tRNA synthetases	Amino acylation of tRNAs

[a] Adapted from Tan [224], with permission.

Proliferating Cell Nuclear Antigen

Autoantibody to a nuclear antigen in proliferating cells was initially detected by positive immunofluorescence staining of scattered cells in the interstitial tissue of kidney and liver and negative reactions with nuclei of renal tubular, glomerular, or hepatic parenchymal cells [207]. The autoantibody did not react with nondividing interphase cells, but did react with rapidly proliferating or mitogen-stimulated cells, and the nuclear antigen was called *proliferating cell nuclear antigen* (PCNA). This antigen was not restricted to a specific cell type and was found in activated T and B lymphocytes [208]. PCNA was soluble in saline and was immunologically distinct from DNA and other known nuclear antigen–antibody systems such as Sm, U1 RNP, or SS-B/La.

Early studies focused on characterization of the antigen and the discovery that PCNA was identical to a protein referred to as cyclin [209–212]. It is important to note that the usage of the term "cyclin" in the current literature is reserved for a different family of proteins with regulatory roles for the cell cycle [213]. After that, the pure protein was isolated, the amino terminus was sequenced [214], and monoclonal antibodies were produced [215–217]. Eventually a rat cDNA [218] and a full-length cDNA encoding human PCNA [219] were isolated. Shortly after the description of a 36-kDa protein that was required for chain elongation in DNA synthesis mediated by DNA polymerase δ [220], it was reported that PCNA is identical to the auxiliary protein of DNA polymerase δ [221].

The determinants recognized by human autoantibodies and monoclonal antibodies directed against PCNA protein are different [222]. Other studies have shown that human antibodies can inhibit oligonucleotide synthesis *in vitro* whereas monoclonal antibodies cannot [223] (Table 4). These observations are concordant with observations that other autoantibodies directed against nonhistone proteins are able to inhibit the function of the cognate antigens (Table 4) and this feature is not generally shared by monoclonal antibodies binding to the same protein [224].

Antibodies to PCNA are detected in less than 5% of patients with SLE [207, 208, 225]. However, despite its low frequency, it is regarded as a highly specific marker antibody for SLE [1, 6]. Because of the somewhat low frequency of these antibodies, it has been difficult to determine if they serve as a marker for a distinct clinical subset of patients. One study has suggested a high frequency of diffuse proliferative glomerulonephritis in anti-PCNA-positive patients, and the somewhat low frequency of the antibody may be due to high sensitivity to immunosuppressive therapy [225]. Another study [208] did not identify unique clinical features, except for a higher frequency of lymphadenopathy.

Ku, Sp1

Different investigators in the same laboratory [226, 227] identified the Ku and Ki antibody systems almost simultaneously, and initially it was thought that Ki and Ku were identical [228, 229] (see Fig. 4). Subsequently, distinct antigens were cloned and identified [230]. In immunoprecipitation with [^{35}S]methionine labeled cells, the Ku antigen system has been shown to consist of a pair of ~70- and 80-kDa proteins designated p70/p80. Unlike other nonhistone proteins (i.e., Sm, U1 RNP, SS-A/Ro), p70/p80 are not bound tightly to RNA but form heterodimers and bind tightly to free ends of DNA [229, 231] where they serve as an anchor for a 350-kDa catalytic peptide with DNA-dependent protein kinase activity [232]. This complex appears to be involved in the repair of DNA double helix breaks and in VDJ recombination [233]. The association of these proteins

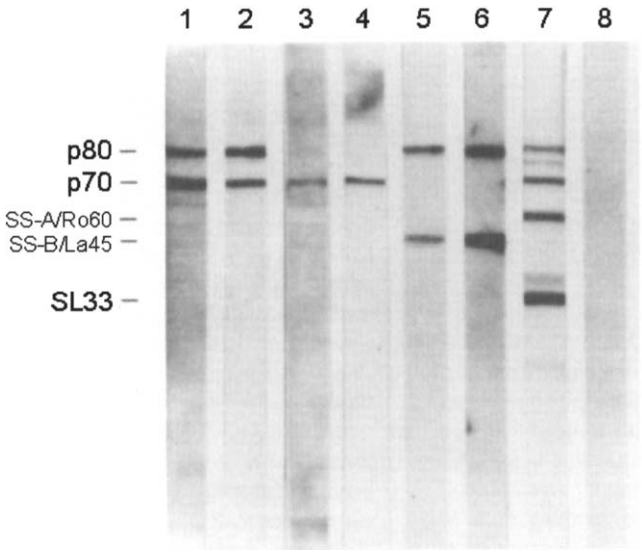


FIGURE 4 Immunoblotting studies showing five autoantigen systems. The HeLa cell extract was separated in SDS polyacrylamide gels and transferred to nitrocellulose. The first two antibody systems are represented in lanes 1 and 2 and show reactivity with both of the Ku p70 and p80 proteins. As illustrated in lanes 3 and 4, some sera react only with the p70 Ku antigen. In lanes 5 and 6, sera with reactivity to the p80 antigen but not the p70 Ku antigen are shown. In lane 7, a serum with reactivity with both Ku antigens and the Ki/SL (SL33) antigen is shown. Some sera with anti-Ku activity also bind SS-A/La (lanes 5 and 6) and 60-kDa SS-A/Ro (lane 7). Lane 8 is normal serum.

in vivo was demonstrated by colocalization of the antigen on early prophase and late telophase chromosomes. Curiously, these antigens appear to dissociate from chromosomes in metaphase and anaphase of the cell cycle, although they are reassociated to the nucleoplasm and nucleolus in interphase [229]. Analysis of anti-Ku sera by IIF demonstrated a speckled pattern of nuclear staining, and the nucleoli were stained in the G1 phase of the cell cycle [234, 235].

Patients with anti-Ku were initially characterized clinically by a predominance of arthritis, pericarditis, and pulmonary hypertension, but since then, antibodies to the Ku antigen have been described in a variety of conditions [230, 236]. In Japanese patients, anti-Ku is commonly associated with overlap syndromes, especially polymyositis/scleroderma and also in SLE/scleroderma [226]. One study used immunopurified p70/p80 antigen in sensitive ELISA or immunoblotting assays to identify Ku antibodies in 20–40% of SLE patients, 15–40% of scleroderma patients [229, 235], ~10% of myositis patients [235], 20% of Sjögren's syndrome patients [235], and 55% of MCTD patients [229]. The variation in antibody frequency in these studies is likely related to differences in the selection of sera, the assays employed, and the racial background of the patient [198].

Most patient sera recognize both Ku polypeptides in immunoblots, but the ratio of the intensity of reactivity of antibodies to the p80 and p70 antigens vary from serum to serum [235]. This suggested that the two proteins were the product of separate genes [237, 238], a theory that was supported when it was demonstrated that the cDNA sequence of the two proteins shows no amino acid sequence homology [239]. Nonetheless, some evidence has suggested that there are both unique and shared determinants on the p70 and p80 proteins. Because there is no amino acid homology between these two peptides, it is suggested that the shared determinants are conformational epitopes on these proteins. A study has shown that some autoantibodies bind to a presumably conformational epitope on the p70/p80 complex [240].

Many autoantibodies tend to be associated as "linked sets" [11], and in that context, it is interesting that there is a high frequency of anti-Sm antibodies in anti-Ku-positive sera [228, 235]. Although patients with anti-Sm frequently have concomitant anti-U1RNP antibodies, it appears that the "linked set" of anti-Ku/anti-Sm is to the exclusion of anti-U1RNP [235].

Sp1 and Ku are components of macromolecular complexes referred to as terminal repeat binding proteins (TRBP) [241]. Sp1 is phosphorylated by Ku, is characterized by a zinc finger domain, and serves as an RNA polymerase II transcription activator [241]. A study of eight patients who had antibodies directed against Sp-1 as determined by an electrophoretic mobility shift assay and immunoprecipitation showed that the majority had features of SLE (i.e., arthritis, fatigue, photosensitivity) but only one met the diagnostic criteria for SLE [241]. In addition, antibodies to a phosphorylated form of RNA polymerase II has also been shown to be an autoantigenic target in SLE [242].

Ki/SL

The Ki antigen is a ~31-kDa protein [238, 243, 244] that has sequence homology with topoisomerase IIα [245]. The sequence is highly conserved and has a predicted nuclear localization sequence [244]. ELISA has been used to detect anti-Ki/SL antibodies in up to 12% of SLE sera and overlap syndrome sera [246]. More recently, it was shown that an epitope recognized by SLE sera has sequence identity with the SV40 large T antigen nuclear localization signal [247]. This 31-kDa Ki antigen should not be confused with the nuclear proliferation-associated Ki-67 antigen [248, 249].

Ribosomal RNP and P Proteins

The mammalian ribosome is a cytoplasmic organelle consisting of 28S, 18S, 5.8S, 5S RNA, and some 80 different proteins [250, 251]. Because ribosomal RNP, (rRNP) antigens are cytoplasmic and nucleolar in location, a discussion of their biochemical characteristics and clinical relevance is included in this chapter. Although many of the ribosomal proteins are basic, the rRNP-P antigens (P0, P1, P2) are acidic phosphoproteins of 36, 19, and 17 kDa [252, 253]. The phosphoprotein moiety may account for observations that some high-affinity antibodies to dsDNA can also bind to these P proteins [254, 255]. P1 and P2 are involved in three steps of polypeptide synthesis: initiation, translocation, and termination [256]. Autoantibodies to other constituents, such as L12, the 20-kDa 60S subunit, S10 the 20-kDa 40S subunit, L5 the 35-kDa protein of the 5S subunit, and 28S ribosomal RNA have been described in SLE [257–260].

Autoantibodies from SLE patients cross-react with similar antigens present in rodent, brine shrimp, and yeast cells, demonstrating that the proteins and their epitopes have been conserved. Although the major epitope recognized by SLE sera resides within a 22 amino acid domain in the carboxyl-terminal region common to P0, P1, and P2, additional epitopes are recognized because the carboxyl peptides do not absorb the reactivity of some human sera to the native protein [253, 261]. It is possible that conformational epitopes not detected using short peptides are also recognized by SLE sera [261]. A mouse monoclonal antibody [262] and spontaneously arising autoantibodies in MRL/lpr mice recognize the same spectrum of rRNP-P proteins that are recognized by human autoantibodies [263].

The IIF staining pattern on HEp-2 cells is characterized by homogeneous or finely granular cytoplasmic, with or without nucleolar, staining [264] but it should be noted that at least 50% of sera with anti-P proteins do not show any staining on conventionally prepared tissue substrates. Immunoassays employing the recombinant P2 fusion protein or synthetic constructs of the dominant epitope have been developed and were found to correlate with immunoblotting and ELISA of the intact proteins [261, 265]. Also, anti-rRNP antibodies are associated more frequently with anti-Sm antibodies (present in 20% of sera containing anti-Sm) than with any other autoantibodies of the lupus profile [252].

Antibodies to ribosomal P proteins, ribosomal ribonucleoprotein (rRNP), 28S ribosomal RNA, and the L12/S10 subunits have been identified in the sera of 3–70% of SLE patients. Antibodies to the L12 subunit were found in higher frequency in juvenile SLE [266]. Antibodies to the S10 subunit appear to be less common than anti-L12 [258, 266, 267] and may be related to anti-Sm activity [266]. Reichlin and collaborators showed that patients with anti-rRNP appear to identify a subset of SLE patients with liver and kidney involvement, although some of their patients also had concomitant antibodies to native DNA [268–270]. It has been reported that 30–90% of patients, including children, with neuropsychiatric lupus have P protein antibodies [270–277] and the titers fluctuate in concert with disease activity [278]. Other studies [41, 271, 277] have not confirmed these clinical correlates.

HIGH MOBILITY GROUP PROTEINS

High mobility group proteins (HMG) are chromosomal proteins that are defined by their extractability with 0.35 *M* NaCl, solubility in 2–5% perchloric acid, a high content of charged amino acids, and a molecular mass of less than 30 kDa. They possess an extraordinary amino acid composition of 50% acidic and basic residues and a somewhat high content of proline. There are three distinct families of HMG proteins; the HMG-1/-2 family, the HMG-14/-17 family, and the HMG-I family. HMG-1 and HMG-2 comprise most HMG protein in the cell and have four domains: an unstructured N terminus, two basic folded regions containing many hydrophobic amino acids (DNA-binding domains), and a highly acidic carboxyl terminus (histone-binding domain). HMG-17 and HMG-14 are characterized by a highly basic amino terminus and a highly acidic carboxyl terminus. HMG-1 and HMG-2 bind to the linker DNA in chromatin, whereas HMG-17 and HMG-14 bind primarily to nucleosomes, especially those associated with transcriptionally active chromatin [279]. Of interest, binding of these HMGs may increase the susceptibility of chromatin to digestion by DNase I. The HMG-I family includes the HMG-I protein (also called a-protein) and its isoform HMG-Y. The amino acid composition of these proteins resembles HMG-14/-17 proteins but they appear to bind preferentially to A-T-rich DNA and are found in highest concentrations in rapidly dividing cells.

In one study, antibodies to calf thymus HMGs were found in 13/29 (45%) SLE sera and of these, 10 reacted with HMG-17, 3 with HMG-1, and 2 with HMG-2 [280]. In contrast, HMG antibodies were found in only 1/11 MCTD and in 0/14 RA patients. This study agrees with studies showing that antibodies to HMG-17 are prominent in SLE [281]. Of interest, there was a high correlation between the presence of HMG antibodies and histone antibodies, an observation that lends additional

evidence to the concept that intact nucleosomes serve as the autoantigenic stimulus in some SLE patients [282]. Further, some studies have suggested that HMG-17 resides on a subset of nucleosomes that are located in transcribable regions of the genome [283–285]. This suggests that one of the immunogens in SLE may be a subset of nucleosomes that contain specialized DNA sequences involved in the processing of messenger RNA. This possibility brings into sharper focus the recurring "theme" of autoantibodies in SLE that are characterized by their binding to various macromolecules involved in gene expression. Other studies have shown that HMG antibodies are found in drug-induced lupus [286], systemic sclerosis [287], idiopathic pulmonary fibrosis [288], and juvenile rheumatoid arthritis where they are correlated with a positive ANA [289, 290]. A study using ELISA and immunoblotting techniques showed that over 28% of SLE sera react with the HMG-associated protein known as structure specific recognition protein 1 (SSRP1) [291].

Ubiquitin and Proteasome

Ubiquitin is a low molecular weight, highly conserved eukaryotic protein that exists as a free molecule or as a protein linked covalently to a variety of cytoplasmic, nuclear, and cell membrane proteins [292, 293]. Most proteins that are degraded in the cytosol are delivered to a large protein complex called the proteasome [294]. In this organelle, degradation proceeds by a ubiquitin- and ATP-dependent mechanism. Histones H2A and H2B, which are targets of SLE autoantibodies [295, 296], are ubiquitinated during the cell cycle and during stress responses. Antibodies to ubiquitin and a synthetic fragment of ubiquitin were found in up to 80% of SLE patients and in 16% of other systemic rheumatic diseases [297, 298]. Further analysis of antibody reactivity to ubiquitin–protein conjugates has shown that 95% of SLE sera that bind ubiquitin contain antibodies specific for the branched ubiquitin-H2A peptide [299] and may have a role in the pathogenesis of lupus nephritis [300]. The α type subunit C9 of the 20S proteasome complex has been shown to be a SLE target antigen as well [294, 301].

Nuclear Lamins

The nuclear envelope is composed of four major structural components: a double membrane consisting of an inner laminar layer, an outer layer that is continuous with the endoplasmic reticulum, nuclear pores where the outer layer abuts the inner layer, and the interlaminar "space," also known as the *nuclear lamina* [302–304]. Electron microscopic analysis of the nuclear lamina reveals a meshwork of intermediate filaments that line the inner surface of the inner nuclear membrane [304, 305]. The nuclear lamina is rich in three structural proteins of 74, 68, and 60 kDa also called A, B, and C proteins. It is thought that lamins A and C serve as anchoring sites for interphase chromatin, whereas lamin B binds to the inner nuclear membrane [306]. Analysis of the amino acid sequence of the lamins shows similarity to the cytoskeletal intermediate filament proteins [307, 308].

Autoantibodies to nuclear lamins are characterized by linear staining of the nuclear envelope that clearly delineates the nucleus from the cytoplasm [309], and in some laboratories this is called a *peripheral* or *rim* pattern of staining [310]. After the introduction of IIF for the identification of antinuclear antibodies, it was felt that the rim pattern of staining represented antibodies to dsDNA [311]. Although these sera often contained anti-DNA antibodies, it is probable that the rim pattern of staining represented antibodies directed against other components of the nuclear membrane. It was suggested that sera containing monospecific antibodies to DNA can be differentiated from sera containing anti-lamin antibodies based on these staining patterns on IIF [309]. However, if the DNA and lamin antibodies occur in the same serum, a distinctive staining pattern is less apparent. Because most studies of the frequency of lamin antibodies have been based on sera selected for specific staining patterns, the frequency of lamin antibodies in systemic rheumatic diseases may be underestimated.

Nuclear lamin antibodies have been described in a variety of autoimmune diseases, including systemic lupus erythematosus [312–314], linear scleroderma [315], autoimmune (lupoid) hepatitis [312, 316, 317], chronic fatigue syndrome [318], rheumatoid arthritis [319], and primary biliary cirrhosis [320, 321]. An analysis of the patients in these reports suggests that common clinical features in patients with lamin antibodies are the presence of "lupoid" or chronic active hepatitis [317, 320] and the lupus anticoagulant [322]. In addition, diseases may be differentiated based on antibodies that bind to specific lamin proteins and epitopes [80]. For example, sera from SLE patients (particularly those with cytopenias) react predominantly to a unique domain on the B protein, whereas patients with autoimmune liver diseases bind to A and C proteins. This is of interest because the sequences of lamins A and C differ only at the carboxyl terminus, whereas the B protein appears to have less sequence similarity to the others. However, the observation that monoclonal antibodies can recognize all three lamins suggests that they bear similar epitopes [323].

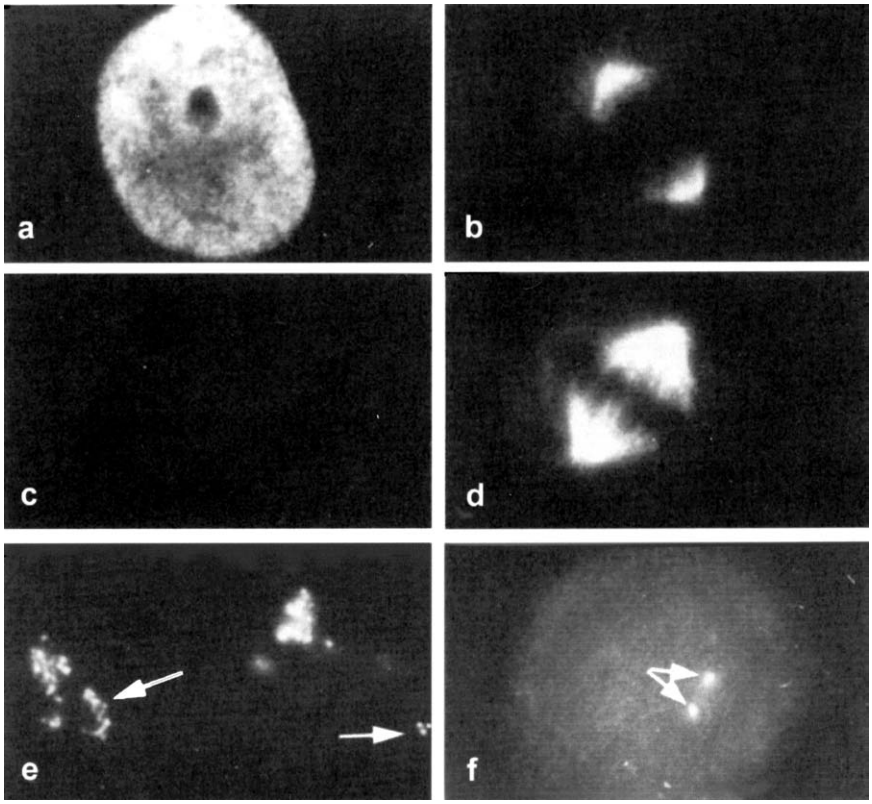


FIGURE 5 A comparison of the IIF patterns of human antibodies to NuMA (**a** and **b**), HsEg5 (**c** and **d**), and ASE-1 (**e** and **f**). In interphase cells, NuMA is represented by a fine speckled staining pattern (**a**) and bright staining of the centrosome and the spindle fibers proximal to the centrosomes (**b**). In comparison, antibodies to HsEg5 show no staining of interphase cells (**c**) but bright staining that extends along kinetochore-to-pole microtubules in metaphase cells (d). ASE-1 antibodies stain discrete foci in the fibrillar centers of interphase nucleoli (**e**, arrows) and the nucleolar organizer regions of condensed metaphase chromosomes (**f**, arrows).

Mitotic Spindle Apparatus

A number of autoantigens that reside in the mitotic spindle apparatus (MSA) have been described [324]. These include the nuclear mitotic apparatus (NuMA) [325] and the human spindle kinesin-like (HsEg5) [326] proteins. NuMA, also referred to as mitotic apparatus antigen I (MA-I) by Andrade *et al.* [325], is a 235-kDa protein characterized by IIF as a finely speckled nuclear pattern in interphase cells and bright staining of the spindle poles and associated fibers during metaphase (Figs. 5a and 5b). By comparison, HsEg5 is a ~130-kDa protein that does not appear in interphase nuclear IIF staining but is characterized by intense staining of the spindle fibers at metaphase, the interpolar fibers at anaphase, and intercellular bridge staining in telophase (Figs. 5c and 5d) [326]. When autoantibodies directed against HsEg5 occur in isolation, the distinctive IIF pattern is easily identified. However, in a few patients, autoantibodies to HsEg5 and NuMA have been found to coexist, making the distinction of the two autoantibodies in the same serum difficult. A study of 7 patients who had antibodies to HsEg5 showed that 6 had SLE and 1 had Sjögren's syndrome, and the autoantibody to HsEg5 was only found in 6% of an unselected cohort of SLE patients [326]. In this same study, only 1 of 52 SLE patients had anti-NuMA. In another study of 17 patients with antibodies to NuMA, 9 (53%) had Sjögren's syndrome and none had SLE [325]. Antibodies to NuMA have also been reported in patients with systemic sclerosis and other diseases [327, 328].

Other Antigens

Autoantibodies in SLE directed against other nonhistone intracellular antigens include the Golgi apparatus [329–331], endosomes [332, 333], mitochondria [334–341], a multicatalytic protease [342], red blood cell carbonic anhydrase [343, 344], RNA polymerase I [160],

TABLE 5 Comparison of ANA Profiles in Five Systemic Rheumatic Diseases

	Systemic rheumatic disease				
Antibodies to	**SLE**	**Sjögren's syndrome**	**MCTD**	**Systemic sclerosis**	**Polymyositis–dermatomyositis**
dsDNA	+	–	–	–	–
U1 RNP	+	–	+	+	+
Sm	+	–	–	–	–
SS-A/Ro	+	+	–	–	–
SS-B/La	+	+	–	–	–
Scl-70	–	–	–	+	–
Centromere	–	–	–	+	–
DM/PM antigens[a]	–	–	–	–	+

[a] Dermatomyositis/polymyositis antigens include Jo-l, Mi2, and Pl-7.

Alu RNA–protein complex [345], a 90-kDa nucleolar organizer upstream binding factor for RNA polymerase I [346, 347], p53 [348, 349], p57 [350], calreticulin [351, 352], and a number of proteins related to DNA binding and DNA metabolism [241, 353–356]. More recently described autoantibodies in SLE sera include those directed against a 100-kDa RNA helicase (Gu) [357], the DEK oncoprotein [358, 359], and ASE-1, an antigen in the fibrillar center of nucleoli that has a distribution and molecular mass similar to NOR-90 (Figs. 5e and 5f) [360]. In SLE sera, ASE-1 tends to be commonly associated with sera that also have anti-Sm antibodies [360, 361]. Cross-reacting antibodies and molecular mimicry have been areas of some interest, and it has been shown that a subset of anti-DNA antibodies bind to a molecular mimic pentapeptide Asp/Glu-Trp-Asp/Glu-Tyr-Ser/Gly found in the NR2 glutamate receptor [362].

SUMMARY: SLE IS CHARACTERIZED BY DISTINCT AUTOANTIBODY PROFILES

Distinct profiles of ANA have been found in different systemic rheumatic diseases, characteristics of which include the presence or absence of certain antibodies and differences in mean titers of these antibodies. Table 5 outlines the ANA profiles of SLE and four other systemic rheumatic diseases: Sjögren's syndrome, MCTD, myositis, and scleroderma. The following conclusions can be made from these profiles: (1) multiple ANA are found frequently in SLE; (2) anti-Sm, anti-PCNA, and anti dsDNA are distinctive markers for SLE; (3) anti-U1-RNP is present in several diseases but in different frequencies; (4) anti-Scl-70 and anticentromere are distinctive for scleroderma; and (5) anti-Jo-1 and anti-Pl-7 antibodies are distinctive for polymyositis.

References

1. von Muhlen, C. A., and Tan, E. M. (1995). Autoantibodies in the diagnosis of systemic rheumatic disease. *Semin. Arthritis Rheum*. **24**, 323–358.
2. Fritzler, M. J. (1997). Autoantibodies: Diagnostic fingerprints and etiologic perplexities. *Clin. Invest. Med.* **20**, 50–66.
3. Fritzler, M. J. (1996). Clinical relevance of autoantibodies in systemic rheumatic diseases. *Mol. Biol. Rep.* **23**, 133–145.
4. Harley, J. B. (1994). Autoantibodies are central to the diagnosis and clinical manifestations of lupus. *J. Rheumatol.* **21**, 1183–1185.
5. Tan, E. M., *et al*. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271.
6. Tan, E. M. (1989). Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* **44**, 93–151.
7. Tan, E. M., Chan, E. K. L., Sullivan, K. F., and Rubin, R. L. (1988). Antinuclear antibodies (ANAs): Diagnostically specific immune markers and clues toward the understanding of systemic autoimmunity. *Clin. Immunol. Immunopathol.* **47**, 121–141.
8. Tan, E. M. (1991). Autoantibodies in pathology and cell biology. *Cell* **67**, 841–842.
9. Tan, E. M., and Chan, E. K. L. (1993). Molecular biology of autoantigens and new insights into autoimmunity. *Clin. Invest.* **71**, 327–330.
10. Conrad, K., Tan, E. M., Humbel, R.-L., and Shoenfeld, Y. (1997). Autoantibodies: Diagnostic, pathogenic and pathognostic relevance. *Clin. Exp. Rheumatol.* **15**, 457–465.
11. Craft, J., and Hardin, J. A. (1987). Linked sets of antinuclear antibodies: What do they mean? *J. Rheumatol.* **14**(Suppl.), 106–109.
12. Hardin, J. A. (1986). The lupus autoantigens and the pathogenesis of systemic lupus erythematosus. *Arthritis Rheum.* **29**, 457–460.

13. Reichlin, M. (1994). Antibodies to ribonuclear proteins. *Rheum. Dis. Clin. North Am.* **20**, 29–43.
14. Reeves, W. H., and Satoh, M. (1996). Features of autoantigens. *Mol. Biol. Rep.* **23**, 217–226.
15. Van Venrooij, W. J., and Sillekens, P. T. G. (1989). Small nuclear RNA associated proteins: Autoantigens in connective tissue diseases. *Clin. Exp. Rheumatol.* **7**, 635–645.
16. Deutscher, S. L., and Keene, J. D. (1988). A sequence-specific conformational epitope on U1 RNA is recognized by a unique autoantibody. *Proc. Natl. Acad. Sci. USA* **85**, 3299–3303.
17. Uchium, T., Traut, R. R., Elkon, K., and Kominami, R. (1991). A human autoantibody specific for a unique conserved region of 28 S ribososmal RNA inhibits the interaction of elongation factors 1α and 2 with ribosomes. *J. Biol. Chem.* **266**, 2054–2062.
18. Boulanger, C., Chabot, B., Ménard, H. A., and Boire, G. (1995). Autoantibodies in human anti-Ro sera specifically recognize deproteinized hY5 Ro RNA. *Clin. Exp. Immunol.* **99**, 29–36.
19. Granger, D., Tremblay, A., Boulanger, C., Chabot, B., Menard, H.-A., and Boire, G. (1996). Autoantigenic epitopes on hY5 Ro RNA are distinct from regions bound by the 60-kDa Ro and La protiens. *J. Immunol.* **157**, 2193–2200.
20. Hoet, R. M., De Weerd, P., Gunnewiek, J. K., Koornneef, I., and Van Venrooij, W. J. (1992). Epitope regions on U1 small nuclear RNA recognized by anti-U1RNA-specific autoantibodies. *J. Clin. Invest.* **90**, 1753–1762.
21. Van Venrooij, W. J., Hoet, R., and Castrop, J. (1990). Anti(U1) small nuclear RNA antibodies in anti-small nuclear ribonucleoprotein sera from patietns with connective tissue disease. *J. Clin. Invest.* **86**, 2154–2160.
22. Hoffman, R. W., Sharp, G. C., and Deutscher, S. L. (1995). Analysis of anti-U1 RNA antibodies in patients with connective tissue disease. *Arthritis Rheum.* **38**, 1837–1844.
23. Van Venrooij, W. J., and Pruijn, G. J. M. (1995). Ribonucleoprotein complexes as autoantigens. *Curr. Opin. Immunol.* **7**, 819–824.
24. Habets, W. H., DeRooij, J., and Salden, M. H. (1983). Antibodies against distinct nuclear matrix protein are characteristic of mixed connective tissue disease. *Clin. Exp. Immunol.* **54**, 265–271.
25. Fritzler, M. J., Ali, R., and Tan, E. M. (1984). Antibodies from patients with mixed connective tissue disease react with heterogeneous nuclear ribonucleoprotein or ribonucleic acid (hnRNP-RNA) of the nuclear matrix. *J. Immunol.* **132**, 1216–1222.
26. Slobbe, R. L., Pluk, W., Van Venrooij, W. J., and Pruijn, G. J. M. (1992). Ro ribonucleoprotein assembly *in vitro*: Identification of RNA-protein and protein-protein interactions. *J. Mol. Biol.* **227**, 366.
27. Chan, E. K. L., and Andrade, L. E. C. (1992). Antinuclear antibodies in Sjögren's syndrome. *Rheum. Dis. Clin. North Am.* **18**, 551–570.
28. Conner, G. E., Nelson, D., Wiesniewolski, R., Lahita, R. G., Blobel, G., and Kunkel, H. G. (1982). Protein antigens of the RNA-protein complexes detected by anti-Sm and anti-RNP antibodies found in serum of patients with systemic lupus erythematosus and related disorders. *J. Exp. Med.* **156**, 1475–1486.
29. White, P. J., Gardner, W. D., and Hoch, S. O. (1981). Identification of the immunologically active components of the Sm and RNP antigens. *Proc. Natl. Acad. Sci. (USA)* **78**, 626.
30. Pettersson, I., Hinterberger, M., Mimori, T., Gottlieb, E., and Steitz, J. A. (1984). The structure of mammalian small nuclear ribonucleoproteins. *J. Biol. Chem.* **259**, 5907–5914.
31. Craft, J. (1992). Autoantibodies to snRNPs in SLE. *Rheum. Dis. Clin. North Am.* **18**, 311–335.
32. Lerner, M. R., and Steitz, J. A. (1979). Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **76**, 5495–5497.
33. Kambach, C., *et al*. (1999). Crystal structures of two Sm protein complexes and their implications for the assembly of the spliceosomal snRNPs. *Cell* **96**, 375–387.
34. Coppel, R. L., Gershwin, M. E., and Sturgess, A. D. (1989). Cloned antigens in the study and diagnosis in autoimmune diseases. *Mol. Biol. Med.* **6**, 27–34.
35. Williams, D. G. (1990). Recombinant autoantigens. *Ann. Rheum. Dis.* **49**, 445–451.
36. Barakat, S., Briand, J.-C., Weber, J.-C., Van Regenmortel, M. H. V., and Muller, S. (1990). Recognition of synthetic peptides of Sm-D autoantigen by lupus sera. *Clin. Exp. Immunol.* **81**, 256–262.
37. de Rooij, D. J. R. A. M., Habets, W. J., van de Putte, L. B. A., Hoet, M. H., Verbeek, A. L., and Van Venrooij, W. J. (1990). Use of recombinant RNP peptides 70 K and A in an ELISA for measurement of antibodies in mixed connective tissue disease: A longitudinal follow up of 18 patients. *Ann. Rheum. Dis.* **49**, 391–395.
38. Tan, E. M., *et al*. (1982). Reference sera for antinuclear antibodies. I. Antibodies to native DNA, Sm, nuclear RNP, and SS-B/La. *Arthritis Rheum.* **25**, 1003–1005.
39. Nakamura, R. M., and Tan, E. M. (1977). Recent progress in the study of autoantibodies to nuclear antigens. *Hum. Pathol.* **9**, 85–91.
40. Notman, D. D., Kurata, N., and Tan, E. M. (1975). Profiles of antinuclear antibodies in systemic rheumatic diseases. *Ann. Intern. Med.* **83**, 464–469.
41. Boey, M. L., Peebles, C. L., Tsay, G., Feng, P. H., and Tan, E. M. (1988). Clinical and autoantibody correlations in Orientals with systemic lupus erythematosus. *Ann. Rheum. Dis.* **47**, 918–923.
42. Barada, F. A., Andrews, B. S., Davis, J. S., and Taylor, R. P. (1981). Antibodies to Sm in patients with systemic lupus erythematosus. *Arthritis Rheum.* **24**, 1236–1244.
43. Winn, D. M., Wolfe, J. F., Lindberg, D. A., Fristoe, F. A., Kingland, L., and Sharp, G. C. (1979). Identification of a clinical subset of systemic lupus erythematosus by antibodies to Sm antigen. *Arthritis Rheum.* **22**, 1334–1337.
44. Homma, M., *et al*. (1987). Autoantibodies to the Sm antigen: Immunological approach to clinical aspects of systemic lupus erythematosus. *J. Rheumatol.* **14**, 188–193.

45. Takeda, Y., *et al*. (1989). Enzyme-linked immunosorbent assay using isolated (U) small nuclear ribonucleoprotein polypeptides as antigens to investigate the clinical significance of autoantibodies to these peptides. *Clin. Immunol. Immunopathol.* **50**, 213–230.
46. Gulko, P. S., Reveille, J. D., Koopman, W. J., Burgard, S. L., Bartolucci, A. A., and Alarcòn, G. S. (1994). Survival impact of autoantibodies in systemic lupus erythematosus. *J. Rheumatol.* **21**, 224–228.
47. Brahms, H., Raker, V. A., Van Venrooij, W. J., and Lührmann, R. (1997). A major, novel systemic lupus erythematosus autoantibody class recognizes the E, F, and G Sm snRNP proteins as an E-F-G complex but not in their denatured states. *Arthritis Rheum.* **40**, 672–682.
48. Reichlin, M., and Van Venrooij, W. J. (1991). Autoantibodies to the URNP particles: Relationship to clinical diagnosis and nephritis. *Clin. Exp. Immunol.* **83**, 286–290.
49. Salgado-Garrido, J. E., Bragado-Nilsson, E., Kandels-Lewis, S., and Seraphin, B. (1999). Sm and Sm-like proteins assemble in two related complexes of deep evolutionary origin. *EMBO J.* **18**, 3451–3462.
50. Achsel, T., Brahms, H., Kastner, B., Bachi, A., Wilm, M., and Luhrmann, R. (1999). A doughnut-shaped heteromer of human Sm-like proteins binds to the 3′-end of U6 snRNA, thereby facilitating U4/U6 duplex formation *in vitro*. *EMBO J.* **18**, 5789–5802.
51. He, W., and Parker, R. (2002). Functions of Lsm proteins in mRNA degradation and splicing. *Curr. Opin. Cell Biol.* **12**, 346–350.
52. Tharun, S., He, W., Mayes, A. E., Lennertz, P., Beggs, J. D., and Parker, R. (2000). Yeast Sm-like proteins function in mRNA decapping and decay. *Nature* **404**, 515–518.
53. Eystathioy, T., Peebles, C., Hamel, J. C., Vaughan, J. H., and Chan, E. K. L. (2002). Autoantibody to hLSm4 and the hepatameric LSm complex in anti-Sm sera. *Arthritis Rheum*.
54. Pettersson, I., *et al*. (1986). The use of immunoblotting and immunoprecipitation of (U) small nuclear ribonucleoproteins with mixed connective tissue disease and systemic lupus erythematosus. *Arthritis Rheum.* **29**, 986–996.
55. Kinlaw, C. S., Robertson, B. L., and Berget, S. M. (1983). fractionation and characterization of human small nuclear ribonucleoproteins containing U1 and U2 RNAs. *J. Biol. Chem.* **258**, 7181.
56. Mimori, T., Hinterberger, M., Pettersson, I., and Steitz, J. A. (1984). Autoantibodies to the U2 small nuclear ribonucleoprotein in a patient with scleroderma-polymyositis overlap syndrome. *J. Biol. Chem.* **259**, 560–565.
57. Habets, W., Hoet, M., Bringmann, P., Lührman, R., and VanVenrooij, W. J. (1985). Autoantibodies to ribonucleoprotein particles containing U2 small nuclear RNA. *EMBO J.* **4**, 1545–1550.
58. Sharp, G. C., Irwin, W., Tan, E. M., Gould, G., and Holman, H. R. (1972). Mixed connective tissue disease-an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med.* **52**, 148–159.
59. Sharp, G. C., Irvin, W. S., and May, C. M., *et al*. (1976). Association of auto-antibodies to ribonucleoprotein and Sm antigens with mixed connective tissue disease, systemic lupus erythematosus and other rheumatic diseases. *N. Engl. J. Med.* **295**, 1149.
60. Bouffard, P., Laniel, M. A., and Boire, G. (1996). Anti-Ro(SSA) antibodies: Clinical significance and biological relevance. *J. Rheumatol.* **23**, 1838–1841.
61. Fatenjad, S., Mamula, M. J., and Craft, J. (1993). Role of intermolecular/intrastructural B and T cell determinants in the diversification of autoantibodies to ribonucleoprotein particles. *Proc. Natl. Acad. Sci. USA* **90**, 12010–12014.
62. James, J. A., Gross, T., Scofield, R. H., and Harley, J. B. (1997). Immunoglobulin epitope spreading and autoimmune disease after peptide immunization. *J. Exp. Med.* **185**, 453–459.
63. Craft, J., and Fatenejad, S. (1997). Self antigens and epitope spreading in systemic autoimmunity. *Arthritis Rheum.* **40**, 1374–1382.
64. Harley, J. B., and James, J. A. (1995). Autoepitopes in lupus. *J. Lab. Clin. Med.* **126**, 509–516.
65. Hoffman, R. W., *et al*. (1990). Human autoantibodies agaist the 70-kd polypeptide of U1 small nuclear RNP are associated with HLA-DR4 among connective tissue disease patients. *Arthritis Rheum.* **33**, 666–673.
66. Arnett, F. C., Hamilton, R. G., Roebber, M. G., Harley, J. B., and Reichlin, M. (1988). Increased frequencies of Sm and nRNP autoantibodies in American blacks compared to whites with systemic lupus erythematosus. *J. Rheumatol.* **15**, 1773–1776.
67. Houtman, P. M., Kallenberg, C. G. M., Limburg, P. C., van Leeuwen, M. A., van Rijswijk, M. H., and Van Venrooij, W. J. (1986). Fluctuations in anti-nRNP levels in patients with mixed connective tissue disease are related to disease activity as part of a polyclonal B cell response. *Ann. Rheum. Dis.* **45**, 800–808.
68. St. Clair, E. W., *et al*. (1990). Expression of autoantibodies to recombinant (U1) RNP-associated 70 K antigen in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **54**, 266–280.
69. Alarcon-Segovia, D., Ruiz-Arguelles, A., and Fishbein, E. (1979). Antibody to nuclear ribonuclear protein penetrates live human mononuclear cells through Fc receptors. *Nature* **271**, 67–69.
70. Alarcon-Segovia, D., Ruiz-Arguelles, A., and Llorente, L. (1996). Broken dogma: Penetration of autoantibodies into living cells. *Immunol. Today* **17**, 163–164.
71. Gilliam, J. N., Smiley, J. D., and Ziff, M. (1974). Correlation between serum antibody to extractable nuclear antigen and immunoglobulin in localization in epidermal nuclei. *Clin. Res.* **22**, 611–612.
72. Combe, B., Rucheton, M., Graafland, H., Lussiez, V., Brunel, C., and Sany, J. (1989). Clinical significance of anti-RNP and anti-Sm autoantibodies as determined by immunoblotting and immunoprecipitation in sera from patients with connective tissue diseases. *Clin. Exp. Immunol.* **75**, 18–24.

73. Ehrfeld, H., Renz, M., Seelig, H. P., Hartung, K., Deicher, H., and Coldeway, R. (1991). Antibodies to recombinant U1-70K and U1-a protein in systemic lupus erythematosus (SLE). *Mol. Biol. Rep.* **15**, 190.
74. Habets, W. J., Hoet, M. H., and van Venrooij, W. (1990). Epitope patterns of anti-RNP antibodies in rheumatic diseases: Evidence for an antigen-driven autoimmune response. *Arthritis Rheum.* **33**, 834–841.
75. Lundberg, I., Nyman, U., Pettersson, I., and Hedfors, E. (1992). Clinical manifestations and anti-(U1). snRNP antibodies: A prospective study of 29 anti-RNP positive patients. *Br. J. Rheumatol.* **31**, 811–817.
76. Dreyfuss, G., Hentze, M., and Lamond, A. I. (1996). From transcript to protein. *Cell* **85**, 963–972.
77. Mayeda, A., Munroe, S. H., Caceres, J. F., and Krainer, A. R. (1994). Function of conserved domains of hnRNP A1 and other hnRNP A/B proteins. *EMBO J.* **13**, 5483–5495.
78. Steiner, G., Skriner, K., and Smolen, J. S. (1996). Autoantibodies to the A/B proteins of the heterogeneous nuclear ribonucleoprotein complex: Novel tools for the diagnosis of rheumatic diseases. *Int. Arch. Allergy Immunol.* **111**, 314–319.
79. Skriner, K., *et al*. (1997). Anti-A2/RA33 autoantibodies are directed to the RNA binding region of the A2 protein of the heterogeneous nuclear ribonucleoprotein complex: Differential epitope recognition in rheumatoid arthritis, systemic lupus erythematosus, and mixed connective tissue disease. *J. Clin. Invest.* **100**, 127–135.
80. Montecucco, C., *et al*. (1990). Antibodies from patients with rheumatoid arthritis and systemic lupus erythematosus recognize different epitopes of a single heterogeneous nuclear RNP core protein. *Arthritis Rheum.* **33**, 180–186.
81. Montecucco, C., Caporali, R., Cobianchi, F., Negri, C., and Astaldi-Ricotti, G. C. B. (1992). Antibodies to hn-RNP protein A1 in systemic lupus erythematosus: Clincial association with Raynaud's phenomenon and esophageal dysmotility. *Clin. Exp. Rheumatol.* **10**, 223–227.
82. Hassfeld, W., *et al*. (1995). Autoimmune response to the spliceosome: An immunologic link between rheumatoid arthritis, mixed connective tissue disease, and systemic lupus erythematosus *Arthritis Rheum.* **38**, 777–785.
83. Mediwake, R., Isenberg, D. A., Schellekens, G. A., and Van Venrooij, W. J. (2001). Use of anti-citrullinated peptide and anti-RA33 antibodies to distinguish erosive arthritis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Ann. Rheum. Dis.* **60**, 67–68.
84. Van Venrooij, W. J., and van Gelder, C. W. G. (1994). B cell epitopes on nuclear autoantigens: What can they tell us? *Arthritis Rheum.* **37**, 608–616.
85. Craft, J., Mimori, T., Olsen, T. L., and Hardin, J. A. (1988). The U2 smallribonucleoprotein particle as an autoantigen: Analysis of sera from patients with overlap syndromes. *J. Clin. Invest.* **81**, 1716–1724.
86. Guerrier-Takada, C., and Altman, S. (1984). Catalytic activity of an RNA molecule prepared by transcription *in vitro*. *Science* **223**, 285–286.
87. Altman, S., Baer, M., Guerrier-Takada, C., and Vioque, A. (1986). Enzymatic cleavage of RNA by RNA. *Trends Biol. Sci.* 515–518.
88. Thurlow, D. L., Shilowski, D., and Marsh, T. L. (1991). Nucleotides in precursor tRNAs that are required intact for catalysis by RNase P RNAs. *Nucleic Acids Res.* **19**, 885.
89. Gold, H. A., Craft, J., Hardin, J. A., Bartkiewicz, M., and Altman, S. (1988). Antibodies in human serum that precipitate ribonuclease P. *Proc. Natl. Acad. Sci. USA* **85**, 5483–5487.
90. Gold, H. A., Topper, J. N., Clayton, D. A., and Craft, J. (1989). The RNA processing enzyme RNase MRP is identical to the Th RNP and related to RNase P. *Science* **245**, 1377–1380.
91. Eder, P. S., Kekuda, R., Stolc, V., and Altman, S. (1997). Characterization of two scleroderma autoimmune antigens that copurify with human ribonuclease P. *Proc. Natl. Acad. Sci. USA* **94**, 1101–1106.
92. Ohosone, Y., *et al*. (1998). Spectrum and clinical significance of autoantibodies against transfer RNA. *Arthritis Rheum.* **41**, 1625–1631.
93. Satoh, T., *et al*. (1997). Systemic lupus erythmatosus associated with autoimmune hepatitis two cases with novel autoantibodies to transfer RNA-related antigens. *Clin. Rheumatol.* **16**, 305–309.
94. Mimori, T., *et al*. (1991). Newly identified autoantibodies to U4/U6 small nuclear ribonuclearprotein particle in a patient with primary Sjögren's syndrome. *Arthritis Rheum.* **34**, S46.
95. Okano, Y., and Medsger, T. A. (1991). Newly identified U4/U6 snRNP-binding proteins by serum antibodies from a patient with systemic sclerosis. *J. Immunol.* **146**, 535–542.
96. Okano, Y., *et al*. (1996). Anti-U5 small nuclear ribonucleoprotein (snRNP) antibodies: A rare anti-U snRNP specificity. *Clin. Immunol. Immunopathol.* **81**, 41–47.
97. Pironcheva, G., and Russev, G. (1994). Characterization of a protein moiety of U7 small nuclear RNP particles. *Microbios* **77**, 41–46.
98. Gilliam, J. N., and Steitz, J. A. (1993). Rare scleroderma autoantibodies to the U11 small nuclear ribonucleoprotein and t the trimethyl guanosine cap of U small nuclear RNAs. *Proc. Natl. Acad. Sci. USA* **90**, 6781–6785.
99. Tye, K., and Steitz, J. A. (1989). U3, U8, and U13 comprise a new class of mammalian snRNPs localized to the cell nucleolus. *EMBO J.* **8**, 3113–3119.
100. Alspaugh, M. A., and Tan, E. M. (1975). Antibodies to cellular antigens in Sjögren's syndrome. *J. Clin. Invest.* **55**, 1067–1073.
101. Akizuki, M., Powers, R., and Holman, H. R. (1977). A soluble acidic protein of the cell nucleus which reacts with serum from patients with systemic lupus erythematosus and Sjogren's syndrome. *J. Clin. Invest.* **59**, 254.
102. Mattioli, M., and Reichlin, M. (1974). Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. *Arthritis Rheum.* **17**, 421–429.

103. Alspaugh, M. A., and Maddison, P. (1979). Resolution of the identity of certain antigen-antibody systems in systemic lupus erythematosus and sjogren's syndrome: An interlaboratory collaboration. *Arthritis Rheum.* **22**, 796–798.
104. Fritzler, M. J., Pauls, J. D., Kinsella, T. D., and Bowen, T. J. (1985). Antinuclear, anticytoplasmic and anti-Sjogren's syndrome antigen-A (SS-A/Ro) antibodies in female blood donors. *Clin. Immunol. Immunopathol.* **36**, 120–128.
105. Maddison, P. J., Mogavero, H., and Provost, T. T., *et al*. (1979). The clinical significance of autoantibodies to a soluble cytoplasmic antigen in systemic lupus erythematosus and other connective tissue diseases. *J. Rheumatol.* **6**, 189–195.
106. Ben-Chetrit, E., Chan, E. K. L., Sullivan, K. F., and Tan, E. M. (1988). A 52 kD protein is a novel component of the SS-A/Ro antigenic particle. *J. Exp. Med.* **167**, 1560–1571.
107. Reichlin, M. (1991). Molecular definition of the Ro(SSA) particle(s): A frequent target of autoimmunity in systemic lupus erythematosus and Sjogren's syndrome. *Br. J. Rheumatol.* **30**(Suppl. 1), 58–62.
108. Hendrick, J. P., Wolin, S. L., Rinke, J., Lerner, M. R., and Steitz, J. A. (1981). Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: Further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. *Mol. Cell Biol.* **1**, 1138–1148.
109. Wolin, S. L., and Steitz, J. A. (1984). The Ro small cytoplasmic ribonucleoproteins: Identification of the antigenic protein and its binding site on the Ro RNAs. *Proc. Natl. Acad. Sci. USA* **81**, 1996–2000.
110. Boire, G., and Craft, J. (1989). Biochemical and immunological heterogeneity of the Ro ribonucleoprotein particles: Analysis with sera specific for the Ro^{hy5} particle. *J. Clin. Invest.* **84**, 270–279.
111. Boire, G., and Craft, J. (1990). Human Ro ribonucleoprotein particles: Characterization of native structure and stable association with the La polypeptide. *J. Clin. Invest.* **85**, 1182–1190.
112. Buyon, J. P., Slade, S. G., Chan, E. K. L., Tan, E. M., and Winchester, R. (1990). Effective separation of the 52 kD SS-A/Ro polypeptide from the 48 kD SS-B/La polypeptide by altering conditions of polyacrylamide gel electrophoresis. *J. Immunol. Methods* **129**, 207–210.
113. Chan, E. K. L., Hamel, J. C., Buyon, J. P., and Tan, E. M. (1991). Molecular definition and sequence motifs of the 52-kD component of human SS-A/Ro autoantigen. *J. Clin. Invest.* **87**, 68–76.
114. Itoh, K., Itoh, Y., and Frank, M. B. (1991). Protein heterogeneity in the human Ro/SSA ribonucleoproteins. *J. Clin. Invest.* **87**, 177–186.
115. Ben Chetrit, E., Gandy, B. J., Tan, E. M., and Sullivan, K. F. (1989). Isolation and characterization of a cDNA clone encoding the 60 kD component of the human SS-A/Ro ribonucleoprotein autoantigen. *J. Clin. Invest.* **83**, 1284–1292.
116. Deutscher, S. L., Harley, J. B., and Keene, J. D. (1988). Molecular analysis of the 60 kDa human ribonucleoprotein. *Proc. Natl. Acad. Sci. USA* **85**, 9479–9483.
117. Chan, E. K. L., Tan, E. M., Ward, D. C., and Matera, A. G. (1994). Human 60-kD SS-A/Ro ribonucleoprotein autoantigen gene (SSA2). localized to 1q31 by fluorescence in situ hybridization. *Genomics* **23**, 300.
118. Frank, M. B., Itoh, K., Fujisaku, A., Pontarotti, P., Mattei, M. G., and Neas, B. R. (1993). The mapping of the human 52-kD Ro/SSA autoantigen gene to human chromosome 11, and its polymorphisms. *Am. J. Hum. Genet.* **52**, 183–191.
119. Wang, D. R., Buyon, J. P., and Chan, E. K. L. (1996). Cloning and expression of mouse 60 kDa ribonucleoprotein SS-A/Ro. *Mol. Biol. Rep.* **23**, 205–210.
120. O'Brien, C. A., Margelot, K., and Wolin, S. L. (1993). Xenopus Ro ribonucleoproteins: Members of an evolutionarily conserved class of cytoplasmic ribonucleoproteins. *Proc. Natl. Acad. Sci. USA* **90**, 7250–7254.
121. O'Brien, C. A., and Wolin, S. L. (1994). A possible role for the 60-kD Ro autoantigen in a discard pathway for defective 5 S rRNA precursors. *Genes Dev.* **8**, 2891–2903.
122. Wang, D., Buyon, J. P., Yang, Z., DiDonato, F., Miranda-Carus, M. E., and Chan, E. K. L. (2001). Leucine zipper domain of 52 kDa SS-A/Ro promotes protein dimer foramtion and inhibits *in vitro* transcription activity. *Biochim. Biophys. Acta* **1568**, 161.
123. Chan, E. K. L., Di Donato, F., Hamel, J. C., Tseng, C.-E., and Buyon, J. P. (1995). 52-kD SS-A/Ro: Genomic structure and identification of altenatively spliced transcript encoding a novel leucine zipper-minus autoantigen expressed in fetal and adult heart. *J. Exp. Med.* **182**, 983–992.
124. Buyon, J. P., Tseng, C. E., DiDonato, F., Rashbaum, W., Morris, A., and Chan, E. K. L. (1997). Cardiac expression of 52β, an alternative transcript of the congenital heart block-associated 52-kd SS-A/Ro autoantigen, is maximal during fetal development. *Arthritis Rheum.* **40**, 655–660.
125. Huang, S.-C., Yu, H., Scofield, R. H., and Harley, J. B. (1995). Human anti-Ro autoantibodies bind peptides accessible to the surface of the native Ro autoantigen. *Scand. J. Immunol.* **41**, 220–228.
126. Buyon, J. P., Slade, S. G., Reveille, J. D., Hamel, J. C., and Chan, E. K. L. (1994). Autoantibody responses to the "native" 52-kDa SS-A/Ro protein in neonatal lupus syndromes, systemic lupus erythematosus, and Sjögren's syndrome. *J. Immunol.* **152**, 3675–3684.
127. Itoh, Y., Itoh, K., Frank, M. B., and Reichlin, M. B. (1992). Autoantibodies to the Ro/SSA autoantigen are conformation dependent. II. Antibodies to the denatured form of 52 kD Ro/SSA are a cross-reacting subset of antibodies to the native 60 kD Ro/SSA molecule. *Autoimmun.* **14**, 89–95.
128. Keech, C. L., Gordon, T. P., and McCluskey, J. (1996). The immune response to 52-kDa Ro and 60-kDa Ro is linked in experimental autoimmunity. *J. Immunol.* **157**, 3694–3699.

129. Tseng, C. E., Chan, E. K. L., Miranda, E., Gross, M., Di Donato, F., and Buyon, J. P. (1997). The 52-kd protein as a target of intermolecular spreading of the immune response to components of the SS-A/Ro-SS-B/La complex. *Arthritis Rheum.* **40**, 936–944.
130. Boire, G., Gendron, M., Monast, N., Bastin, B., and Menard, H. A. (1995). Purification of antigenically intact Ro ribonucleoproteins; biochemical and immunological evidence that the 52-kD protein is not a Ro protein. *Clin. Exp. Immunol.* **100**, 489–498.
131. Ricchiuti, V., Pruijn, G. J. M., Thijssen, J. P. H., Van Venrooij, W. J., and Muller, S. (1997). Accessibility of epitopes on the 52-kD Ro/SSA protein (Ro52) and on the RoRNP associated Ro52 protein as determined by anti-peptide antibodies. *J. Autoimmun.* **10**, 181–191.
132. Harley, J. B., Alexander, E. L., and Bias, W., *et al*. (1986). Anti-Ro/SSA and anti-La/SSB in Sjogren's syndrome. *Arthritis Rheum.* **29**, 196–206.
133. Garcia-de la Torre, I., Sanchez-Guerrero, S. A., Salmon-de la Torre, G., and Hernandez-Vazquez, L. (1987). Prevalence of anti-SS-A/Ro antibodies in a Mexican population of patients with various systemic rheumatic diseases. *J. Rheumatol.* **14**, 479–481.
134. Watson, R. M. (1984). Neonatal lupus erythematosus: A clinical, serological and immunogenetic study with review of the literature. *Medicine (Balt.)* **63**, 362–378.
135. Franco, H. L., Weston, W. L., Peebles, C., Forstot, S. L., and Phanuphak, P. (1981). Autoantibodies directed against sicca syndrome antigens in the neonatal lupus syndrome. *J. Am. Acad. Dermatol.* **4**, 67–72.
136. Scott, J. S., Maddision, P. J., Taylor, P. V., Escher, E., Scott, O., and Skinner, R. P. (1983). Connective tissue disease, antibodies to ribonucleoprotein and congenital heart block. *N. Engl. J. Med.* **309**, 209–212.
137. Maddison, P. J., Mogavero, H., and Reichlin, M. (1978). Antibodies to nuclear ribonucleoprotein. *J. Rheumatol.* **5**, 407–411.
138. Maddison, P. J., Mogavero, H., and Provost, T. T. (1979). The clinical significance of autoantibodies to a soluble cytoplasmic antigen in systemic lupus erythematosus and other connective tissue diseases. *J. Rheumatol.* **6**, 189–195.
139. Mond, C. B., Peterson, M. G. E., and Rothfield, N. F. (1989). Correlation of anti-Ro antibody with photosensitivity rash in systemic lupus erythematosus patients. *Arthritis Rheum.* **32**, 202–204.
140. Zappi, E., and Sontheimer, R. (1993). Clinical relevance of antibodies to Ro/SS-A and La/SS-B in subacute cutaneous lupus erythematosus and related conditions. *Clin. Dermatol.* **10**, 431–441.
141. Hedgpeth, M. T., and Boulware, D. W. (1988). Interstitial pneumonitis in antinuclear antibody-negative systemic lupus erythematosus: A new clinical manifestation and possible association with anti-Ro (SS-A) antibodies. *Arthritis Rheum.* **31**, 545–548.
142. Boulware, D. W., and Hedgpeth, M. T. (1989). Lupus pneumonitis and anti-SSA(Ro) antibodies. *J. Rheumatol.* **16**, 479–481.
143. Provost, T. T., Arnett, F. G., and Reichlin, M. (1983). Homozygous C2 deficiency, lupus erythematosus and anti-Ro/SSA antibodies. *Arthritis Rheum.* **26**, 1279–1282.
144. Meyer, O., Hauptmann, G., Tappeiner, G., and Ochs, H. D. (1985). Genetic deficiency of C4, C2 or Clq and lupus syndromes: Association with anti-Ro (SS-A) antibodies. *Clin. Exp. Immunol.* **62**, 678–684.
145. Morley, K. D., *et al*. (1981). Thrombocytopenia and anti-Ro. *Lancet* **2**, 940.
146. Anderson, M. J., Peebles, C. L., McMillar, R., and Curd, J. G. (1985). Fluorescent antinuclear antibodies and anti-SS-A/Ro in patietns with immune thrombocytopenia developing systemic lupus erythematosus. *Ann. Intern. Med.* **103**, 548–511.
147. Adachi, M., Mita, S., Obana, M., Matsuoka, Y., Harada, K., and Irimajiri, S. (1990). Thrombocytopenia subsequently develops systemic lupus erythematosus: Can anti-SSA predict the next event? *Jpn. J. Med.* **29**, 481–486.
148. Alexander, E. L., Arnett, F. C., Provost, T. T., and Stevens, M. B. (1983). Sjogren's syndrome: Association of anti-Ro(SS-A) antibodies with vasculitis, hematologic abnormalities and serologic hyperreactivity. *Ann. Intern. Med.* **98**, 155–159.
149. Harley, J. B., Sestak, A. L., Willis, L. E., Fu, S. M., Hansen, J. A., and Reichlin, M. (1989). A model for disease heterogeneity in sytemic lupus erythematosus: Relationships between histocompatability antigens, autoantibodies, and lymphopenia or renal disease. *Arthritis Rheum.* **32**, 826–836.
150. Wasicek, C. A., and Reichlin, M. (1982). Clinical and serolgoical differences between systemic luipus erythematosus patients with antibodies to Ro versus patients with antibodies to Ro and La. *J. Clin. Invest.* **69**, 835–843.
151. Hamilton, R. G., Harley, J. B., and Bias, W. B. (1988). Two Ro(SS-A) autoantibody responses in systemic lupus erythematosus: Correlation of HLA-DR/DQ specificities with quantitative expression of Ro(SS-A) autoantibody. *Arthritis Rheum.* **31**, 496–505.
152. Praprotnik, S., Bozic, B., Kveder, T., and Rozman, B. (2002). Fluctuation of anti-Ro/SS-A antibody levels in patitns with systemic lupus erythematosus and Sjögren's syndrome: A prospective study. *Clin. Exp. Rheumatol.* **17**, 63–68.
153. Wahren, M., Tengner, P., and Gunnarsson, I. (1998). Ro/SS-A and La/SS-B antibody level variation in patietns with Sjögren's syndrome and systemic lupus erythematosus. *J. Autoimmun.* **11**, 29–38.
154. Wang, D. R., Buyon, J. P., Zhu, W. G., and Chan, E. K. L. (1999). Defining a novel 75-kDa phosphoprotein associated with SS-A/Ro and identification of distinct human autoantibodies. *J. Clin. Invest.* **104**, 1265–1275.
155. Ben-Chetrit, E., Fox, R. I., and Tan, E. M. (1990). Dissociation of immune responses to the SS-A/Ro 52-kd and 60-kd polypeptides in systemic lupus erythematosus and Sjogren's syndrome. *Arthritis Rheum.* **33**, 349–355.

156. Michaelson, M., and Engle, M. A. (1972). Congenital complete heart block: An international study of the natural history. *Cardiovasc. Clin. North Am.* **4**, 85–101.
157. Buyon, J. P., Ben-Chetrit, E., and Karp, S., *et al*. (1989). Acquired congenital heart block: Pattern of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La system in neonatal lupus. *J. Clin. Invest.* **84**, 627–634.
158. Tseng, C. E., Caldwell, K., Feit, S., Chan, E. K. L., and Buyon, J. P. (1996). Subclass distribution of maternal and neonatal Anti-Ro(SSA) and La(SSB) antibodies in congenital heart block. *J. Rheumatol.* **23**, 925–932.
159. Silverman, E., Mamula, M., Hardin, J. A., and Laxer, R. (1991). Importance of the immune response to the Ro/La particle in the development of congenital heart block and neonatal lupus erythematosus. *J. Rheumatol.* **18**, 120–124.
160. Andrade, L. E., Chan, E. K. L., Raska, I., Peebles, C. L., Roos, G., and Tan, E. M. (1991). Human autoantibody to a novel protein of the nuclear coiled body immunological characteristics and cDNA cloning of p80-coilin. *J. Exp. Med.* **173**, 1407–1419.
161. Alexander, E., Buyon, J. P., Provost, T. T., and Guarnieri, T. (1992). Anti-Ro/SS-A antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model: In vitro electrophysiologic and immunocytochemical studies. *Arthritis Rheum.* **35**, 176–189.
162. Garcia, S., *et al*. (1994). Cellular mechanism of the conduction abnormalities induced by serum from anti-Ro/SSA-positive patients in rabbit hearts. *J. Clin. Invest.* **93**, 718–724.
163. McCauliffe, D. P., Yin, H., Wang, L. X., and Lucas, L. (1994). Autoimmune sera react with multiple epitopes on recombinant 52 and 60 kDa Ro(SSA) proteins. *J. Rheumatol.* **21**, 1073–1080.
164. Scofield, R. H., Zhang, F. C., Kurien, B. T., and Harley, J. B. (1997). Anti-Ro fine specificity defined by multiple antigenic peptides identifies components of tertiary epitopes. *Clin. Exp. Immunol.* **109**, 480–487.
165. Scofield, R. H., and Harley, J. B. (1991). Autoantigenicity of Ro/SSA antigen is related to a nucleocapsid protein of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. USA* **88**, 3343–3347.
166. Scofield, R. H., Dickey, W. D., Jackson, K. W., James, J. A., and Harley, J. B. (1991). A common autoepitope near the carboxyl terminus of the 60 kD Ro ribonucleoprotein: Sequence similarity with a viral protein. *J. Clin. Immunol.* **11**, 378–388.
167. Hardgrave, K. L., Neas, B. R., Scofield, R. H., and Harley, J. B. (1993). Antibodies to vesicular stomatitis virus proteins in patients with systemic lupus erythematosus and in normal subjects. *Arthritis Rheum.* **36**, 962–970.
168. Huang, S.-C., Pan, Z., Kurien, B. T., James, J. A., Harley, J. B., and Scofield, R. H. (1995). Immunization with vesicular stomatitis virus nucleocapsid protein induces autoantibodies to the 60 kD Ro ribonucleoprotein particle. *J. Invest. Med.* **43**, 151–158.
169. Maddison, P. J., Provost, T. T., and Reichlin, M. (1982). ANA-negative systemic lupus erythematosus: serological analysis. *Medicine (Balt.)* **60**, 87–94.
170. Provost, T. T., Razzaque, A., Maddison, P. J., and Reichlin, M. (1977). Antibodies to cytoplasmic antigens in lupus erythematosus. *Arthritis Rheum.* **20**, 1457–1463.
171. Tan, E. M., *et al*. (1997). Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum.* **40**, 1601–1611.
172. Harmon, C. E., Deng, J. S., Peebles, C. L., and Tan, E. M. (1984). The importance of tissue substrate in the SS-A/Ro antigen-antibody system. *Arthritis Rheum.* **27**, 166–173.
173. Raska, I., *et al*. (1991). Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. *Exp. Cell Res.* **195**, 27–37.
174. Keech, C. L., McCluskey, J., and Gordon, T. P. (1994). Transfection and overexpression of the human 60-kDa Ro/SS-A autoantigen in HEp-2 cells. *Clin. Immunol. Immunopathol.* **73**, 146–151.
175. Fritzler, M. J., and Miller, B. J. (1995). Detection of autoantibodies to SS-A/Ro by indirect immunofluorescence using a transfected and overexpressed human 60 kD Ro autoantigen in HEp-2 cells. *J. Clin. Lab. Anal.* **9**, 218–224.
176. Pollock, W., and Toh, B. H. (1999). Routine immunofluorescence detection of Ro/SS-A autoantibody using HEp-2 cells transfected with human 60 kDa Ro/SS-A. *J. Clin. Pathol.* **52**, 684–687.
177. Peene, I., Van Ael, W., Vandenbossche, M., Vervaet, T., Veys, E., and De Keyser, F. (2000). Sensitivity of the HEp-2000 substrate for the detection of anti-SSA/Ro60 antibodies. *Clin. Rheumatol.* **19**, 291–295.
178. Morozzi, G., *et al*. (2000). Comparison of differrent methods for the detection of anti-Ro/SSA antibodies in connective tissue diseases. *Clin. Exp. Rheumatol.* **18**, 729–731.
179. Bossuyt, X., Meurs, L., Mewis, A., Marien, G., and Blanckaert, N. (2000). Screening for autoantibodies to SS-A/Ro by indirect immunofluorescence using HEp-2000TM cells. *Ann. Clin. Biochem.* **37**, 216–219.
180. Fritzler, M. J., Hanson, C., Miller, J., and Eystathioy, T. (2002). Specificity of autoantibodies to SS-A/Ro on a transfected and overexpressed human 60 kDa Ro autoantigen substrate. *J. Clin. Lab. Anal.*
181. McCauliffe, D. P., Wang, L. X., Satoh, M., Reeves, W. H., and Small, D. (1997). Recombinant 52 kDa Ro(SSA) ELISA detects autoantibodies in Sjogren's syndrome sera that go undetected by conventional serologic assays. *J. Rheumatol.* **24**, 860–866.
182. Emlen, W., and O'Neill, L. (1997). Clinical significance of antinuclear antibodies. *J. Rheumatol.* **40**, 1612–1618.
183. Tan, E. M., *et al*. (2002). A critical evaluation of enzyme immunoassay kits for the detection of antinuclear antibodies of defined specificities. II. Potential for quantitation of antibody content. *J. Rheumatol.* **29**, 68–74.
184. Pizer, L. I., Deng, J.-S., Sternberg, R. M., and Tan, E. M. (1983). Characterization of a phosphoprotein associated

with the SS-B/La nuclear antigen in adenovirus-infected and uninfected KB cells. *Mol. Cell Biol.* **3**, 1235–1245.
185. Chan, E. K. L., and Tan, E. M. (1987). Human autoantibody-reactive epitopes of SS-B/La are highly conserved in comparison with epitopes recognized by murine monoclonal antibodies. *J. Exp. Med.* **166**, 1627–1640.
186. Rother, R. P., and Thomas, P. S. (1991). La/SSB ribonucleoprotein levels increased in transformed cells. *Clin. Exp. Immunol.* **83**, 369–374.
187. Francoeur, A. M., and Mathews, M. B. (1982). Interaction between VA-RNA and the lupus antigen La: Formaton of a ribonucleoprotein particle *in vitro*. *Proc. Natl. Acad. Sci. USA* **79**, 6772–6776.
188. Lerner, M. R., Boyle, J. A., Hardin, J. A., and Steitz, J. A. (1981). Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* **211**, 400–402.
189. Lerner, M. R., Andrews, N. C., Miller, G., and Steitz, J. A. (1981). Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **78**, 805–809.
190. McNeilage, L. J., Wittingham, S., Jack, I., and Mackay, I. R. (1985). Molecular analysis of the RNA and protein components recognized by anti-La(SS-B) autoantibodies. *Clin. Exp. Immunol.* **62**, 685–695.
191. Wilusz, J., Kurilla, M. G., and Keene, J. D. (1983). A host protein (La) binds to unique species of minus-sense leader RNA during replication of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. USA* **80**, 5827–5831.
192. Wilusz, J., and Keene, J. D. (1984). Interactions of plus and minus strand leader RNAs of the New Jersey serotype of vesicular stomatitis virus with the cellular La protein. *Virology* **135**, 65–73.
193. Bachmann, M., Pfeifer, K., Schroeder, H. C., and Muller, W. E. G. (1989). The La antigen shuttles between the nucleus and cytoplasm in CV-1 cells. *Mol. Cell. Biochem.* **85**, 103–114.
194. Bachmann, M., Pfeifer, K., Schroder, H.-C., and Muller, W. E. G. (1990). Characterization of the autoantigen La as a nucleic acid-dependent ATPase/dATPase with melting properties. *Cell* **60**, 85–93.
195. Belsham, G. J., Sonenberg, N., and Svitkin, Y. V. (1995). The role of the La autoantigen in internal initiation. *Curr. Top. Microimmunol.* **203**, 85–98.
196. Gottlieb, E., and Steitz, J. A. (1989). Function of the mammalian La protein: Evidence for this action in transcription termination by RNA polymerase III. *EMBO J.* **8**, 851–861.
197. Meerovitch, K., *et al*. (1998). La autoantigen enhances and corrects aberrant translation of polivirus RNA in reticulocyte lysate. *J. Virol.* **67**, 3798–3807.
198. Svitkin, Y. V., Meerovitch, K., Lee, H. S., Dholakia, J. N., Kenan, D. J., and Agol, V. I. (1994). Internal translation initiation on poliovirus RNA: Further characterization of La function in poliovirus translation *in vitro*. *J. Virol.* **68**, 1544–1550.
199. Chang, Y. N., Kenan, D. J., Keene, J. D., Gatignol, A., and Keang, K. T. (1994). Direct interactions between La and human immunodeficiency virus leader RNA. *J. Virol.* **68**, 7008–7020.
200. Chambers, J. C., and Keene, J. D. (1985). Isolation and analysis of cDNA clones expressing human lupus La antigen. *Proc. Natl. Acad. Sci. USA* **82**, 2115–2119.
201. Kohsaka, H., *et al*. (1990). Fine epitope mapping of the human SS-B/La protein: Identification of a distinct autoepitope homologous to viral Gag protein. *J. Clin. Invest.* **85**, 1566–1574.
202. St. Clair, E. W., Pisetsky, D. S., Reich, C. F., and Keene, J. D. (1988). Analysis of autoantibody binding to different regions of the human La antigen expressed in recombinant fusion proteins. *J. Immunol.* **141**, 4173–4180.
203. Rischmueller, M., McNeilage, L. J., McCluskey, J., and Gordon, T. (1995). Human autoantibodies directed against the RNA recognition motif of La (SS-B) bind to a conformational epitope present on the intact La (SS-B)/Ro (SS-A) ribonucleoprotein particle. *Clin. Exp. Immunol.* **101**, 39–44.
204. Sturgess, A. D., Peterson, M. G. E., McNeilage, L. J., and Whittingham, S. (1988). Characteristics and epitope mapping of a cloned human autoantigen La. *J. Immunol.* **140**, 3212–3218.
205. Fox, R. I., Chan, E. K., and Kang, H. I. (1992). Laboratory evaluation of patients with Sjögren's syndrome. *Clin. Biochem.* **25**, 213–222.
206. St. Clair, E. W., Pisetsky, D. S., Reich, C. F., Chambers, J. C., and Keene, J. D. (1988). Quantitative immunoassay of anti-La antibodies using purified recombinant La antigen. *Arthritis Rheum.* **31**, 506–514.
207. Miyachi, K., Fritzler, M. J., and Tan, E. M. (1978). Autoantibody to a nuclear antigen in proliferating cells. *J. Immunol.* **121**, 2228–2234.
208. Takasaki, Y., Deng, J. S., and Tan, E. M. (1981). A nuclear antigen associated with cell proliferation and blast transformation: Its distribution in synchromized cells. *J. Exp. Med.* **154**, 1899–1909.
209. Bravo, R., Fey, S. J., Bellatin, J., Larson, P. M., Arvelo, J., and Celis, J. E. (1981). Identification of a nuclear and of a cytoplasmic polypeptide whose reactive proportions are sensitive to changes in rate of cell proliferation. *Exp. Cell Res.* **136**, 311–317.
210. Bravo, R., and Celis, J. E. (1980). A search for differential polypeptide synthesis throughout the cell cycle of HeLa cells. *J. Cell Biol.* **84**, 795–803.
211. Mathews, M. B., Bernstein, R. M., Franze, B. R., Jr., and Garrels, J. I. (1984). Identity of the proliferating cell nuclear antigen and cyclin. *Nature* **309**, 374–376.
212. Celis, J. E., Bravo, R., Larsen, P. M., and Fey, S. J. (1984). Review cyclin: An nuclear protein whose level correlates directly with the proliferative state of normal as well as transformed cells. *Leuk. Res.* **8**(2), 143–157.
213. Miller, M. E., and Cross, F. R. (2001). Cyclin specificity: How many wheels do you need on a unicycle? *J. Cell Sci.* **114**, 1820.
214. Ogata, K., Ogata, Y., Nakamura, R. M., and Tan, E. M. (1985). Purification and N-terminal amino acid sequence of proliferating cell nuclear antigen (PCNA)/cyclin and

development of ELISA for anti-PCNA antibodies. *J. Immunol.* **135**, 2623–2627.
215. Ogata, K., Kurki, P., Celis, J. E., Nakamura, R. M., and Tan, E. M. (1987). Monoclonal antibodies to a nuclear protein (PCNA/cyclin) associated with DNA replication. *Exp. Cell Res.* **168**, 475–486.
216. Kurki, P., Vanderlaan, M., Dolbeare, F., Gray, J., and Tan, E. M. (1986). Expression of the proliferating cell nuclear antigen during the cell cycle. *Exp. Cell Res.* **166**, 209–219.
217. Kurki, P., Lotz, M., Ogata, K., and Tan, E. M. (1987). Proliferating cell nuclear antigen (PCNA)/cyclin in activated human T lymphocytes. *J. Immunol.* **138**, 4114–4120.
218. Matsumoto, K., Moriuchi, T., Koji, T., and Nakane, P. K. (1987). Molecular clonging of cDNA coding for rat proliferating cell nuclear antigen (PCNA)/cyclin. *EMBO J.* **6**, ••–637.
219. Almendral, J. M., Huebsch, D., Blundell, P. A., Macdoald-Bravo, H., and Bravo, R. (1987). Cloning and sequence of the human nuclear protein cyclin: Homology with DNA-binding proteins. *Proc. Natl. Acad. Sci. USA* **84**, 1575–1581.
220. Tan, C.-K., Castillo, C., So, A. G., and Downey, K. M. (1986). An auxiliary protein for DNA polymerase delta from fetal calf thymus. *J. Biol. Chem.* **261**, 12310–12316.
221. Prelich, G., *et al*. (1987). Functional identity of proliferating cell nuclear antigen and a DNA polymerase delta auxiliary protein. *Nature* **326**, 517–520.
222. Ogata, K., Ogata, Y., Takasaki, Y., and Tan, E. M. (1987). Epitopes on proliferating cell nuclear antigen recognized by human lupus autoantibody and murine monoclonal antibody. *J. Immunol.* **139**, 2942–2946.
223. Tan, C.-K., Sullivan, K., Li, X., Tan, E. M., Downey, K. W., and So, A. G. (1987). Autoantibody to the proliferating cell nuclear antigen neutralizes the activity of the auxiliary protein for DNA polymerase delta. *Nucleic Acids Res.* **15**, 9299–9308.
224. Tan, E. M. (1997). Autoantibodies and autoimmunity: A three-decade perspective. *Ann. N. Y. Acad. Sci.* **815**, 1–14.
225. Fritzler, M. J., McCarty, G. A., Ryan, J. P., and Kinsella, T. D. (1983). Clinical features of patients with antibodies directed against proliferating cell nuclear antigen (PCNA). *Arthritis Rheum.* **26**, 140–145.
226. Mimori, T., Akizuki, M., Yamagata, H., Inada, S., Yoshida, S., and Homma, M. (1981). Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. *J. Clin. Invest.* **68**, 611–620.
227. Tojo, T., Kaburaki, J., Hayakawa, M., Okamato, T., Tomii, M., and Homma, M. (1981). Precipitating antibody to a soluble nuclear antigen "Ki" with specificity for systemic lupus erythematosus. *Ryumachi* **21**(Suppl.), 129–137.
228. Francoeur, A. M., Peebles, C. L., Gomper, P. T., and Tan, E. M. (1986). Identification of Ki(Ku, p70/p80). autoantigens and analysis of anti-Ki autoantibody reactivity. *J. Immunol.* **136**, 1648–1653.
229. Reeves, W. H. (1985). Use of monoclonal antibodies for the characterization of novel DNA-binding proteins recognized by human autoimmune sera. *J. Exp. Med.* **161**, 18–39.
230. Reeves, W. H., Satoh, M. S., Stojanov, L., and Wang, J. (1996). Ku and Ki autoantibodies. *In* "Autoantibodies" (J. B. Peter and Y. Shoenfeld, eds.), pp. 449–455. Elsevier Science, The Netherlands.
231. Mimori, T., Hardin, J. A., and Steitz, J. A. (1986). Characterization of the DNA-binding protein Ku recognized by autoantibodies from patients with rheumatic disorders. *J. Biol. Chem.* **261**, 2274–2278.
232. Gottlieb, T. M., and Jackson, S. P. (1993). The DNA-dependent protein kinase: Requirement for DNA ends and association with Ku antigen. *Cell* **72**, 131–142.
233. Jeggo, P. A., Taccioli, G. E., and Jackson, S. P. (1995). Menage a trois: Double strand break repair, V(D)J recombination and DNA-PK. *Bioessays* **17**, 949–957.
234. Yaneva, M., Ochs, R., McRorie, D. K., Zweig, S., and Busch, H. (1985). Purification of an 86–70 kDa nuclear DNA-associated protein complex. *Biochim. Biophys. Acta* **841**, 22–29.
235. Yaneva, M., and Arnett, F. C. (1989). Antibodies against Ku protein in sera from patients with autoimmune diseases. *Clin. Exp. Immunol.* **76**, 366–372.
236. Birdi, N., Laxer, R. M., Thorner, P., Fritzler, M. J., and Silverman, E. D. (1993). Localized scleroderma progressing to systemic disease. *Arthritis Rheum.* **36**, 410–415.
237. Yaneva, M., Wen, J., Ayala, A., and Cook, R. (1989). cDNA-derived amino acid sequence of the 86-kda subunit of the Ku antigen. *J. Biol. Chem.* **264**, 13407–13411.
238. Reeves, W. H., *et al*. (1991). Epitopes of the p70 and p80 (ku) lupus autoantigens. *J. Immunol.* **146**, 2678–2686.
239. Mimori, T., *et al*. (1990). Isolation and characterization of cDNA encoding the 80-kDA subunit protein of the human autoantigen Ku (p70/p80). recognized by autoantibodies from patients with scleroderma-polymyositis overlap syndrome. *Proc. Natl. Acad. Sci. USA* **87**, 1777–1781.
240. Wang, J. S., Dong, X. W., Stojanov, L., Kimpel, D., Satoh, M., and Reeves, W. H. (1997). Human autoantibodies stabilize the quaternary structure of Ku antigen. *Arthritis Rheum.* **40**, 1344–1353.
241. Spain, T. A., Sun, R., Gradzka, M., Lin, S. F., Craft, J., and Miller, G. (1997). The transcriptional activator Sp1, a novel autoantigen. *Arthritis Rheum.* **40**, 1085–1095.
242. Satoh, M., *et al*. (1994). Autoantibodies to RNA polymerase II are common in systemic lupus erythematosus and overlap syndrome: Specific recognition of the phosphorylated (IIO) form by a subset of human sera. *J. Clin. Invest.* **94**, 1981–1989.
243. Sakamoto, M., Takasaki, Y., Yamanaka, K., Kodama, A., Hashimoto, H., and Hirose, S. (1989). Purification and characterization of Ki antigen and detection of anti-Ki antibody by enzyme-linked immunosorbent assay in patients with systemic lupus erythematosus. *Arthritis Rheum.* **32**, 1554.
244. Nikaido, T., *et al*. (1990). Cloning and nucleotide sequence of cDNA for Ki antigen, a highly conserved nuclear protein detected with sera from patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **79**, 209–214.

245. Boege, F., Andersen, A., Jensen, S., Zeidler, R., and Kreipe, H. (1995). Proliferation-associated nuclear antigen Ki-S1 is identical with topoisomerase IIa: Delineation of a carboxyl-terminal epitope with peptide antibodies. *Am. J. Pathol.* **146**, 1302–1308.
246. Yamanaka, K., Takasaki, Y., Nishida, Y., Shimada, K., Shibata, M., and Hashimoto, H. (1992). Detection and quantification of anti-Ki antibodies by enzyme-linked immunosorbent assay using recombinant Ki antigen. *Arthritis Rheum.* **35**, 667.
247. Takasaki, Y., *et al*. (1996). An epitope on Ki antigen recognized by autoantibodies from lupus patients shows homology with the SV40 large T antigen nuclear localization signal. *Arthritis Rheum.* **39**, 855–862.
248. Gerlach, C., Kubbutat, M., Schwab, U., Key, G., Flad, H. D., and Gerdes, J. (1998). Proliferation-associated Ki-67 protein is a target for autoantibodies in the human autoimmune disease systemic lupus erythematosus. *Lab. Invest.* **78**, 129–130.
249. Muro, Y., Kano, T., Suguira, K., and Hagiwara, M. (1997). Low frequency of antibodies against Ki-67 antigen in Japanese patients with systemic autoimmune diseases. *J. Autoimmun.* **10**, 499–503.
250. Matheson, A. T., Möller, W., Amons, W., and Yaguchi, M. (1979). Comparative studies on the structure of ribosomal proteins with emphasis on the alanine-rich, acidic, ribosomal "A" protein. *In* "Ribosomes: Structure, Function and Genetics" (G. Chambliss, G. R. Craven, J. Davies, K. Davis, L. Kahan, and M. Nomura, eds.), p. 297. University Park Press, Baltimore.
251. Warner, J. R. (1990). The nucleolus and ribosome formation. *Curr. Opin. Cell Biol.* **2**, 521–527.
252. Francoeur, A. M., Peebles, C. L., Heckman, K. J., Lee, J. C., and Tan, E. M. (1985). Identification of ribosomal protein autoantigens. *J. Immunol.* **135**, 2378–2384.
253. Rich, B. E., and Steitz, J. A. (1987). Human acidic ribosomal phosphoproteins P0, P1 and P2: Analysis of cDNA clones, *in vitro* synthesis and assembly. *Mol. Cell Biol.* **7**, 4065–4074.
254. Tsuzaka, K., *et al*. (1996). Lupus autoantibodies to double-stranded DNA cross-react with ribosomal protein S1. *J. Immunol.* **156**, 1668–1675.
255. Takeda, I., Rayno, K., Movafagh, F. B., Wolfson-Reichlin, M., and Reichlin, M. (2001). Dual binding capabilities of anti-double-stranded DNA antibodies and anti-ribosomal phosphoprotein (P) antibodies. *Lupus* **10**, 857–865.
256. MacConnell, W. P., and Kaplan, N. O. (1982). The activity of the acidic phosphoproteins from the 80 S rat liver ribosome. *J. Biol. Chem.* **257**, 5359–5366.
257. Sato, T., Uchium, T., Kominami, R., and Arakawa, M. (1990). Autoantibodies specific for the 20-kDa ribosomal large subunit protein. *Biochem. Biophys. Res. Commun.* **172**, 496–502.
258. Bonfa, E., Parnassa, A. P., Rhoads, D. D., Roufa, D. J., Wool, I. G., and Elkon, K. B. (1989). Antiribosomal S10 antibodies in humans and MRL/lpr mice with systemic lupus erythematosus. *Arthritis Rheum.* **32**, 1252–1261.
259. Chu, J. L., Brot, N., Weissbach, H., and Elkon, K. (1991). Lupus antiribosomal P antisera contain antibodies to a small fragment of 28 S rRNA located in the proposed ribosomal GTPase center. *J. Exp. Med.* **174**, 507–514.
260. Elkon, K., Bonfa, E., Skelly, S., Weissbach, H., and Brot, N. (1987). Ribosomal protein autoantibodies in systemic lupus erythematosus. *Bioessays* **7**, 258–261.
261. Elkon, K., *et al*. (1986). Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **83**, 7419–7423.
262. Towbin, H., Ramjoue, H. P., Kuster, H., Liverani, D., and Gordon, J. (1982). Monoclonal antibodies against eukaryotic ribosomes: Use to characterize a ribosomal protein not previously idetnified and antigenically related to the acidic phosphoproteins P1/P2. *J. Biol. Chem.* **257**, 12709–12715.
263. Elkon, K. B., Bonfa, E., Llovet, R., and Eisenberg, R. A. (1989). Association between anti-Sm and anti-ribosomal P protein autoantibodies in human systemic lupus erythematosus and MRL/lpr mice. *J. Immunol.* **143**, 1549–1554.
264. Miyachi, K., and Tan, E. M. (1979). Antibodies reacting with ribosomal ribonucleoproteins in connective tissue disease. *Arthritis Rheum.* **22**, 87–93.
265. Magsaam, J., Gharavi, A. E., Parnassa, A. P., Weissbach, H., Brot, N., and Elkon, K. B. (1989). Quantification of lupus anti-ribosome P antibodies using recombinant P2 fusion protein and determination of the predicted amino acid sequence of the autoantigen in patients' mononuclear cells. *Clin. Exp. Immunol.* **76**, 165–171.
266. Anderson, C. J., Neas, B. R., Uchiumi, T., and Stafford, H. A. (2001). Autoantibodies to the 20-kDa ribosomal proteins: Identification, characterization, and new aspects on prevalence in systemic lupus erythematosus. *Clin. Immunol.* **98**, 249–257.
267. Sato, T., *et al*. (1991). Autoantibodies against ribosomal proteins found with high frequency in patients with systemic lupus erythematosus with active disease. *J. Rheumatol.* **18**, 1681–1684.
268. Hulsey, M., Goldstein, R., Scully, L., Surbeck, W., and Reichlin, M. (1995). Anti-ribosomal P antibodies in systemic lupus erythematosus: A case-control study correlating hepatic and renal disease. *Clin. Immunol. Immunopathol.* **74**, 252–256.
269. Arnett, F. C., and Reichlin, M. (1995). Lupus hepatitis: An under-recognized disease feature associated with autoantibodies to ribosomal P. *Am. J. Med.* **99**, 465–472.
270. Bonfa, E., *et al*. (1987). Association between lupus psychosis and anti-ribosomal P protein antibodies. *N. Engl. J. Med.* **317**, 265–271.
271. Bonfa, E., and Elkon, K. B. (1986). Clinical and serological associations of the ribosomal P protein antibody. *Arthritis Rheum.* **29**, 981–985.
272. Hardin, J. A., and Mimori, T. (1985). Autoantibodies to ribonucleoproteins. *Clin. Rheum. Dis.* **11**, 485–505.
273. Schneebaum, A. B., *et al*. (1991). Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am. J. Med.* **90**, 54–62.

274. Nojima, Y., Minota, S., Yamada, A., Takaku, F., Aotsuka, S., and Yokohari, R. (1992). Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 1053–1055.
275. Press, J., *et al*. (1996). Antiribosomal P antibodies in pediatric patients with systemic lupus erythematosus and psychosis. *Arthritis Rheum.* **39**, 671–676.
276. Georgescu, L., Mevorach, D., Arnett, F. C., Reveille, J. D., and Elkon, K. B. (1997). Anti-P antibodies and neuropsychiatric lupus erythematosus. *Ann. N.Y. Acad. Sci.* **823**, 263–269.
277. Hay, E. M., and Isenberg, D. A. (1993). Autoantibodies in central nervous system lupus. *Br. J. Rheumatol.* **32**, 329–332.
278. Elkon, K. B., Bonfa, E., Weissbach, H., and Brot, N. (1994). Antiribosomal antibodies in SLE, infection, and following deliberate immunization. *Adv. Exp. Med. Biol.* **347**, 81–92.
279. Postnikov, Y. V., *et al*. (1991). Distribution of high mobility group proteins 1/2, E and 14/17 and linker histones H1 and H5 on transcribed and non-transcribed regions of chicken erythrocyte chromatin. *Nucleic Acids Res.* **19**, 717–725.
280. Bustin, M., Reisch, J., Einck, L., and Klippel, J. H. (1982). Autoantibodies to nucleosomal proteins: Antibodies to HMG-17 in autoimmune diseases. *Science* **215**, 1245–1247.
281. Tzioufas, A. G., Boumba, V. A., Seferiadis, C., Tsolas, O., and Moutsopoulos, H. M. (1993). Autoantibodies to HMG-17 nucleosomal protein in autoimmune rheumastic diseases: Correlation with systemic lupus erythematosus clinical activity and with antibodies to double-stranded DNA. *Arthritis Rheum.* **36**, 955–961.
282. Fritzler, M. J., and Salazar, M. (1991). The diversity and origin of rheumatologic autoantibodies. *Clin. Microbiol. Rev.* **4**, 256–269.
283. Zlatanova, J. S. (1990). Immunochemical approaches to the study of histone H1 and high mobility group chromatin proteins. *Mol. Cell. Biochem.* **92**, 1–22.
284. Einck, L., and Bustin, M. (1985). The intracellular distribution and function of the high mobility group chromsomal proteins. *Exp. Cell Res.* **156**, 295–310.
285. Bustin, M., Lehn, D. A., and Landsman, D. (1990). Structural features of the HMG chromosomal proteins and their genes. *Biochim. Biophys. Acta* **1049**, 231–243.
286. Ayer, L. M., Senécal, J.-L., Martin, L., Dixon, G. H., and Fritzler, M. J. (1994). Antibodies to high mobility group (HMG) proteins in systemic sclerosis. *J. Rheumatol.* **21**, 2071–2075.
287. Ayer, L. M., Rubin, R. L., Dixon, G. H., and Fritzler, M. J. (1994). Antibodies to HMG proteins in patients with drug-induced autoimmunity. *Arthritis Rheum.* **37**, 98–103.
288. Morse, J. H., Barst, R. J., Fotino, M., Zhang, Y., Flaster, E., and Fritzler, M. J. (1996). Primary pulmonary hypertension: Immunogenetic response to high-mobility group (HMG) proteins and histone. *Clin. Exp. Immunol.* **106**, 389–395.
289. Wittemann, B., Neuer, G., Michels, H., Truckenbrodt, H., and Bautz, F. A. (1990). Autoantibodies to nonhistone chromosomal proteins HMG-1 and HMG-2 in sera of patients with juvenile rheumatoid arthritis. *Arthritis Rheum.* **33**, 1378.
290. Burlingame, R. W., Rubin, R. L., and Rosenberg, A. M. (1993). Autoantibodies to chromatin components in juvenile rheumatoid arthritis. *Arthritis Rheum.* **36**, 836–841.
291. Santoro, P., De Andrea, M., Migliaretti, G., Trapani, C., Landolfo, S., and Gariglio, M. (2002). High prevalence of autoantibodies against the nuclear high mobility group (HMG) protein SSRP1 in sera from patients with SLE, but not other rheumatic diseases. *J. Rheumatol.* **29**, 90–93.
292. Busch, H., and Goldknopf, I. L. (1981). Ubiquitin–protein conjugates. *Mol. Cell Biochem* **40**, 173–187.
293. Finley, D., and Varshavsky, A. (1985). The ubiquitin system: Functions and mechanisms. *Trends Biol. Sci.* 343.
294. Feist, E., Dörner, T., Kuckelkorn, U., Scheffler, S., Burmester, G. R., and Kloetzel, P. M. (2000). Diagnostic importance of anti-proteasome antibodies. *Int. Arch. Allergy Immunol.* **123**, 92–97.
295. Gohill, J., Cary, P. D., Couppez, M., and Fritzler, M. J. (1985). Antibodies from patients with drug-induced and idiopathic lupus erythematosus react with epitopes restricted to the amino and carboxyl termini of histone. *J. Immunol.* **135**, 3116–3121.
296. Burlingame, R. W., and Rubin, R. L. (1996). Autoantibody to the nucleosome subunit (H2A-H2B)-DNA is an early and ubiquitous feature of lupus-like conditions. *Mol. Biol. Rep.* **23**, 159–166.
297. Muller, S., Briand, J. P., and Van Regenmortel, M. H. V. (1988). Presence of antibodies to ubiquitin during the autimmune response associated with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **85**, 8176–8180.
298. Muller, S. (1994). Ubiquitin. *In* "Manual of Biological Markers of Disease" (W. J. Van Venrooij and R. N. Maini, eds.), pp. 1–11. Kluwer, Boston.
299. Plaue, S., Muller, S., and Van Regenmortel, M. H. V. (1989). A branched, synthetic octapeptide of ubiquitinated histone H2A as target of autoantibodies. *J. Exp. Med.* **169**, 1607–1617.
300. Stockl, F., *et al*. (1994). A role for histones and ubiquitin in lupus nephritis? *Clin. Nephrol.* **41**, 10–17.
301. Feist, E., *et al*. (1996). Proteasome alpha-type subunit C9 is a primary target of autoantibodies in sera of patients with myositis and systemic lupus erythematosus. *J. Exp. Med.* **184**, 1313–1318.
302. Franke, W. W. (1987). Nuclear lamins and cytoplasmic intermediate filament proteins: A growing multigene family. *Cell* **48**, 3–4.
303. Krohne, G., and Benavente, R. (1986). The nuclear lamins: A multigene family of proteins in evolution and differentiation. *Exp. Cell Res.* **162**, 1.
304. Gerace, L., and Burke, B. (1988). Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* **4**, 335–374.
305. Bosman, F. T. (1996). Nuclear structure and the lamins. *J. Pathol.* **178**, 3–4.

306. Collard, J.-F., Senécal, J.-L., and Raymond, Y. (1990). Differential accesability of the tail domain of nuclear lamin A in interphase and mitotic cells. *Biochem. Biophys. Res. Commun.* **173**, 636–369.
307. Fisher, D. Z., Chaudhary, N., and Blobel, G. (1986). cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proc. Natl. Acad. Sci. USA* **83**, 6450–6454.
308. Goldman, A. E., Maul, G., Steinert, P. M., Yank, H.-Y., and Goldman, R. D. (1986). Keratin-like proteins that coisolate with intermediate filaments of BHK-21 cells are nuclear lamins. *Proc. Natl. Acad. Sci. USA* **83**, 3839–3843.
309. Senécal, J.-L., and Raymond, Y. (1991). Autoantibodies to DNA, lamins, and pore complex proteins produce distinct peripheral pattern fluorescent atninuclear antibody patterns on the HEp-2 substrate. *Arthritis Rheum.* **34**, 249–251.
310. Dagenais, A., Bibo-Hardy, V., and Senécal, J.-L. (1988). A novel autoantibody causing a peripheral fluorescent antinuclear antibody pattern is specific for nuclear pore complexes. *Arthritis Rheum.* **31**, 1322–1327.
311. Casals, S. P., Friou, G. J., and Teague, P. O. (1963). Specific nuclear reaction pattern of antibody to DNA in lupus erythematosus sera. *J. Lab. Clin. Med.* **62**, 625–631.
312. Lassoued, K., *et al*. (1988). Antinuclear antibodies specific for lamins: Characterization and clinical significance. *Ann. Intern. Med.* **108**, 829–833.
313. Reeves, W. H., Chaudhary, N. L., Salerno, A., and Blobel, G. (1987). Lamin B autoantibodies in sera of patients with systemic lupus erythematosus. *J. Exp. Med.* **165**, 750–762.
314. Guilly, M. N., Danon, F., Brouet, J. C., Bornens, M., and Courvalin, J. C. (1987). Autoantibodies to nuclear lamin B in a patient with thrombocytopenia. *Eur. J. Cell Biol.* **43**, 266–272.
315. McKeon, F. D., Tuffanelli, D. L., Fukuyama, K., and Kirschner, M. W. (1983). Autoimmune response directed against conserved determinants of nuclear envelope proteins in a patient with linear scleroderma. *Proc. Natl. Acad. Sci. USA* **80**, 4374–4378.
316. Wesierska-Gadek, J., Penner, E., Hitchman, E., and Sauermann, G. (1988). Antibodies to nuclear lamins in autoimmune liver disease. *Clin. Immunol. Immunopathol.* **49**, 107–115.
317. Lassoued, S., *et al*. (1988). Antinuclear antibodies specific for lamins. *Ann. Intern. Med.* **108**, 829–833.
318. Konstantinov, K., Von Mikecz, A., Buchwald, D., Jones, J., Gerace, L., and Tan, E. M. (1996). Autoantibodies to nuclear envelope antigens in chronic fatigue syndrome. *J. Clin. Invest.* **98**, 1888–1896.
319. Lassoued, S., Oksman, F., Fournie, B., Danon, F., Fournie, A., and Lassoued, K. (1990). Autoantibodies to lamins in rheumatoid arthritis. *Arthritis Rheum.* **33**, 877–879.
320. Worman, H. J., and Courvalin, J. C. (1991). Autoantibodies against nuclear envelope proteins in liver disease. *Hepatology* **14**, 1269–1279.
321. Courvalin, J. C., and Worman, H. J. (1997). Nuclear envelope protein autoantibodies in primary biliary cirrhosis. *Semin. Liver Dis.* **17**, 79–90.
322. Senécal, J.-L., *et al*. (1999). Strong association of autoantibodies to human nuclear lamin B1 with lupus anticoagulant antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 1347–1353.
323. Burke, B., Tooze, J., and Warren, G. (1983). A monoclonal antibody which recognizes each of the nuclear lamin polypeptides in mammalian cells. *EMBO J.* **2**, 361.
324. Fritzler, M. J., and Rattner, J. B. (1996). Mitotic spindle apparatus autoantibodies. *In* "Autoantibodies" (J. B. Peter, and Y. Shoenfeld, eds.), pp. 501–506. Elsevier Science, The Netherlands.
325. Andrade, L. E., Chan, E. K. L., Peebles, C. L., and Tan, E. M. (1996). Two major autoantigen-antibody systems of the mitotic spindle apparatus. *Arthritis Rheum.* **39**, 1643–1653.
326. Whitehead, C. M., Winkfein, R. J., Fritzler, M. J., and Rattner, J. B. (1996). The spindle kinesin-like protein HsEg5 is an autoantigen in systemic lupus erythematosus (SLE). *Arthritis Rheum.* **39**, 1635–1642.
327. McCarty, G. A., Valencia, D. W., Fritzler, M. J., and Barada, F. A. (1981). A unique antinuclear antibody staining only the mitotic spindle apparatus. *N. Engl. J. Med.* **305**, 703.
328. Auer-Grumbach, P., and Achleitner, B. (1994). Epidemiology and clinical associations of NuMA (nuclear mitotic apparatus protein) autoantibodies. *J. Rheumatol.* **21**, 1779–1781.
329. Fritzler, M. J., Etherington, J., Sokoluk, C., Kinsella, T. D., and Valencia, D. W. (1984). Antibodies from patients with autoimmune disease react with a cytoplasmic antigen in the Golgi apparatus. *J. Immunol.* **132**, 2904–2908.
330. Fritzler, M. J., Hamel, J. C., Ochs, R. L., and Chan, E. K. L. (1993). Molecular characterization of two human autoantigens: Unique cDNAs encoding 95- and 160-kD proteins of a putative family in the Golgi complex. *J. Exp. Med.* **178**, 49–62.
331. Renier, G., Fritzler, M. J., and Chevailler, A. (1996). Golgi apparatus autoantibodies. *In* "Autoantibodies" (J. B. Peter and Y. Shoenfeld, eds.), pp. 325–330. Elsevier Science, The Netherlands.
332. Selak, S., Scheonroth, L., Senécal, J.-L., and Fritzler, M. J. (1999). Early endosome antigen 1: An autoantigen associated with neurological diseases. *J. Invest. Med.* **47**, 311–318.
333. Waite, R. L., Sentry, J. W., Stenmark, H., and Toh, B. H. (1998). *Clin. Immunol. Immunopathol.* **86**, 81–87.
334. Meek, F., Khoury, E. L., Doniach, D., and Baum, H. (1980). Mitochondrial antibodies in chronic liver diseases and connective tissue disorders: Further characterization of the autoantigens. *Clin. Exp. Immunol.* **41**, 43–54.
335. Meyer, O., Abuaf, N., Cyna, L., Homberg, J. C., Kahn, M. F., and Rykewaert, A. (1987). Anti-mitochondrial type 5 antibodies and anti-cardiolipin antibodies in systemic lupus erythematosus and auto-immune diseases. *Clin. Exp. Immunol.* **69**, 485–492.
336. Mouritsen, S., Demant, E., Permin, H., and Wiik, A. (1986). High prevalence of antimitochondrial antibodies among patients with some well-defined connective tissue diseases. *Clin. Exp. Immunol.* **66**, 68–76.

337. Reimer, G., Rubin, R. L., Kotzin, B. L., and Tan, E. M. (1984). Anti-native DNA antibodies from autoimmune sera also bind DNA in mitochondria. *J. Immunol.* **133**, 2532–2536.
338. Tincani, A., *et al*. (1985). Anti-phospholipid and anti-mitochondrial type M5 antibodies in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **3**, 321–326.
339. Trujillo, M. A., Yebra, M., Mulero, J., Gea, J. C., and Ferrer, J. (1990). Antimitochondrial antibodies and the antiphospholipid syndrome. *J. Rheumatol.* **17**, 718–719.
340. Celis, J. E., *et al*. (1987). Anti-mitochondrial protein antibodies in a serum from a patient with systmeic lupus erythematosus: Specificity and comparison with other antimitochondrial antibodies. *Electrophor.* **8**, 238–243.
341. Verrier Jones, J., *et al*. (1991). Antimitochondrial antibodies and antiphospholipid antibodies. *J. Rheumatol.* **18**, 478–479.
342. Arribas, J., Rodriguez, M. L., Alvarez-DoForno, R., and Castano, J. G. (1991). Autoantibodies against mulit-catalytic proteinase in patients with systemic lupus erythematosus. *J. Exp. Med.* **173**, 423–427.
343. Itoh, Y., and Reichlin, M. (1992). Antibodies to carbonic anhydrase in systemic lupus erythematosus and other rheumatic diseases. *Arthritis Rheum.* **35**, 73–82.
344. Ono, M., Ono, M., Watanabe, K., Miyashita, Y., Inagaki, Y., and Ueki, H. (1999). A study of anti-carbonic anhydrase II antibodies in rheumatic autoimmune diseases. *J. Dermatol. Sci.* **21**, 183–186.
345. Kole, R., Fresco, L. D., Keene, J. D., Cohen, P. L., Eisenberg, R. A., and Andrews, P. G. (1985). Alu RNA-protein complexes formed *in vitro* react with a novel lupus autoantibody. *J. Biol. Chem.* **260**, 11781–11786.
346. Chan, E. K. L., Imai, H., Hamel, J. C., and Tan, E. M. (1991). Human autoantibody to RNA polymerase I transcription factor hUBF: Molecular identity of nucleolus organizer region autoantigen NOR-90 and ribosomal RNA transcription upstream binding factor. *J. Exp. Med.* **174**, 1239–1244.
347. Fujii, T., Mimori, T., and Akizuki, M. (1996). Detection of autoantibodies to nucleolar transcription factor nor 90/h UBF in sera of patients with rheumatic diseases, by recombinant autoantigen-based assays. *Arthritis Rheum.* **39**, 1313–1318.
348. Kovacs, B., Patel, A., Hershey, J. N., Dennis, G. J., Kirschfink, M., and Tsokos, G. C. (1997). Antibodies against p53 in sera from patients with systemic lupus erythematosus and other rheumatic diseases. *Arthritis Rheum.* **40**, 980–985.
349. Kuhn, H. M., Kromminga, A., Flammann, H. T., Frey, M., Layer, P., and Arndt, R. (1999). p53 autoantibodies in patients with autoimmune diseases: A quantitative approach. *Autoimmun.* **31**, 229–235.
350. Maddison, P. J., Lee, L., Reichlin, M., Sinclair, A., Wasson, C., and Schemmer, G. (1995). Anti-p57: A novel association with neonatal lupus. *Clin. Exp. Immunol.* **99**, 42–48.
351. McCauliffe, D. P., Zappi, E., Lieu, T.-S., Michalak, M., Sontheimer, R. D., and Capra, J. D. (1990). A human Ro/SS-A autoantigen is the homologue of calreticulin and is highly homologous with Onchocercal RAL-1 antigen and an Aplysia "memory molecule." *J. Clin. Invest.* **86**, 332–335.
352. Eggleton, P., Reid, K. B. M., Kishore, U., and Sontheimer, R. D. (1997). Clinical relevance of calreticulin in systemic lupus erythematosus. *Lupus* **6**, 564–571.
353. Ricotti, G. C. B. A., *et al*. (1987). Autoantibodies to purified nuclear proteins related to DNA metabolism during ageing and in SLE patients. *Immunology* **61**, 375–381.
354. Morrow, W. J. W., *et al*. (1982). Studies on autoantibodies to poly (adenosine diphosphate-ribose) in SLE and other autoimmune diseases. *Ann. Rheum. Dis.* **41**, 396–402.
355. LePage, S. H., Dudeney, C., Shall, S., Shoenfeld, Y., and Isenberg, D. A. (2002). Disease specificity of antibodies to poly (ADP-ribose); their relationships to anti-DNA antibodies and disease activity in lupus. *Autoimmun.* **5**, 167–177.
356. Isonishi, S., and Kanai, Y. (1988). Antibody to poly(ADP-ribose) as a predictor of obstetric complications in autoimmune MRL/Mp-lpr/lpr mice: Basis for its application to pregnant patients with systemic lupus erythematosus. *Immunol. Lett.* **18**, 61–66.
357. Arnett, F. C., Reveille, J. D., and Valdez, B. C. (1997). Autoantibodies to a nucleolar RNA helicase protein in patients with connective tissue disease. *Arthritis Rheum.* **40**, 1487–1492.
358. Dong, X. W., *et al*. (2000). Autoantibodies to DEK oncoprotein in human inflammatory disease. *Arthritis Rheum.* **43**, 85–93.
359. Wichmann, I., Respaldiza, N., Garcia-Lozano, J. R., Montes, M., Sanchez-Roman, J., and Nunez-Roldan, A. (2000). Autoantibodies to DEK oncoprotein in systemic lupus eryuthematosus (SLE). *Clin. Exp. Immunol.* **119**, 530–532.
360. Whitehead, C. M., Fritzler, M. J., and Rattner, J. B. (1998). The relationship of ASE-1 and NOR-90 in autoimmune sera. *J. Rheumatol.* **25**, 2126–2130.
361. Edworthy, S., Fritzler, M., Whitehead, C., Martin, L., and Rattner, J. B. (2000). ASE-1: An autoantigen in systemic lupus erythematosus. *Lupus* **9**, 1–7.
362. DeGiorgio, L. A., Konstantinov, K. N., Lee, S. C., Hardin, J. A., Volpe, B. T., and Diamond, B. (2001). A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nature Med.* **7**, 1189–1193.

14

IMMUNE COMPLEXES

Mark H. Wener
Mart Mannik

Systemic lupus erythematosus (SLE) is the prototype of human diseases that are principally mediated by immune complexes. The evidence for tissue injury by immune complexes in SLE is marshaled from many observations. The recognition of a number of autoantibodies in this disease served as an important initial finding. The presence of immunoglobulin and complement deposits in target organs and the characteristics of pathologic changes allowed the comparison of SLE to experimental models of serum sickness [1]. The conclusive evidence of the involvement of immune complexes in the genesis of tissue lesions, however, was provided by the identification of specific antibodies in the glomeruli of patients with SLE [2].

Injury to specific target organs in immune complex-mediated diseases results from the presence of antigen–antibody complexes in tissue. Immune complex deposits activate complement and interact with cell receptors, leading to the release of cytokines from cells and culminating in tissue damage and organ dysfunction. Antigen–antibody deposits in tissue may arise from deposition in tissues of circulating immune complexes, from formation of immune complexes at the site of their existence in tissues, or a combination of both.

This chapter reviews the nature of immune complexes, considers the biologic properties of immune complexes in relation to pathogenic processes, and discusses the clinical application of tests for the detection of immune complexes.

CHARACTERISTICS OF IMMUNE COMPLEXES

Immune complexes are composed of antigens and antibodies. The chemical features and physical characteristics of antigens can be quite variable, ranging from simple molecules to complex macromolecules or tissue components. The nature of an antigen in immune complexes may influence the biologic properties of the complexes. Antibodies in immune complexes may belong to any of the classes of immunoglobulins and endow the complexes with biologic properties unique to the particular class of immunoglobulin. In addition, the nature of the union between the antigen and the antibody influences the biologic properties of the resultant immune complexes, particularly the number of antibodies in a given immune complex.

Antigens

Antigens are substances that interact specifically with available antibodies or with receptors on sensitized lymphocytes. The term *immunogen* is used for substances that upon administration to a suitable host will elicit an immune response. The two concepts are separated, as antigens that can be shown to react with specific antibodies or with specifically sensitized lymphocytes do not necessarily induce an immune response. The actual portion of an antigen molecule that interacts with the

antibody-combining site or with a specific receptor of a sensitized lymphocyte is an *antigenic determinant* or *epitope*. The epitope is of limited size. For example, an epitope consists of five to six amino acids or of six to seven monosaccharide units. Furthermore, the antigenic determinant may consist of a sequence of amino acids or of a particular conformation of the protein so that the actual epitope is formed by amino acids from various areas of the polypeptide chain, or the epitope may be formed by the proximity of amino acids from separate polypeptide chains. Antigens may be proteins, polysaccharides, nucleic acids, lipoproteins, phospholipids, or other chemicals.

The number of antigenic determinants on a given molecule defines its valence for the interaction with specific antibodies. Small molecules with a single antigenic determinant are called *haptens.* Proteins or other substances of larger molecular size may possess multiple epitopes. The number of antigenic determinants on the molecule for a given antibody specificity defines the valence of that antigen for that antibody specificity. Most proteins and other macromolecules possess multiple epitopes with variable valence. As discussed later, the valence of antigens profoundly alters the nature of immune complexes formed upon interaction with antibodies.

The chemical features of an antigen may significantly influence the biologic properties of immune complexes, independent of the properties provided by antibodies in the complexes. This point can best be made by several specific examples. Highly cationic (positively charged) antigens can interact with fixed negative charges in glomeruli and thereby influence the localization of immune complexes in these structures. The clearance of immune complexes from the circulation can be altered by the nature of antigens present in immune complexes. For example, small-latticed immune complexes prepared with orosomucoid persisted in circulation similar to other small-latticed immune complexes. In contrast, when similar small-latticed immune complexes were prepared with asialo-orosomucoid with exposed galactose, the removal of these small-latticed immune complexes from circulation was hastened by the interaction of galactose with galactose receptors on hepatocytes [3]. In a similar manner, large molecular weight DNA is removed quickly from circulation by the liver because of the presence of specific receptors for DNA on nonparenchymal cells [4]. As a consequence, immune complexes containing such DNA are also removed faster from circulation than immune aggregates with comparable numbers of antibody molecules and protein antigens [5]. These studies performed in experimental animals emphasize the potential role of antigens on the biologic properties of immune complexes.

Whereas in the classic serum sickness immune complex model, the antigen in the immune complex (often heterologous serum albumin in experimental systems) bears little relevance to the resultant pathology, in SLE and other human immune complex diseases the antigen constituents within the immune complex could influence the pattern of clinical sequelae. For example, immune complexes containing lipids could augment the risk for cardiovascular disease. The uptake of lipids and the formation of foam cells is enhanced if the lipids are bound to IgG, i.e., as an immune complex [6]. This uptake is mediated, at least in part, by Fcγ receptors (FcγR) [7–9]. The FcγR-mediated uptake of lipids can lead to the formation and activation of the lipid-laden macrophage [10], including promotion of an atherogenic, oxidative phenotype of the macrophage [11]. Serum from patients with SLE has been reported to contain LDL-bearing immune complexes, which enhanced the uptake of lipids into macrophages [12], although similar findings were also found in patients with coronary artery disease [13].

Some antiphospholipid antibodies bind to other families of lipids, including oxidized low-density lipoproteins, (LDL) [14]. Immune complexes containing antibodies to lipoproteins known to be associated with atherogenesis could play a role in the development of coronary artery disease. Hasunama *et al*. [15] found that the anticardiolipin cofactor β_2-glycoprotein I (β_2-GPI) bound preferentially to oxidized plasma lipoproteins, i.e., oxidized (ox)VLDL, oxLDL, and oxHDL, in comparison with the native forms of the lipoproteins [15]. Antibodies to β_2-GPI bound to the β_2-glycoprotein I–oxLDL complex. Whereas binding of β_2-GPI to oxLDL inhibited the uptake of oxLDL by macrophages, the uptake was enhanced in the presence of immune complexes containing anti-β_2-GPI and β_2-GPI–oxLDL. Uptake of oxLDL by macrophages predisposes to the formation of foam cells, leading to intimal disease and atherosclerosis, thus the enhanced uptake caused by lipoprotein-containing immune complexes could contribute to accelerated atherosclerosis [16] as well las immune complex disease. Given the growing importance of coronary disease and of the antiphospholipid syndrome in SLE, the role of immune complexes in those manifestations bears further investigation.

Evidence has accumulated that immune complexes containing nucleosomes are important in the pathogenesis of lupus nephritis, and the antigen within those complexes may play a critical role, leading to the localization of immune complexes in the glomerular basement membrane. Renal biopsies from patients with diffuse proliferative lupus nephritis and membranous lupus

nephritis were found to contain nucleosomes and histones [17]. DNA found in the blood of patients with SLE is probably nucleosomal [18]. Furthermore, immune complexes containing nucleosomes are found circulating in MRL/lpr mice [19]. Clearance of chromatin by the liver is mediated in part by charge–charge interactions with heparan sulfate proteoglycans on hepatic Kuffper cells [20]. It has been suggested that nucleosomes can also interact with heparan sulfate proteoglycans in the kidney, promoting the glomerular deposition of circulating nucleosome–antinucleosome immune complexes or *in situ* formation of immune complexes after the initial deposition of nucleosomes in the glomerulus [21, 22]. Reasoning that heparin injections can compete with glomerular basement membrane structural heparan sulfate for binding to nucleosomes, van Bruggen and colleagues [23] treated MRL mice with heparin and demonstrated that heparin treatment led to less severe nephritis compared with untreated MRL mice [23]. Collectively, these data suggest that nucleosomes within immune complexes of lupus patients could influence the deposition of those immune complexes and again demonstrate that the antigen within an immune complex can contribute to the pathogeneticity of an immune complex.

Antibodies

Antibodies are the other essential constituent of antigen–antibody complexes. Detailed discussion of the antibody structure is beyond the scope of this chapter. Antibodies may belong to the IgG, IgA, IgM, IgD, or IgE classes of immunoglobulins. Most antibody molecules have two combining sites and hence a valence of 2 (IgG, monomeric IgA, IgD, and IgE). Dimeric IgA has a valence of 4, and IgM molecules exhibit a valence of 10 for small antigenic molecules or a valence of 5 for some large antigenic molecules. The distance between the two antibody-combining sites can also alter the nature of the formed complexes. In a given antibody molecule, this distance is somewhat variable, depending on the flexibility of the two Fab arms at the hinge region. The flexibility of the hinge region depends substantially on the isotype of the immunoglobulin, with human IgG3 having a long, flexible hinge region and IgG2 having a shorter, more constrained hinge region. Effector functions of the antibody molecules are localized in the constant regions of the two heavy chains. The important effector functions in relation to immune complexes are complement activation and interaction with specific cell receptors. Detailed reviews of immunoglobulin structure and effector functions are available [24, 25].

Nature of the Antigen–Antibody Union

The interaction of antigens and antibodies leads to the formation of immune complexes. A variety of immune complexes can be formed, ranging from the union of one antigen molecule and one antibody molecule to interactions of many molecules of each of the reactants. The *lattice* of immune complexes is defined as the number of antigens and the number of antibody molecules in a given immune complex. Such unions may be designated Ag_1Ab_1, Ag_2Ab_1, Ag_1Ab_2, Ag_2Ab_2, and so forth. A number of variables alter the lattice formation of immune complexes. In turn, the lattice of immune complexes influences the biologic properties of the complexes.

The valence of antibodies and the valence of antigens, among other variables, dictate the nature of the formed lattice. As already pointed out, most antibodies have a valence of 2. A monovalent antigen can maximally form Ag_2Ab_1 complexes, and larger lattices and immune precipitates cannot be formed. Bivalent antigens, depending on the distance between the antigenic determinants, may form Ag_1Ab_1, Ag_2Ab_2, circular Ag_3Ab_3, or larger open or closed complexes. Multivalent antigens may form increasingly larger complexes with high degrees of lattice formation, and immune precipitates may form. The presence of multiple different antigenic determinants allows the formation of complex and large lattices as well as precipitation. When an antigen is polyvalent with an appropriately spaced, repeating antigenic determinant, both antibody-combining sites of a bivalent antibody molecule preferentially react with the same antigenic molecule because of the formation of two antigen–antibody bonds between the reactants. This type of reaction is termed *monogamous bivalent reaction* and does not favor the formation of immune complexes with many antigen and many antibody molecules [26]. Antibodies to DNA, for example, may bind with monogamous bivalent interactions to DNA of appropriate size, and the cross-linking of DNA strands by antibody molecules is not favored by this interaction [27]. Of considerable interest is the finding that the strand of DNA bound with antibodies to DNA by monogamous bivalent binding was protected from degradation by DNase. The protected length of DNA corresponded to the distance between the two combining sites of an IgG molecule [28].

Another variable in the nature of antigen–antibody union is the molar ratio of the reactants; this is well illustrated by the classical precipitation curves. For example, when an increasing amount of antigen is added to a constant amount of antibody, then an increasing amount of precipitate is formed in the antibody excess zone. The maximum amount of precipitate is formed at the point

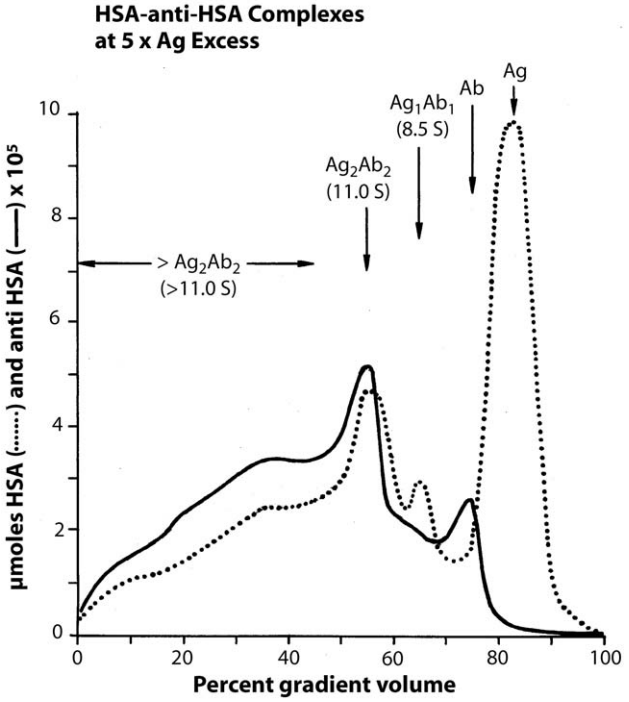


FIGURE 1 An example of the lattice of immune complexes. These soluble immune complexes were prepared with ^{131}I-labeled human serum albumin (HSA) and ^{125}I-labeled rabbit anti-HSA (IgG) at five times antigen excess, as determined by a precipitation curve. The size distribution of the complexes upon sucrose density gradient ultracentrifugation is shown. The micromoles of antigen and micromoles of antibody in each fraction are plotted against the percent gradient volume, with the top of the gradient being to the right. Starting from the right, the free excess antigen (Ag) is evident, and some unreactive or denatured antibody (Ab) is present. A small amount of Ag_1Ab_1 complexes is present, and a distinct peak of Ag_2Ab_2 complexes is evident with a sedimentation constant of 11 units. Ag_1Ab_1 and Ag_2Ab_2 complexes are collectively called *small-latticed immune complexes*. These complexes are inefficient in complement activation and interactions with FcγR and do not deposit in tissues. Beyond the peak of Ag_2Ab_2 complexes is a spectrum of larger lattices, ranging up to 22 units at the bottom of the tube. Discrete sizes of complexes cannot be distinguished, and therefore these complexes are collectively called *large-latticed immune complexes* (>Ag_2Ab_2). These complexes can activate complement, interact with FcγR, and deposit in tissues.

of equivalence. At this molar ratio of the interactants, free antigen and free antibody are not detectable in the supernatant. The addition of antigen beyond the point of equivalence leads to the formation of soluble immune complexes, and the amount of formed precipitate is decreased. At low degrees of antigen excess the soluble complexes have relatively large lattices (Fig. 1). With the addition of higher degrees of antigen excess, the formed soluble immune complexes become smaller. With very high degrees of antigen excess, only Ag_1Ab_1, Ag_2Ab_2, or Ag_2Ab_1 is formed, depending on the characteristics of the antigen. Furthermore, the absolute concentration of antigen and antibody influences lattice formation independent of the antigen–antibody molar ratio. Very low concentrations of antigens and antibodies tend to form more small-latticed complexes than are formed at higher antigen and antibody concentrations at the same antigen–antibody molar ratio. Finally, the association constant between the antigen and the antibody influences the lattice formed by these interactants. In general, low-affinity antibodies form smaller lattices than high-affinity antibodies when all other variables remain constant. With increasing lattice formation, biologic properties such as complement activation and interaction with cell receptors increase, as is discussed further in a subsequent section.

The initial interaction of an antigen and antibody depends on the antibody-combining site and the antigenic determinant of the antigen molecule. Interactions between other parts of antibody molecules, such as the Fc regions, are thought to contribute to nonspecific aggregation, to cryoprecipitability, and to formation of precipitates of the immune complexes. These events are slower than the initial antigen–antibody interaction.

Complement components have two significant influences on antigen–antibody complexes. First, when small amounts of immune complexes at equivalence are formed in the presence of fresh serum, then precipitation is prevented and only soluble complexes are generated (Fig. 2). When complement components are inactivated, then immune precipitates are formed under the same conditions. In this prevention of precipitation, the classical complement pathway plays a significant role [29]. Second, small quantities of already formed immune precipitates can be converted to soluble immune complexes by activation of the complement system. The alternative complement pathway is important in this complement-dependent solubilization of immune complexes [30]. The solubilized immune complexes contain covalently bound C3b and no longer are able to form immune precipitates. These observations, in conjunction with the observed increased prevalence of immune complex-mediated diseases in patients with deficiencies of the components of the classical pathway, have suggested an important role for complement components in retarding the deposition of immune complexes in tissues [31]. Thus, the possibility exists that the absence of complement components or the decrease of complement components due to the activation by immune complexes may contribute to the formation of immune complexes with increased pathogenic potential in tissues or in the circulation. However,

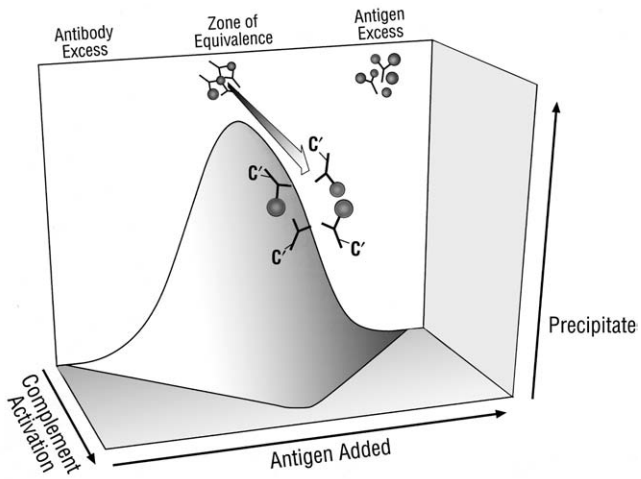


FIGURE 2 Schematic representation of immune precipitation and the effect of complement on this process. The classical precipitin curve demonstrates the effect of adding increasing amounts of antigen to a constant amount of antibody. With increasing amounts of antigen, the amount of precipitate increases until the zone of equivalence. Upon the addition of even more antigen, the amount of precipitate decreases due to the formation of soluble immune complexes. This relationship is shown by the two-dimensional precipitin curve shown as the "back" of this schematic. This three-dimensional schematic also illustrates the effect of complement on the amount of immune precipitate. Increasing amounts of added complement prevent the formation of immune precipitates and large immune complexes via the classical pathway, and the alternate pathway solubilizes preformed immune precipitates. The complement protein fragment C3b interferes with formation of the immune complex lattice or disrupts the lattice that has been formed. The large arrow represents solubilization of immune complexes by increasing amounts of added complement.

inflammatory cells may be recruited to the sites of immune deposits less efficiently in the presence of complement deficiency. These possibilities and the association between genetic abnormalities of the complement system and SLE are considered further in Chapters 5 and 6.

BIOLOGIC PROPERTIES OF IMMUNE COMPLEXES

Among the many properties of immune complexes, activation of the complement systems, interaction with cell receptors, and deposition in tissues are most relevant to the present discussion. Interactions with cells are mediated largely through Fc and complement receptors present on a variety of cell types. The degree of these biologic activities depends in part on the class or subclass of antibodies in the immune complexes and on the degree of lattice formation in the complexes.

Several biologic properties of immune complexes form the basis of tests that have been designed to detect and quantify immune complexes in serum or other body fluids. The use of these tests for clinical evaluation is discussed in the last section of this chapter.

Complement Activation by Immune Complexes

Activation of the complement systems by immune complexes may proceed through the classical or the alternative pathways. The interested reader should refer to standard texts or reviews of the complement system.

Antibodies of the IgG and IgM class activate the complement by the classical pathway, if sufficient lattice formation is present. The IgG1, IgG2, and IgG3 subclasses are effective in complement activation, and the IgG4 subclass is ineffective. As the initial step of complement activation through the classical pathway, globular regions of the C1q molecules bind to the Cγ2 domain of the IgG molecule in immune complexes. The exact site for binding C1q, however, remains controversial, and differences in the binding site may depend on the species and isotype of immunoglobulin studied [32–35]. Even monomeric IgG molecules bind weakly to isolated C1q, as revealed by analytic ultracentrifugation. Activation of the system, however, requires that the immune complexes containing IgG form a multivalent ligand for interaction with C1. In general, immune complexes become increasingly more effective in binding C1 and activation of the complement system with increasing numbers of IgG molecules [36]. The number of IgM molecules required in soluble immune complexes for complement activation has not been carefully defined, but on the cell surface, one IgM antibody suffices to activate the complement system. The other classes of antibody molecules in immune complexes do not activate complement. The alternative complement pathway is also activated by immune complexes that activate the classical pathway by generating C3b and thereby activating the C3b-dependent loop of the alternative pathway. Immune complexes containing IgA as antibodies activate the alternative pathway directly and efficiently [36].

Complement activation by immune complexes has several consequences in relation to disease processes. For example, the generation of chemotactic factors leads to an influx of phagocytic cells to tissues that contain deposits of immune complexes. The phagocytic cells ingest the immune deposits and, during this process, release enzymes and other factors that contribute to the damaging effects of inflammation. In

addition, the Fc receptor-mediated interaction of IgG-containing immune complexes with phagocytic cells is enhanced by the presence of complement components on immune complexes and complement receptors on these cells. The binding of complement components to immune deposits contributes to tissue damage and organ malfunction. For instance, complement components of the membrane-attack complex have been identified in skin and in glomeruli of patients with SLE [37, 38]. Studies in experimental animals have shown that the binding of complement components to the subepithelial deposits in glomeruli initiates the proteinuria associated with this lesion [39]. There is, however, a critical role of FcγRs in the mediation of experimental murine immune complex nephritis, as discussed later. The role of complement in the solubilization of immune precipitates was discussed in a preceding section and in Chapter 5.

The pivotal role of complement in causing immune complex disease has been challenged by experiments involving animals without functional complement, caused by induced defects in either complement proteins or complement receptors. In mice lacking complement proteins due to experimental manipulation, immune-mediated renal disease may be less severe, more severe, or essentially unchanged in comparison with mice with genetically normal complement proteins. The acute renal disease caused by administration of antibodies to glomerular basement membrane (GBM) antigens is less severe in mice deficient in C3 or C4 [40], whereas the chronic immune complex phase, mediated by antibodies to the administered anti-GBM, is little different or possibly slightly more severe in mice with complement deficiency. In MRL/lpr mice with C3 deficiency, a larger number of glomerular immune deposits were observed but the renal disease was no different histopathologically than found in MRL/lpr mice with normal C3 genes [41]. Together, these data indicate that an intact complement system is not necessary for many forms of immune complex tissue damage. Nevetheless, other evidence indicates that complement deficiency can ameliorate immune complex disease and decrease tissue damage (see Chapter 5 and 6).

Interaction of Immune Complexes with Cell Receptors: Binding and Functional Consequences

Specific receptors exist on cells that interact with the antibody molecules in immune complexes. Other receptors react specifically with complement components that have bound to immune complexes. Cellular receptors have important roles in the clearance of immune complexes from the circulation and from tissues, in recruitment of inflammatory cells into an area of immune deposits, and in regulation of cells function, as discussed later.

Fcγ receptors reacting with IgG molecules and not with other classes of immunoglobulins exist in three major forms, termed FcγRI, FcγRII, and FcγRIII [42–44]. Each of these major types has subtypes, with three genes encoding FcγRI, three genes encoding FcγRII, and two genes encoding FcγRIII; gene loci are on the long arm of chromosome 1 [1q21–23]. In addition, there are splice variants of several of the FcγRs (see Table 1). The FcγRI, FcγRII, and FcγRIII on Kupffer cells of the liver all may have a role in the removal of circulating IgG-containing immune complexes, and FcγRs on liver endothelial cells may also have a role in immune complex binding [45–47]. FcγRI have high affinity for monomeric IgG1 and IgG3 on cell surfaces and function in antibody-dependent cellular cytotoxicity [42, 47]. Genetic polymorphisms exist for FcγRII and FcγRIII and possess different functions. Most of the FcγRs are transmembrane protein, but FcγRIIIb, which is expressed only on neutrophils and activated eosinophils, is a phosphatidylinositol glycan-anchored form of the CD16 molecule. Several clinical studies have implicated allelic polymorphisms of FcγR II and FcγR III as risk factors for SLE. In addition, mice genetically engineered to delete FcγRI and FcγRIII have been shown to be protected from experimental immune complex disease, whereas FcγRII knockout mice have more severe immune complex disease [42–44].

The lattice of immune complexes significantly influences the interaction of complexes with FcγR. Monomeric IgG molecules have a weak interaction with FcγRII and FcγRIII and these interactions do not trigger interiorization of the attached molecules by the cells. The attachment of immune complexes with sufficient lattice, however, results in phagocytosis of the complexes. Small-latticed immune complexes are not efficiently phagocytized by neutrophils, monocytes, or Kupffer cells. Such small complexes could be formed with monovalent or divalent antigens or formed by high degrees of antigen excess with polyvalent antigenic molecules. In contrast, large-latticed immune complexes are attached to the FcγR and then phagocytized by the cell. Investigations have suggested that the interaction of large-latticed complexes with receptors results in an increased local concentration of immune complexes that leads to further condensation or rearrangement of the complexes to even larger lattices before interiorization [48].

The interaction of immune complexes with cell receptors provides the principal basis for their removal from circulation by the mononuclear phagocyte system. This topic is considered later. In addition, immune

TABLE 1 Characteristics of Fc gamma receptors

Receptor class, CD designation	Affinity for IgG (M^{-1})	Genes	Cell linkage	IgG form	Effect on cells	Cells bearing receptor	Clinically important allelic isoforms	IgG subclass specificity	Functional importance	Relevance to SLE
FcγRI, CD64	10^8–10^9 (high)	FcγRIA	TM	monomeric	Activation	Monocytes, macrophages, activated PMNs		1,3 >> 4 >> 2	Binds monomeric IgG as well as immune complexes	KO mice protected
		FcγRIB, FcγRIC	TM	complexes	Activation	Monocytes, macrophages, activated PMNs				
FcγRII, CD32	$<10^7$ (low)	FcγRIIA	TM	complexes	Activation	PMNs, macrophages, monocytes, platelets, mast cell	FcγRIIa-131H (Low responder)	3 > 1,2> 4	Only FcR with significant binding to IgG2 subclass	
							FcγRIIa-131R (high responder)	3 > 1 >> 2,4		Associated with SLE (especially in African-Americans); lupus nephritis with antiC 1 g
		FcγRIIB	TM		Inhibition	monocytes, macrophages, B-cells, PMNs, mast cell		3,1 > 4 >>> 2	Immunoregulatory and inhibitory role	
		FcγRIIC	TM		Activation	NK				
FcγRIII, CD 16	10^7–10^8 (low)	FcγRIIIA	TM	complexes > monomeric	Activation	NK cells, monocytes, macrophages	FcγRIIIa-F176			SLE protected KO mice (Caucasians) protected
		FcγRIIIB	GPI-linked	complexes	Activation	PMN's activated eosinophils	NA1-FcγRIIIb	1, 3 >>> 2,4		
							NA2-FcγRIIIb	3 >> 1 >> 2,4		
							SH-FcγRIIIb	3,1 >> 2,4		

GPI = glycosyl phosphatidylinositol.
TM = transmembrane protein.
KO mice = knock-out mice.

complexes have a role in regulating lymphocytes and inflammatory cells.

Immune complexes can alter the immune response and lymphocyte functions. For example, immune complexes are 100 to 1000-fold more effective in inducing the proliferation of primed T-cells-than antigen alone [49]. FcγRs may have an important role in modulating T-cell-mediated responses by targeting immune complexes to antigen-presenting dendritic cells, leading to internalization of antigen, maturation of dendritic cells, activating or inhibiting dendritic cell function, and antigen presentation to T cells [50]. Polyclonal B-cell activation by immune complexes is of considerable interest in autoimmune diseases such as SLE. In experimental animals, immune complexes have been demonstrated to stimulate the proliferation of B cells and the synthesis of antibodies that are not related to the constituents of immune complexes [51]. Binding of immune complexes to Fc receptors leads to aggregation of those receptors and triggers intracellular signalling pathways [42]. Immune complexes binding via the FcγRIIB receptor have been shown to exert a downregulatory immunomodulatory effect, counterbalancing the activation induced by immune complexes binding to cells via the FcγRI and FcγRIII receptors [42]. Soluble and insoluble immune complexes appear to activate neutrophils by different mechanisms and via somewhat different FcγR involvment. Activation by soluble complexes is mediated by binding to both FcγRII and FcγRIIIb and leads to the release of secreted inflammatory mediators from cytokine-primed neutrophils, whereas insoluble complex activation was mediated by FcγRIIIb without FcγRII and led to the production of intracellular reactive oxygen species and the release of granule enzymes from both cytokine-primed and -unprimed neutrophils [52].

Information indicates that FcγR may have a more important role in mediating immune complex disease than had been appreciated previously. These data have been reported in a series of papers, largely using mice generated by Ravetch, that lack the transmembrane, signal-transducing γ chain that is found on IgG FcγRI and FcγRIII. In cutaneous Arthus reaction models in those mice, as well as experiments with autoimmune mice crossbred with the Fc receptor knock out mice, neutrophil infiltration and organ dysfunction required intact Fc receptors, despite the presence of immune complexes in tissues [53]. Furthermore, inflammation was not altered by complement deficiency. The susceptibility to murine lupus nephritis [54, 55] and to collagen-induced arthritis was also found to be altered in FcγR-deficient mice [56], indicating that similar mechanisms were important in these diseases. In a murine model of immune complex peritonitis, neutrophil migration was attenuated after complement depletion, but was totally abolished in mice lacking the FcR γ chain [57]. Additional data suggested that engagement of FcγRIII did not lead to neutrophil recruitment, but that engagement of FcγRI was most important in inducing inflammatory exudates [57]. It has been proposed that local microenvironments within different tissues could influence the expression of FcR on macrophages at different sites, thus modulating the local inflammation and other tissue effects of circulating or deposited immune complexes [58].

Investigations have explored the implications of polymorphisms in various Fcγ receptors with regard to their potential role in clearing immune complexes from the circulation and causing a predisposition to SLE. Lack of the H131 allele of the FcγRIIA, which is responsible for the efficient clearance of IgG_2-containing immune complexes, has been associated with lupus nephritis in American blacks [59]. While a large meta-analysis confirmed this association [60], studies in other populations have failed to find an association between SLE and this polymorphism [61]. A report has implicated a functionally important genetic polymorphism of FcγRIIIA as a risk factor for SLE in a genetically diverse group of patients [62]. FcγRIIIa-F176 is associated with SLE in some populations, particularly Caucasians [60].

As already pointed out, human erythrocytes possess receptors for C3b, termed *complement receptor type I* (CR1). The average number of CR1 molecules per erythrocyte varies considerably among normal persons and also among erythrocytes of an individual. CR1 molecules exist in clusters, rendering them highly effective in interacting with immune complexes that contain multiple molecules of C3b or C4b [63, 64]. Therefore, because of the red cell mass, these receptors effectively bind the very large, complement-containing immune complexes and thus prevent them from depositing in tissues. *In vitro* experiments have shown that immune complexes with complement components transfer effectively from erythrocytes to monocytes with FcγR and CR1 receptors. This reaction is much faster than the uptake of the same complexes from a fluid phase [65]. Experiments in monkeys suggest that binding of very large, complement-containing immune complexes to erythrocytes may diminish immune deposits in glomeruli [66]. It must be emphasized, however, that this system is applicable only to very large immune complexes that have bound complement components. In rodents and rabbits, CR1 does not exist on erythrocytes, but is present primarily on platelets. In patients with SLE, the number of CR1 molecules per erythrocyte is decreased [67].

Complement receptors have also been described in tissues. Of particular interest has been the presence of

C3b receptors in glomeruli. Several investigations have indicated that these receptors are present on the epithelial cells of glomeruli [68, 69]. These receptors have been found to be decreased in glomeruli of patients with proliferative lupus glomerulonephritis, but not with membranous lupus glomerulonephritis, in whom the deposits are adjacent to the epithelial cells [68]. The role of C3b receptors in the localization of immune complexes in glomeruli has not been established. In experimental animals, immune deposits form in the same location in species that do not have demonstrable C3b receptors in glomeruli. These observations suggest that the presence of C3b receptors in glomeruli do not play a significant role in the deposition of immune complexes.

Fate of Circulating Immune Complexes

Studies in experimental animals have indicated that with increasing loads of circulating immune complexes, deposition in tissues is enhanced. Protective mechanisms, however, exist to remove complexes from circulation, thereby decreasing the possibility of deposition in tissues. The principles involved in the removal of circulating immune complexes have been examined in experimental animals. Once immune complexes are formed in circulation or gain access to circulation, the removal by protective mechanisms is influenced by the lattice of immune complexes, the status of the mononuclear phagocyte system, the nature of antibodies in the complexes, and, to some degree, the nature of the antigen molecules in the complexes.

Studies in mice, rabbits, and rhesus monkeys showed that large-latticed immune complexes composed of lattices greater than Ag_2Ab_2 (ranging from 14 to 22 S units) and containing IgG molecules were removed from the circulation by the mononuclear phagocyte system. The interaction of complexes with the FcγR of Kupffer cells in the liver mediated this removal from circulation. In contrast, small-latticed complexes composed of Ag_2Ab_2 and Ag_1Ab_1 complexes persisted longer in circulation but were removed faster than antibodies alone. The spleen in these animals accounted for only a relatively small proportion of the large-latticed immune complexes removed from circulation [70]. Furthermore, complement components were not involved in this rapid uptake of these large-latticed immune complexes in mice and rabbits. The rapid removal of immune complexes by Kupffer cells is aided by the fact that these cells in hepatic sinusoids are not covered by endothelial cells and thus are directly exposed to circulating materials.

The mononuclear phagocyte system in the liver, previously termed the reticuloendothelial system, is involved in the removal of a variety of substances from the circulation independent of the FcγR. This nonspecific uptake is a saturable process. Also, the FcγR-specific uptake of large-latticed circulating immune complexes is saturable, as established by injecting increasing does of immune complexes. Saturation of the hepatic uptake of immune complexes caused prolonged circulation of the large-latticed immune complexes and increased deposition in glomeruli [70].

Several studies of soluble immune complex clearance have been conducted in humans. In the first study, immune complexes were formed with tetanus toxoid and antibodies to tetanus toxoid [71]. The injected complexes were quite large (45 S units or larger) and rapidly incorporated complement components. In normal persons, these complexes were cleared with a single exponential component, ranging from 9.9 to 18.7% of the injected complexes per minute. In 11 of 15 patients with SLE or other immune complex diseases, an initial very rapid component of removal was present. This was thought to result from trapping in tissues, but proof for this was not obtained. The second phase of clearance in patients ranged from 8.6 to 32.2% per minute. The binding of injected complexes to erythrocytes during the first minute correlated with the average number of CR1 molecules per erythrocyte. Furthermore, when immune complexes with complement components were bound to autologous erythrocytes and then injected, a large fraction of the complexes were dissociated from erythrocytes during the first minute. This release was inversely proportional to the average number of CR1 molecules per erythrocyte. Thus, these studies demonstrate that patients with SLE have neither excessive persistence of immune complexes in circulation nor a decreased hepatic uptake of immune complexes. This is in contrast to the previously described decreased splenic uptake and prolonged circulation of IgG-coated red blood cells in patients with SLE [72]. The splenic uptake of a probe, however, is highly dependent on splenic blood flow [73].

A second study examined the clearance from circulation and uptake by the liver and spleen of immune complexes, prepared with hepatitis B surface antigen (HBsAg) and antibodies to this protein, in normal persons ($n = 12$) and in patients with SLE ($n = 10$) [74]. The polymeric HBsAg had a molecular mass of about 3000 kDa and the formed immune complexes were quite large and fixed complement effectively. Upon intravenous injection, some of the very large but soluble immune complexes made in antibody excess bound to erythrocytes at a higher percentage in normal persons than in patients with SLE. The clearance from blood, however, was faster in patients with SLE than in normal persons. The median half-life in patients was 2.15 min (range 1.3 to 6.6) and in normal persons 5.15 min (range

3.6 to 14). Sucrose density gradient analysis showed that in patients with SLE the very largest immune complexes were not removed selectively from plasma. In contrast, the same analysis of plasma from normal persons showed that the very largest immune complexes had been selectively removed. External counting of the ^{123}I on HBsAg in immune complexes demonstrated that the radioactive probe was taken up more rapidly in the liver in SLE patients than in normal persons. The median time in minutes to 90% of maximal uptake was 9 min (range 4.05 to 18) in SLE patients and 16 min (range 13 to 23) in normal persons. In contrast, the splenic uptake at 60 min was decreased in patients with SLE; 9.03% of injected dose (range 4.05 to 23.7) as compared to normal persons with 23.9% of injected dose (range 17.9 to 30.7]. In the SLE patients a high correlation was found between the half-life of immune complexes and the C4 concentration. These findings clearly indicate the role of the complement system in the clearance from circulation of the very large immune complexes. Once complement components are bound to the complexes, they become attached to the erythrocytes via the CR1, are no longer in plasma, and are then delivered mainly to the spleen. The large complexes that are less effective in activating complement are mainly removed by the liver. In patients with SLE and with decreased complement levels the binding of the very large immune complexes to erythrocytes is decreased and these complexes are diverted to the liver. This accounts for the observed initial rapid removal from blood, increased hepatic uptake, and decreased splenic uptake. This interpretation is also consistent with the already discussed decreased hepatic uptake by the spleen of IgG-coated erythrocytes [72]. In addition, this interpretation was confirmed by a study in a patient with hereditary homozygous C2 deficiency [75]. The study was conducted with immune complexes prepared as described earlier. The initial clearance of the complexes was rapid without binding to erythrocytes, the hepatic uptake was rapid, and no uptake was observed in the spleen. After administration of fresh frozen plasma the initial rapid removal was decreased, immune complexes bound to erythrocytes, hepatic uptake was decreased, and splenic uptake was enhanced.

In another investigation, immune complexes were formed *in vivo* in humans during cancer immunotherapy [76]. The formed complexes consisted of monoclonal mouse IgG and purified human antibodies to mouse IgG. The formed complexes had sedimentation coefficients of 19–25 S units. These complexes were cleared by the liver independent of the CR1 on red blood cells, as only a small portion of the complexes bound to erythrocytes. Immune complexes of comparable size, formed between bovine serum albumin and rhesus monkey IgG antibodies to bovine serum albumin and with lattices similar to those illustrated in Fig. 1, were cleared by the liver and did not bind to rhesus erythrocytes *in vivo* or *in vitro* [77].

Radiolabeled, aggregated human IgG has been used in normal persons and small numbers of patients with SLE to determine its clearance from circulation and uptake by the liver. The material used, however, was a mixture of aggregated and monomeric IgG, thereby complicating the analysis of collected data. The study involved 22 patients with SLE, and the kinetics of the removal of the aggregates was analyzed by a model assuming homogeneity of the material and a distribution phase faster than the elimination phase. The authors concluded that the initial phase of removal of the IgG aggregates was faster in patients with SLE than in normals and that the amount of aggregates bound to red cells was higher in normals [78].

Studies in humans and nonhuman primates led to the following concepts on the removal of circulating immune complexes. The very large immune complexes in circulation that have already bound complement components bind effectively to erythrocytes. These complexes thus are no longer available for deposition in tissues and obviously are not detectable by assays for immune complexes in serum specimens. A significant proportion of the erythrocyte-bound immune complexes is delivered to the spleen. As pointed out earlier, in patients with SLE and decreased complement levels or in patients with absent early complement components, the delivery of immune complexes to the spleen is defective. This defect in patients with SLE was also detected by IgG-coated erythrocytes that are removed primarily by the spleen [72]. Small immune complexes that still contain several IgG molecules do not bind to erythrocytes and are cleared by the liver, mainly by Kupffer cells via FcγR. When the binding of very large immune complexes to erythrocytes is defective, these complexes are also cleared rapidly by the liver. Small immune complexes, containing one or two IgG molecules, persist in circulation and are not deposited in tissues (see Fig. 3). In C1q-deficient mice, the clearance of experimental immune complexes in the spleen was shown to be decreased, whereas the initial phase of immune complex clearance in the liver was enhanced [79]. Because mice lack CR1-dependent immune complex binding to erythrocytes, these experiments indicate that activation of the classical pathway of complement may have additional roles in the clearance of immune complexes, at least in mice.

In mice, immune complexes containing the IgA class of antibodies were taken up rapidly by the liver when eight or more IgA antibodies were present in the immune complexes. This critical number of eight or

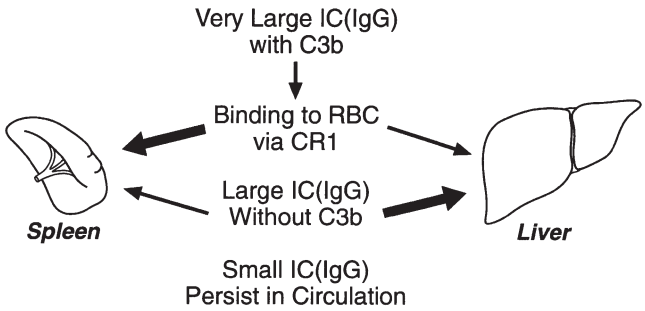


FIGURE 3 Schematic representation of the fate of circulating immune complexes (IC). Very large IC formed in antibody excess activate complement effectively, bind C3b, and become attached to red blood cells (RBC) via complement receptor 1 (CR1). IC bound to RBC do not deposit in tissues and are delivered principally to the spleen. The splenic uptake of IC bound to RBC is decreased in patients with SLE and hypocomplementemia, and IC with C3b are diverted to the liver. Large IC without bound C3b are taken up principally by the liver, both in normal persons and in patients with SLE. Small IC (e.g., Ag_1Ab_1, Ag_2Ab_1, or Ag_2Ab_2) are not removed rapidly and do not deposit in tissues.

more antibody molecules could be achieved either with eight monomeric IgA molecules or by four dimeric IgA molecules [80].

As pointed out under the discussion of antigens, the nature of antigens in the immune complexes can alter the fate of immune complexes. If the antigen alone is removed rapidly from circulation, then the presence of such antigens in the immune complexes can enhance the removal of complexes from circulation. It was demonstrated in experimental animals that the clearance of immune complexes containing glycosylated antigens was governed in part by specific carbohydrate receptors on hepatocytes [3]. A serum carbohydrate-binding protein, mannose-binding protein (MBP), may have an important role in clearing immune complexes containing antigens with selected carbohydrate residues [81]. A member of the collagen motif-containing collectin family of proteins, MBP binds terminal mannose, fucose, glucose, frutose, or *N*-acetylglucosamine residues, can serve as an opsonin, activate the classical or alternative pathways of complement [82], and activate macrophages via the C1q receptor [83]. It has been shown that genetic polymorphisms responsible for depressed function and serum levels of MBP are associated with SLE in African-Americans [84] and some other ethnic and national groups [85–88]. Furthermore, certain ribonucleoprotein autoantigens, including the U1-specific 68-kDa and A proteins and the U2-specific B″ protein, are glycoproteins, with mannose, glucose, and *N*-acetylglucosamine detected on the 68-kDa protein [89]. Thus, it is conceivable that the clearance of glycoprotein antigens or immune complexes containing such antigens, including the U1-RNP particle, could be influenced by MBP polymorphisms. These considerations suggest that MBP polymorphisms could participate in the pathogenesis of SLE by influencing immune complex clearance, analagous to the role of polymorphisms in complement components and FcR.

TISSUE DEPOSITION OF IMMUNE COMPLEXES

In patients with SLE, deposits of immune complexes have been identified in several organs, including glomeruli, renal peritubular capillaries, small and medium blood vessels in several organs, dermal–epidermal junction, and choroid plexus. In most of these sites the presence of immune complexes has been inferred from the presence of immunoglobulins and complement components. Only in the glomeruli and blood vessels have specific antibodies been identified. The presence of immune complexes at various sites may arise from the deposition of circulating immune complexes or from the local formation of antigen–antibody complexes at the site of their presence. The local formation of immune complexes can arise from selective deposition or presence of an antigen at a given location, followed by binding of specific antibodies to the localized antigens. At this time, information is not available to distinguish in human diseases and in patients with SLE the immune complexes that have arisen in the given location by deposition from circulation or by local formation. The presence of circulating immune complexes in association with tissue deposition should not be considered unequivocal evidence for deposits of circulating immune complexes. Studies in experimental animals have clarified the concepts of how immune deposits may arise in glomeruli. Less progress has occurred in the understanding of immune deposit formation in other organs. For this reason, emphasis is given to information concerning immune deposit formation in glomeruli.

Localization of Immune Complexes in Glomeruli

A number of studies in experimental animals have indicated that immune deposits in glomeruli may arise from the deposition of immune complexes from the circulation or immune deposits in glomeruli may develop by the local formation of antigen–antibody complexes. Several unique structural features of glomeruli may well contribute to these processes. First, large volumes of

plasma are filtered through the capillary wall with transient retention of macromolecules, including immune complexes. Second, the glomerular capillary wall contains a fenestrated endothelium. Third, fixed negative charges are present in the glomerular basement membrane in the lamina rara interna (subendothelial area) and in the lamina rara externa (subepithelial area).

In lupus nephritis, in other forms of glomerulonephritides, and in chronic serum sickness models in experimental animals, the renal lesions may contain mesangial, subendothelial, and subepithelial immune deposits (Fig. 4). Experiments with the injection of preformed immune complexes into unimmunized animals have indicated that the complexes in circulation deposit principally in the subendothelial and mesangial areas. In a number of studies of this nature, immune complexes injected into circulation caused no deposits in the subepithelial area [70, 90].

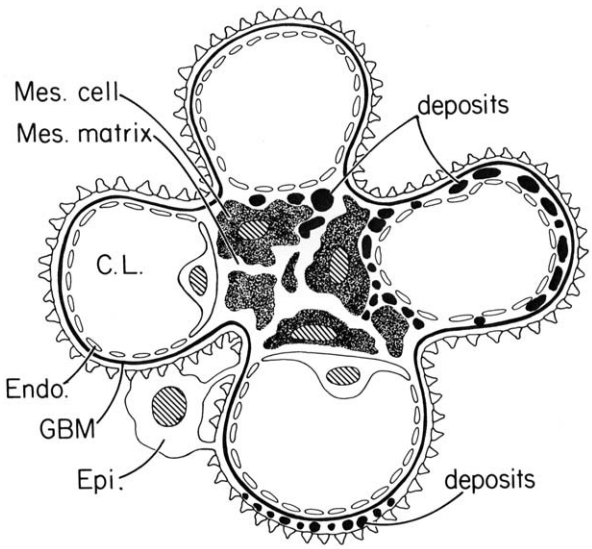


FIGURE 4 Schematic representation of the location of immune complexes in glomeruli. Glomerular capillaries cut in cross section are shown. The capillary lumen (CL) is lined with fenestrated endothelium (Endo). The glomerular basement membrane (GBM) is a size and charge barrier to circulating macromolecules. Negative charges are present in the subendothelial area (lamina rara interna) and in the subepithelial area (lamina rara externa). Beyond the GBM are the foot processes of the epithelial cells (Epi). The area between the capillary loops is the mesangium, consisting of mesangial matrix (Mes. matrix) and mesangial cells (Mes. cell). Immune complexes, recognized as electron-dense deposits by electron microscopy, may be only in the mesangium. Immune complexes may exist in the subendothelial area, but these are accompanied by immune complexes in the mesangium. Immune complexes may be present in the subepithelial area, as seen in membranous glomerulonephritis.

Several lines of evidence have indicated that the lattice of circulating immune complexes is highly important to their deposition in glomeruli. First, when mixtures of large-latticed and small-latticed immune complexes in antigen excess were injected into mice, the glomerular deposition of complexes progressed only while the large-latticed complexes remained in circulation [70]. Second, the injection of only small-latticed immune complexes into the circulation of mice caused no deposits in the glomeruli [91]. Third, when large-latticed immune complexes were deposited in glomeruli, the injection of a large excess of antigen resulted in the complete removal of the antigen–antibody deposits within hours, presumably by the conversion of immune deposits into small-latticed immune complexes [92]. All these studies have indicated that the lattice of immune complexes has a pivotal role in their deposition in the glomeruli.

Immune deposits in the subendothelial or mesangial area, as visualized by electron microscopy, are considerably larger than immune complexes that have been injected into experimental animals. This suggests that after the initial deposition in glomeruli, the immune complexes must undergo further condensation or rearrangement into even larger deposits. The need for further condensation or rearrangement of immune complexes to persist in glomeruli and to become visible as electron-dense deposits was demonstrated by the use of covalently cross-linked immune complexes. Covalent cross-linking established a fixed lattice in the immune complexes so that they could not rearrange into large lattices or precipitate. When such complexes were injected into mice, only transient deposits in glomeruli were identified by immmunofluoresence microscopy, and large electron-dense deposits did not evolve. In contrast, when similar complexes were prepared without covalent bonds and administered to mice, then deposits evolved that persisted and became visible as electron-dense deposits [93]. These studies suggest that large-latticed immune complexes containing more than two antibody molecules become locally concentrated in glomeruli, either as a result of filtration of plasma or because of interactions with glomerular structures. As a consequence of this increased local concentration, condensation occurs into larger lattices. Thus, nonprecipitating antigen–antibody systems would not form persisting immune deposits in glomeruli and would not lead to the formation of electron-dense deposits.

Electrostatic interactions constitute one possible mechanism for the attachment to glomeruli and to the local increase in concentration of immune complexes. When chemically cationized (positively charged) antibodies were used to prepare soluble immune complexes, the injection of these complexes caused

extensive subendothelial and mesangial deposits in glomeruli. In contrast to immune complexes prepared with unaltered antibodies, complexes with cationic antibodies caused extensive subendothelial deposits that persisted in this location for several days, even when injected in small doses. The lattice of the complexes was still highly important with the cationized antibodies. When only small-latticed immune complexes were prepared with these antibodies and injected into mice, the deposits persisted in glomeruli by immunofluorescence microscopy comparable to antibodies alone [94]. These results suggest that when large-latticed immune complexes with more than two antibody molecules attach to the fixed negative charges in the lamina rara interna, their local concentration is increased, leading to rearrangement and formation of larger deposits that become visible as electron-dense deposits in the subendothelial area. Small-latticed immune complexes would initially attach in a comparable manner, but they would not condense into larger deposits and therefore remained in glomeruli only for a few hours. The initial interaction of cationic immune complexes with the fixed negative sites on the glomerular basement membrane is a charge–charge interaction and was disrupted by unrelated polycationic molecules. Once the condensation to larger deposits occurred, then the unrelated polycationic molecules no longer displace the deposits [95]. The mechanism for the removal of immune complexes from the basement membrane and other organs is not well understood. The absence of C3 in experimental immune-mediated renal disease may lead to an alteration in the distribution of immune deposits within the glomerulus, suggesting that complement may play a role in immune complex rearrangement within the kidney [96]. The complement system, particularly C3, probably plays a role not only in the solublization of immune precipitates, but also in the removal and trafficking of immune deposits within the basement membrane of the kidney [97].

The formation of mesangial deposits must also involve other yet unknown factors. Mesangial deposits occurred with large-latticed immune complexes containing cationized antibodies. Similar deposits, however, also occurred with immune complexes prepared with anionic (negatively charged) antibodies and anionic antigens, suggesting that charge–charge interactions were not important in the formation of mesangial deposits [98]. It is of interest that FcγR were not expressed by unstimulated human mesangial cells in culture. Upon stimulation with IL-1 and lipopolysacharide, however, these cells contained mRNA of FcγRIII [99]. The expression of receptors on mesangial cells may enhance localization and retention of immune complexes in the glomerular mesangium.

Several experimental models exist for local immune complex formation in glomeruli due to antigens that become attached or planted in glomeruli [90]. Intravenously injected aggregated IgG or aggregated albumin becomes entrapped in the mesangial matrix. When this event is followed by the injection of specific antibodies, immune deposits form and cause an inflammatory response in the mesangial area of glomeruli, as reviewed by Michael *et al*. [100]. Concanavalin A binds to the endothelium and subendothelial area, and when antibodies to concanavalin A are administered, lumpy-bumpy immune deposits evolve on the glomerular basement membrane [101]. Considerable evidence indicates that subepithelial immune deposits, as seen in membranous lupus glomerulonephritis, are formed locally and are not deposited from the circulation as immune complexes. The initial evidence for local or *in situ* immune complex formation was obtained in rats with the model of passive Hayman nephritis. In this model the antigen is the so-called Fx1A antigen, which is derived from the brush border of proximal tubules but also exists on the epithelial cells of glomeruli. When antibodies to the Fx1A antigen were perfused into the renal artery in a nonrecirculating system, immune deposits formed within hours in the subepithelial area. In these experiments the possibility of immune complexes arising in circulation was excluded [102]. It is now recognized that the involved antigens are part of the epithelial cell surface, and upon interaction with antibodies the formed immune complexes are released into the subepithelial area [103]. The local formation of immune complexes was also achieved by the repeated perfusion of rat kidneys with an bovine serum albumin alternating with an infusion of antibodies to bovine serum albumin [104]. The high density of fixed negative charges in the lamina rara externa (subepithelial area) has served as a mechanism for planting positively charged antigens in glomeruli. The administration of cationized antigens followed by the administration of specific antibodies leads to persisting immune deposits in the subepithelial area and to the formation of electron-dense deposits [105]. Similar results have been achieved with the chronic administration of cationic antigens, leading to an endogenous immune response and to the development of extensive membranous glomerulonephritis with subepithelial deposits [106]. The formation of persisting immune deposits in the subepithelial area and the formation of electron-dense deposits also required precipitating antigen–antibody systems. With nonprecipitating antigen–antibody systems, only transient deposits formed in the subepithelial area as detected by immunofluorescence microscopy, and electron-dense deposits were not formed [107].

The described studies in experimental animals suggest that the formation of subepithelial immune deposits in human disorders, including SLE, should involve cationic antigens or cationic antibodies that become localized in this area due to electrostatic interactions. The subsequent access to this area of glomeruli by specific antibodies or antigens, respectively, would then lead to the formation of immune deposits. The sequence of events may be even more complicated. Studies in experimental animals have shown that cationic histones bind to glomeruli by charge–charge interactions, followed by DNA, and finally antibodies to histones and to DNA bind to these antigens in glomeruli [108]. In support of this concept was the identification of histone H3 and a complex of ubiquitin and histone H2A in the majority of glomeruli in renal biopsies of patients with SLE [109]. Whereas in the study of 70 biopsies from other forms of glomerular disease, these molecules were present only in two specimens.

In patients with lupus the presence of a number of serum autoantibodies has been associated with renal disease, reviewed by Lefkowith and Gilkeson [110]. Serum antibodies to dsDNA have a strong association with renal disease. In addition, antibodies to histones and to chromatin share this association. The extraction of antibodies from glomeruli of patients with SLE has demonstrated the presence of antibodies to dsDNA, ssDNA, and nucleoproteins [2, 111]. In a subsequent investigation, the presence of antibodies to SS-A(Ro) was identified in eluates of renal cortical tissue in two patients with SLE [112]. In patients with proliferative lupus glomerulonephritis (WHO class IV lesions), autoantibodies were identified in serum to the collagen-like region of C1q (anti-C1q-CLR) [113, 114]. Subsequent studies showed that the development of these antibodies in circulation served as a better predictor of developing proliferative glomerulonephritis than a rise in the level of antibodies to dsDNA [115]. Anti-C1q-CLR has been extracted from isolated glomerular basement membrane fragments from four of five patients with proliferative lupus glomerulonephritis [116]. Anti-C1q-CLR do not react with circulating C1 and do not bind well to C1q in the fluid phase. Anti-C1q-CLR, however, bind well to C1q attached to immune complexes *in vitro* or *in vivo* [117]. Therefore, it is likely that anti-C1q-CLR contribute to the glomerular inflammation by binding to already deposited immune complexes with bound C1q. Alternatively, these antibodies may bind to C1q attached to DNA because DNA in glomeruli may bind C1q directly without the presence of antibodies [118]. Further studies have shown that multiple autoantibody specificities, including anti-dsDNA, anti-C1q-CLR, anti-SSA, anti-Sm, anti-SSB, antihistone, and antichromatin, are frequently and concurrently present and enriched in the glomerular basement membranes from kidneys of patients with lupus [119]. In contrast, control antibodies such as antibodies to tetanus and to Epstein–Barr virus antigens are present in concentrations that reflected only the serum concentration of those antibodies and were not enriched in the kidneys. Furthermore, elution of the antibodies from lupus kidneys required strong denaturants and even disruption of covalent bonds, indicating that in chronic human immune complex disease the immune complexes form covalent bonds with components in the kidney.

The mechanisms, however, of how the various autoantibodies deposit in glomeruli of patients with SLE have not been established with certainty. Perhaps the main reason for the absence of this information is the fact that very little is known about the nature and the source of antigens that are present in the glomerular immune deposits of patients with lupus renal disease.

Localization of Immune Complexes in Other Organs

The localization of immune complexes in other organs has not been examined in the detail described for glomeruli. The existence of immunoglobulins and complement components at the dermal–epidermal junction of patients with SLE is well established. Antibodies to nuclear antigens and antibodies to DNA have been recovered from these deposits, suggesting that indeed the detected deposits exist as immune complexes at this location. Some evidence indicates that keratinocytes at the basal layer of skin contain the Ro (SS-A) antigen. As this antigen is extruded, immune complexes form at the dermal–epidermal junction with antibodies to Ro [120].

Studies with preformed immune complexes have suggested that charge–charge interactions contribute to the formation of immune complexes at this site. First, cationic antibodies alone bound to the dermal–epidermal junction of mice, as detected by immunofluorescence microscopy. This finding raises the possibility that *in situ* immune complex formation may occur with the initial deposition of, for example, cationic antibodies to DNA and subsequent immune complex formation with DNA. Second, large-latticed circulating immune complexes, prepared with cationic antibodies, deposited at this location in the skin. The deposited immune complexes persisted in this location longer than the cationic antibodies alone [121]. Third, the chronic injection of a cationized antigen caused immune deposits at the dermal–epidermal junction, whereas the injection of the

unaltered antigen according to the same schedule and dose did not cause these deposits [122].

In patients with SLE, immune complexes have been described in the choroid plexus, between the basement membrane and the base of the epithelial cells. Similar lesions have been observed in NZB/NZW mice and in patients with chronic serum sickness. The injection of very large doses of preformed immune complexes has resulted in some localization of the injected immune complexes in the choroid plexus [123]. The injection of cationic antigens has resulted in deposits of the antigen in the choroid plexus. When antibodies were injected later, immune complexes were formed in the choroid plexus and persisted in this area. Of interest was the observation that a cationic antigen persisted for many days in the choroid plexus, whereas the bulk of cationized antigens lasted in glomeruli for less than a day. These findings suggest differences in the nature or the turnover of the fixed negative charges in glomeruli compared with the choroid plexus [124].

The immune deposits in blood vessel walls clearly are associated with vasculitis in patients with SLE. The mechanisms for the formation of these deposits are not known. In experimental animals, serum sickness is associated with vascular deposits of immune complexes; increased permeability of the vessels facilitates the development of these deposits [125]. Injection of a single bolus of preformed immune complexes into mice also caused vascular deposits, as recognized by immunofluorescence microscopy. These deposits, however, tended to persist for a much shorter period in dermal, myocardial, and peritubular renal small vessels than in glomeruli [121].

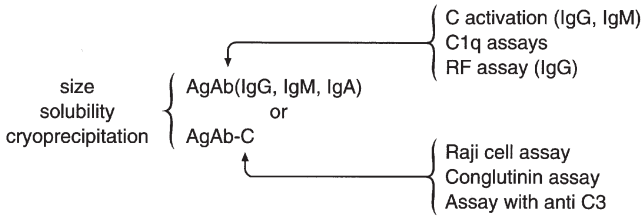


FIGURE 5 A diagrammatic representation of the tests used for the detection and measurement of immune complexes. Immune complexes (AgAb) may contain antibodies belonging to IgG, IgA, or IgM classes. Other immune complexes may have bound complement components *in vivo*. Physical methods for the detection of immune complexes can detect both categories of immune complexes by their size, decreased solubility, or cryoprecipitability. Some biologic assays for immune complexes, as depicted, distinguish immune complexes from free antibodies, as the antibodies in complexes have acquired new or more pronounced properties (i. e., complement activation, binding to C1q or to IgM rheumatoid factors). Other tests recognize immune complexes, as components of complement have bound to the immune complexes (e.g., Raji cell assay, conglutinin assay, or assays with antibodies to C3 components).

MEASUREMENT OF IMMUNE COMPLEXES AND USE OF THESE TESTS IN PATIENTS WITH SLE

Because the clinical manifestations of SLE and other systemic immune complex diseases are related at least in part to circulating immune complexes, it seems desirable to quantify these materials in serum. The principles involved in the tissue deposition of circulating immune complexes, as established in experimental animals, were reviewed previously. A number of methods have been developed to detect and to quantify immune complexes in the serum or other body fluids.

Methods for the detection of immune complexes rely on either the physical or the biologic properties of immune complexes. As already pointed out, both of these categories of properties of immune complexes are highly variable because of the variations in antigens, antibodies, and numbers of reactants that are present in a given complex. The physical methods rely largely on the fact that immune complexes differ from antibodies alone by size, solubility, or cryoprecipitability. Tests based on biologic properties recognize immune complexes because antibodies have acquired properties different from free antibodies or because complement components have been attached to the immune complexes. These principles are presented schematically in Fig. 5.

Physical Methods

Physical methods for the detection of immune complexes are based on the fact that complexes are larger than antibodies alone; the solubility of immune complexes is less than that of antibodies alone, and some immune complexes are precipitable by exposure to cold. Antibodies alone have defined size; therefore, if a serum specimen is submitted to ultracentrifugation or to gel filtration, the obtained fractions can be analyzed for the presence of various classes of immunoglobulins. Identification of immunoglobulins in fractions that normally do not contain antibodies suggests the presence of immune complexes. The normal distribution of antibody molecules upon size separation, however, is a function of the concentration of these molecules. Therefore, in SLE and other diseases in which hypergammaglobulinemia is common, the interpretation of data becomes difficult. Furthermore, small immune complexes are difficult to distinguish from the normal distribution of

antibodies. This type of analysis is time-consuming, and therefore these methods are not used by clinical laboratories but remain tools for research laboratories.

The addition to serum specimens of low concentrations of polyethylene glycol precipitates some immune complexes. At the same time, however some normal serum proteins are also precipitated, particularly large serum proteins. Therefore, the quantitation of precipitated protein alone is not reliable, and the immunologic quantitation of the precipitated proteins is fraught with additional problems. Small amounts of cryoglobulins have been identified in patients with SLE and other disorders with immune complexes. Not all immune complexes, however, are precipitated at a low temperature. Therefore, this approach has not provided a reliable and easy tool for the quantitation of circulating immune complexes.

Methods Based on Biologic Properties

Several assays for the measurement of immune complexes are based on recognizing antibody molecules that have acquired properties that distinguish them from free antibody molecules. This category of assays includes complement activation, C1q assays, and rheumatoid factor assays and assays that depend on the presence of complement components. Because the activation of complement depends on the lattice and on the class of immunoglobulin molecules in the complexes, only immune complexes with IgG and IgM are recognized, and they must have attained sufficient lattice to activate complement. Because the amount of complement activity present in the serum is variable, this activity must first be destroyed before activation of a known amount of added complement is quantified. These tests are not readily employed in clinical laboratories because of their complexity.

C1q assays depend on the ability of immune complexes to interact with the globular regions of C1q. IgG- and IgM-containing immune complexes are recognized by this test, provided sufficient lattice is present. Two major types of assays are used with C1q. In the C1q-binding assay, isolated radiolabeled C1q is added to the serum. Upon the addition of low concentrations of polyethylene glycol, the radiolabeled C1q bound to immune complexes is precipitated, whereas unbound radiolabeled C1q remains in the supernatant. This assay does not distinguish the binding of immune complexes to C1q from the binding of other serum proteins in C1q. The presence of polyanionic molecules in serum and the presence of fibrinogen will lead to positive results. In the solid-phase C1q assay, isolated C1q is absorbed to the walls of the test tubes or microtiter wells. Immune complexes in the specimen bind to the C1q, other serum proteins are removed by washing, and the IgG or IgM bound to the C1q is quantified immunochemically. When the sizes of immune complexes in patients with SLE were examined, those with proliferative lupus glomerulonephritis had IgG-containing complexes but also had monomeric IgG that bound to C1q in the solid-phase assay [113]. This reactivity was shown to result from the presence of autoantibodies to C1q, directed to the collagen-like region of these molecules [114]. Therefore, when the solid-phase C1q assay is used, the presence of immune complexes and autoantibodies to the collagen-like region of C1q must be distinguished. This can be achieved optimally by the use of purified collagen-like regions of C1q for identification of the autoantibodies [126]. Although some authors have measured binding of IgG to C1q in the presence of high salt concentrations as a surrogate for the measurement of autoantibodies to C1q, the two experimental approaches are not identical [127].

Assays using rheumatoid factors detect only immune complexes that contain IgG molecules as antibodies. The main principle of the test is that the IgM rheumatoid factor binds with a much higher affinity to polymeric IgG than to monomeric IgG. The technical conduct of these and other tests for immune complexes is beyond the scope of this discussion, and several reviews are available [128–131].

Some major problems exist in these categories of tests for immune complexes. First, as already pointed out, these biologic properties of immune complexes depend on the lattices of the complexes. Thus, C1q assays and rheumatoid factor assays detect large-latticed complexes more efficiently than small-latticed complexes. The same degree of positivity in a clinical specimen can arise from the presence of a relatively small amount of large-latticed immune complexes or a relatively large amount of small-latticed immune complexes. Furthermore, the presence of monomeric IgG can interfere with these tests to some degree. Second, no ideal standard exists for the detection of immune complexes with these test methods. Heat-aggregated IgG is the most commonly used standard. The degree and the size of the aggregates are usually not defined, and therefore large variations can exist from laboratory to laboratory, as documented by a World Health Organization study [132]. If a standardized method and preparation were adopted and defined, then at least this potential problem could be surmounted.

Other tests for immune complexes depend on the fact that complement components have bound to immune complexes *in vivo*. The prototypic assay in this category is the Raji cell assay [129]. Raji cells are lymphoblastoid B cells derived from a patient with Burkitt's lymphoma. These cells lack surface immunoglobulins

and have low-affinity receptors for IgG and high-affinity receptors for activated complement components. Accordingly, this assay detects immune complexes that have C3b and other complement components bound to them. When the serum specimen with immune complexes is incubated with these cells, the immune complexes with C3b or other components of complement bind to the cells, the cells are washed, and the amount of complexes bound to the cells are quantified by specific radiolabeled or enzyme-labeled antibodies to IgG or to other immunoglobulins. Aggregated IgG is used as a standard for this test. Because the amount of IgG on the cell is quantified, the presence of lymphocytotoxic antibodies to B cells can lead to false-positive results. Some evidence also indicates that even antibodies to nuclear antigens may give false-positive tests, as nuclear antigens either bind to cell surface molecules or are extruded by the cells [129].

The conglutinin assay detects only immune complexes that have activated complement and contain C3bi. If C3b is present before inactivation by the C3b inactivator, the complexes are not detected. Furthermore, once C3bi is cleaved into C3d and C3c, the immune complexes are no longer effectively detected by this method. Another method has been developed by taking advantage of specific antibodies to C3b or C3d that have been absorbed on the surface of plastic tubes. The binding of immune complexes with these complement components to the test tubes is then detected and quantified by antibodies to specific immunoglobulins, either radiolabeled or enzyme linked. Because the solubilization of immune complexes by complement leads to the formation of IgG–C3d complexes without the presence of antigen molecules, this assay also detects material in the size range of normal IgG that does not represent immune complexes.

Assays for the detection and quantification of immune complexes described earlier are not specific for any given antigen; therefore, these tests have no specificity to a given disease and provide no assistance in reaching a specific diagnosis. Research laboratories have shown that antigens can be recognized with appropriate antisera in the complexes detected by these assays. Therefore, it is possible that tests become available for specific disease, including SLE.

In experimental models the load of circulating immune complexes increase the likelihood of tissue deposition and induction of lesions; therefore one would expect that in patients with SLE the manifestations of SLE caused by circulating immune complexes would have a relationship to the measured level of immune complexes. If this were the case, then the measurement of circulating immune complexes should have a predictive value and assist in therapeutic decisions. At this time, however, conclusive data are not available to use these tests in therapeutic decisions, and the clinical utility of tests of circulating immune complexes is limited [133].

In experimental animals, as reviewed earlier, mesangial and subendothelial immune deposits are formed by circulating immune complexes. Using a C1q solid-phase assay for immune complexes in conjunction with size separation of the immune complexes by ultracentrifugation, large immune complexes were found in serum or plasma of patients with SLE who had subendothelial and mesangial immune deposits [113]. These patients also had autoantibodies to the collagen-like region of C1q molecules [114, 115, 126].

Tests for Immune Complexes and Clinical Activity of SLE

Relationships between the clinical activity of SLE and the concentrations of immune complexes by one or more tests have been examined by several laboratories [134]. Positive correlations have been found between the clinical activity of disease in patients with SLE and immune complexes detected by the fluid-phase C1q-binding assay, solid-phase C1q assay, Raji cell assay, and conglutinin assay. Other observers have, however, reached the conclusion that a definite relationship does not exist between disease activity or renal involvement with one form or another of lupus nephritis and the measured levels of immune complexes. One retrospective study concluded that the level of total hemolytic complement or complement components (C4) had a better relationship to disease activity than the fluid-phase C1q-binding assay for circulating immune complexes [135]. A clear and uniform definition of disease activity has not been used in all investigations that would permit critical comparisons of the study results. In addition, some of the differences in conclusions may stem from differences in the methods for the detection of immune complexes. Of course, the possibility exists that indeed the currently available tests for immune complexes do not relate to disease activity and thus would be of no predictive value. Ideally, the predictive value of assays for immune complexes should be determined in a prospective manner with defined criteria for disease activity. This type of information, however, is not on hand for tests for immune complexes or, for that matter, for many other tests used in clinical medicine. In one prospective study the solid-phase C1q assay, which also detects autoantibodies to C1q, was found to have predictive value for flare-ups of SLE or for improvements of SLE, but not the fluid-phase C1q-binding assay [136]. In this study the association with clinical improvement or flare-up was thought to exist if

the solid-phase C1q assay changed from positive to negative, or vice versa, within 2 months of the clinical event, an association that has not been validated. Furthermore, the degrees of clinical changes were not related to the quantity of immune complexes; only the change from a negative to a positive result, or vice versa, was related to a clinical event indicative of improvement or worsening of the disease.

For all these reasons the recommendation has been made that tests for immune complexes are not essential in any clinical condition, but that the detection of immune complexes may be helpful for the assessment and monitoring of disease activity in some disorders, including SLE [133]. Obviously more information and new developments are needed. Meanwhile, clinicians familiar with the interpretation of these tests may use the tests for the measurement of immune complexes as an adjunct with other tests or in place of other tests (e.g., total hemolytic complement or complement component levels) along with clinical information to make therapeutic decisions.

DEVELOPMENT OF THERAPIES BASED ON THE IMMUNE COMPLEX MODEL

The immune complex model for the cause of tissue damage in SLE has been the dominant paradigm for several decades, and it remains so. Therapeutic approaches based on this paradigm, however, have been relatively disappointing. For example, whereas plasmapheresis for the treatment of SLE was originally met with great enthusiasm, a controlled clinical trial of plasmapheresis in patients with lupus nephritis was unsuccessful [137].

Affinity columns containing silica-bound staphylococcal protein A (SPA) have been approved for use in patients with severe, refractory rheumatoid arthitis [138]. The rationale for the use of SPA immunoabsorbants arises from the observation that IgG within immune complexes binds preferentially to SPA, compared with mononeric noncomplexed IgG. It has been suggested that other mechanisms besides immune complex removal may play a role in the improvement [139]; nevertheless, this successful therapy was developed because of the immune complex disease model. C1q immunoadsorption has been employed in the treatment of patients with SLE [140, 141]. Further follow-up and evaluation of this investigational approach are needed to substantiate this therapy, however. As noted earlier, animal studies have shown the efficacy of administered heparin and heparinoids to prevent nephritis in MRL/lpr mice, presumably by preventing the binding of nucleosome-containing immune complexes to heparan sulfate in glomerular basement membranes [23]. Thus, therapeutic approaches targeting the antigens in immune complexes, as well as approaches that are not antigen specific, could prove helpful in the treatment of SLE. Immune complexes play a pivotal role in the pathogenesis of lupus nephritis, as well as other manifestations of SLE, and understanding the pathophysiology could provide an important basis for further therapeutic approaches.

References

1. Dixon, F. J. (1963). The role of antigen-antibody complexes in disease. *Harvey Lect.* **58**, 21–52.
2. Koffler, D., Schur, P. H., and Kunkel, H. G. (1967). Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. Exp. Med.* **126**, 607–624.
3. Finbloom, D. S., Magilavy, D. B., Hartford, J. B., Rifai, A., and Plotz, P. H. (1981). The influence of antigen on immune complex behavior in mice. *J. Clin. Invest.* **68**, 214–224.
4. Emlen, W., Rifai, A., Magilavy, D., and Mannik, M. (1988). Hepatic binding of DNA is mediated by a receptor on nonparenchymal cells. *Am. J. Pathol.* **133**, 54–60.
5. Emlen, W., and Mannik, M. (1982). Clearance of circulating DNA-antiDNA immune complexes in mice. *J. Exp. Med.* **155**, 1210–1215.
6. Khoo, J. C., Miller, E., Pio, F., Steinberg, D., and Witztum, J. L. (1992). Monoclonal antibodies against LDL fu rther enhance macrophage uptake of LDL aggregates. *Arterioscler. Thromb.* **12**, 1258–1266.
7. Gisinger, C., Virella, G. T., and Lopes-Virella, M. F. (1991). Erythrocyte-bound low-density lipoprotein immune complexes lead to cholesteryl ester accumulation in human monocyte-derived macrophages. *Clin. Immunol. Immunopathol.* **59**, 37–52.
8. Morganelli, P. M., Rogers, R. A., Kitzmiller, T. J., and Bergeron, A. (1995). Enhanced metabolism of LDL aggregates mediated by specific human monocyte IgG Fc receptors. *J. Lipid Res.* **36**, 714–724.
9. Lopes-Virella, M. F., Binzafar, N., Rackley, S., Takei, A., La Via, M., and Virella, G. (1997). The uptake of LDL-IC by human macrophages: Predominant involvement of the Fcγ RI receptor. *Atherosclerosis* **135**, 161–170.
10. Huang, Y., Jaffa, A., Koskinen, S., Takei, A., and Lopes-Virella, M. F. (1999). Oxidized LDL-containing immune complexes induce Fcγ receptor I-mediated mitogen-activated protein kinase activation in THP-1 macrophages. *Arterioscler. Thromb. Vasc. Biol.* **19**, 1600–1607.
11. Kiener, P. A., Rankin, B. M., Davis, P. M., Yocum, S. A., Warr, G. A., and Grove, R. I. (1995). Immune complexes of LDL induce atherogenic responses in human monocytic cells. *Arterioscler. Thromb. Vasc. Biol.* **15**, 990–999.
12. Kabakov, A. E., Tertov, V. V., Saenko, V. A., Poverenny, A. M., and Orekhov, A. N. (1992). The atherogenic effect of lupus sera: Systemic lupus erythematosus-derived

immune complexes stimulate the accumulation of cholesterol in cultured smooth muscle cells from human aorta. *Clin. Immunol. Immunopathol.* **63**, 214–220.
13. Tertov, V. V., Orekhov, A. N., Kacharava, A. G., Sobenin, I. A., Perova, N. V., and Smirnov, V. N. (1990). Low density lipoprotein-containing circulating immune complexes and coronary atherosclerosis. *Exp. Mol. Pathol.* **52**, 300–308.
14. Vaarala, O., Alfthan, G., Jauhiainen, M., Leirisalo-Repo, M., Aho, K., and Palosuo, T. (1993). Crossreaction between antibodies to oxidized low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. *Lancet* **341**, 923–925.
15. Hasunuma, Y., Matsuura, E., Makita, Z., Katahira, T., Nishi, S., and Koike, T. (1997). Involvement of β2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. *Clin. Exp. Immunol.* **107**, 569–573.
16. Puurunen, M., Manttari, M., Manninen, V., *et al*. (1994). Antibodies against oxidized low density lipoprotein predicting myocardial infarction. *Arch. Intern. Med.* **154**, 2605–2609.
17. van Bruggen, M. C., Kramers, C., Walgreen, B., *et al*. (1997). Nucleosomes and histones are present in glomerular deposits in human lupus nephritis. *Nephrol. Dial. Transplant.* **12**, 57–66.
18. Rumore, P. M., and Steinman, C. R. (1990). Endogenous circulating DNA in systemic lupus erythematosus: Occurrence as multimeric complexes bound to histone. *J. Clin. Invest.* **86**, 69–74.
19. Licht, R., van Bruggen, M. C., Oppers-Walgreen, B., Rijke, T. P., and Berden, J. H. (2001). Plasma levels of nucleosomes and nucleosome-autoantibody complexes in murine lupus: Effects of disease progression and lipopolyssacharide administration. *Arthritis. Rheum.* **44**, 1320–1330.
20. Du Clos, T. W., Volzer, M. A., Hahn, F. F., Xiao, R., Mold, C., and Searles, R. P. (1999). Chromatin clearance in C57Bl/10 mice: Interaction with heparan sulphate proteoglycans and receptors on Kupffer cells. *Clin. Exp. Immunol.* **117**, 403–411.
21. van Bruggen, M. C., Kramers, C., Hylkema, M. N., Smeenk, R. J., and Berden, J. H. (1994). Pathophysiology of lupus nephritis: The role of nucleosomes. *Neth. J. Med.* **45**, 273–279.
22. Kramers, C., Hylkema, M. N., van Bruggen, M. C., *et al*. (1994). Anti-nucleosome antibodies complexed to nucleosomal antigens show anti-DNA reactivity and bind to rat glomerular basement membrane in vivo. *J. Clin. Invest.* **94**, 568–577.
23. van Bruggen, M. C., Walgreen, B., Rijke, T. P., *et al*. (1996). Heparin and heparinoids prevent the binding of immune complexes containing nucleosomal antigens to the GBM and delay nephritis in MRL/lpr mice. *Kidney. Int.* **50**, 1555–1564.
24. Roux, K. H. (1999). Immunoglobulin structure and function as revealed by electron microscopy. *Int. Arch. Allergy. Immunol.* **120**, 85–99.
25. Burton, D. R., and Woof, J. M. (1992). Human antibody effector function. *Adv. Immunol.* **51**, 1–84.
26. Crothers, D. M., and Metzger, H. (1972). The influence of polyvalency on the binding properties of antibodies. *Immunochemistry* **9**, 341–357.
27. Papalian, M., Lafer, E., Wong, R., and Stollar, B. D. (1980). Reaction of systemic lupus erythematosus antinative DNA antibodies with native DNA fragments from 20 to 1200 base pairs. *J. Clin. Invest.* **65**, 469–477.
28. Emlen, W., Ansari, R., and Burdick, G. (1984). DNA-anti DNA immune complexes: Antibody protection of a discrete DNA fragment from DNase digestion in vitro. *J. Clin. Invest.* **74**, 185–190.
29. Schifferli, J. A., Woo, P., and Peters, D. K. (1982). Complement-mediated inhibition of immune precipitation. I. Role of the classical and alternative pathways. *Clin. Exp. Immunol.* **47**, 555–562.
30. Takahashi, M., Czop, J., Ferreira, A., and Nussenzweig, V. (1976). Mechanism of solubilization of immune aggregates by complement: Implications for immunopathology. *Transplant Rev.* **32**, 121–139.
31. Schifferli, J. A., and Peters, D. K. (1983). Complement, the immune complex lattice, and the pathophysiology of complement-deficiency syndromes. *Lancet* **2**, 957–959.
32. Duncan, A. R., and Winter, G. (1988). The binding site for C1q on IgG. *Nature* **332**, 738–740.
33. Idusogie, E. E., Wong, P. Y., Presta, L. G., *et al*. (2001). Engineered antibodies with increased activity to recruit complement. *J. Immunol* **166**, 2571–2575.
34. Idusogie, E. E., Presta, L. G., Gazzano-Santoro, H., *et al*. (2000). Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc. *J. Immunol.* **164**, 4178–4184.
35. Kishore, U., and Reid, K. B. (2000). C1q: Structure, function, and receptors. *Immunopharmacology* **49**, 159–170.
36. Valim, Y. M. L., and Lachmann, P. J. (1991). The effect of antibody isotype and antigenic epitope density on the complement-fixing activity of immune complexes: A systematic study using chimaeric anti-NIP antibodies with human Fc regions. *Clin. Exp. Immunol.* **84**, 1–8.
37. Biesecker, G., Lavin, L., Ziskind, M., and Koffler, D. (1982). Cutaneous localization of the membrane attack complex in discoid and systemic lupus erythematosus. *N. Engl. J. Med.* **306**, 264–270.
38. Biesecker, R. G., Katz, S., and Koffler, D. (1981). Renal localization of the membrane attack complex (MAC) in SLE nephritis. *J. Exp. Med.* **154**, 1779–1794.
39. Salant, D. J., Belok, S., Madaio, M. P., and Couser, W. G. (1980). A new role for complement in experimental membranous nephropathy in rats. *J. Clin. Invest.* **66**, 1339–1350.
40. Sheerin, N. S., Springall, T., Carroll, M. C., Hartley, B., and Sacks, S. H. (1997). Protection against anti-glomerular basement membrane (GBM)-mediated nephritis in C3- and C4-deficient mice. *Clin. Exp. Immunol.* **110**, 403.
41. Sekine, H., Reilly, C. M., Molano, I. D., *et al*. (2001). Complement component C3 is not required for full expression of immune complex glomerulonephritis in MRL/lpr mice. *J. Immunol.* **166**, 6444–6451.

42. Ravetch, J. V., and Bolland, S. (2001). IgG Fc receptors. *Annu. Rev. Immunol.* **19**, 275–290.
43. Salmon, J. E., and Pricop, L. (2001). Human receptors for immunoglobulin G: Key elements in the pathogenesis of rheumatic disease. *Arthritis Rheum.* **44**, 739–750.
44. Fossati, G., Bucknall, R. C., and Edwards, S. W. (2001). Fcγ receptors in autoimmune diseases. *Eur. J. Clin. Invest.* **31**, 821–831.
45. Lovdal, T., and Berg, T. (2001). Transcription of Fcγ receptors in different rat liver cells. *Cell. Biol. Int.* **25**, 821–824.
46. Lovdal, T., Andersen, E., Brech, A., and Berg, T. (2000). Fc receptor mediated endocytosis of small soluble immunoglobulin G immune complexes in Kupffer and endothelial cells from rat liver. *J. Cell. Sci.* **113**, 3255–3266.
47. Unkeless, J. C., Scigliano, E., and Freedman, V. H. (1988). Structure and function of human and murine receptors for IgG. *Annu. Rev. Immunol.* **6**, 251–281.
48. Dower, S. K., Delisi, C., Titus, J. A., and Segal, D. M. (1981). Mechanism of binding of multivalent immune complexes to Fc receptors. 1. Equilibrium binding. *Biochemistry* **20**, 6326–6334.
49. Marusic-Galesic, S., Pavelic, K., and Pokric, B. (1991). Cellular immune response to antigen administered as an immune complex. *Immunology* **72**, 526–531.
50. Amigorena, S. (2002). Fcγ receptors and cross-presentation in dendritic cells. *J. Exp. Med.* **195**, F1–F3.
51. Morgan, E. L., and Weigle, W. O. (1983). Polyclonal activation of murine B lymphocytes by immune complexes. *J. Immunol.* **130**, 1066–1070.
52. Fossati, G., Bucknall, R. C., and Edwards, S. W. (2002). Insoluble and soluble immune complexes activate neutrophils by distinct activation mechanisms: Changes in functional responses induced by priming with cytokines. *Ann. Rheum. Dis.* **61**, 13–19.
53. Sylvestre, D., Clynes, R., Ma, M., Warren, H., Carroll, M. C., and Ravetch, J. V. (1996). Immunoglobulin G-mediated inflammatory responses develop normally in complement-deficient mice. *J. Exp. Med.* **184**, 2385–2392.
54. Ravetch, J. V., and Clynes, R. A. (1998). Divergent roles for Fc receptors and complement in vivo. *Annu. Rev. Immunol.* **16**, 421–432.
55. Clynes, R., Dumitru, C., and Ravetch, J. V. (1998). Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* **279**, 1052–1054.
56. Yuasa, T., Kubo, S., Yoshino, T., *et al*. (1999). Deletion of Fcγ receptor IIB renders H-2(b) mice susceptible to collagen-induced arthritis. *J. Exp. Med.* **189**, 187–194.
57. Heller, T., Gessner, J. E., Schmidt, R. E., Klos, A., Bautsch, W., and Kohl, J. (1999). Cutting edge: Fc receptor type I for IgG on macrophages and complement mediate the inflammatory response in immune complex peritonitis. *J. Immunol.* **162**, 5657–5661.
58. Bhatia, A., Blades, S., Cambridge, G., and Edwards, J. C. (1998). Differential distribution of FcγRIIIa in normal human tissues and co-localization with DAF and fibrillin-1: Implications for immunological microenvironments. *Immunology* **94**, 56–63.
59. Salmon, J. E., Millard, S., Schachter, L. A., *et al*. (1996). Fcγ RIIA alleles are heritable risk factors for lupus nephritis in African-Americans. *J. Clin. Invest.* **97**, 1348–1354.
60. Lehrnbecher, T., Foster, C. B., Zhu, S., *et al*. (1999). Variant genotypes of the low-affinity Fcγ receptors in two control populations and a review of low-affinity FcγR polymorphisms in control and disease populations. *Blood* **94**, 4220–4232.
61. Botto, M., Theodoridis, E., Thompson, E. M., *et al*. (1996). Fcγ RIIa polymorphism in systemic lupus erythematosus (SLE): No association with disease. *Clin. Exp. Immunol.* **104**, 264–268.
62. Wu, J., Edberg, J. C., Redecha, P. B., *et al*. (1994). A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest.* **100**, 1059–1070.
63. Chevalier, J., and Kazatchkine, M. D. (1989). Distribution in clusters of complement receptor type one (CR1) on human erythrocytes. *J. Immunol.* **142**, 2031–2036.
64. Paccaud, J. P., Carpentier, J. L., and Schifferli, J. A. (1988). Direct evidence for the clustered nature of complement receptor type 1 on the erythrocyte membrane. *J. Immunol.* **141**, 3889–3894.
65. Emlen, W., Burdick, G., Carl, V., and Lachmann, P. J. (1989). Binding of model immune complexes to erythrocyte CR1 facilitates immune complex uptake by U937 cells. *J. Immunol.* **142**, 4366–4371.
66. Hebert, L. A., Cosio, F. G., Birmingham, D. J., *et al*. (1991). Experimental immune complex-mediated glomerulonephritis in the nonhuman primate. *Kidney Int.* **39**, 44–56.
67. Walport, M. J., and Lachmann, P. J. (1988). Erythrocyte complement receptor type 1, immune complexes, the rheumatic diseases. *Arthritis Rheum.* **31**, 153–158.
68. Kazatchkine, M. D., Fearon, D. T., Appay, M. D., Mandet, C., and Bariety, J. (1982). Immunohistochemical study of the human glomerular C3b receptor in normal kidney and seventy-five cases of renal diseases. Loss of C3b receptor antigen in focal hyalinosis and proliferative nephritis of systemic lupus erythematosus. *J. Clin. Invest.* **69**, 900–912.
69. Burkholder, P. M., Oberley, T. D., Barber, T. A., Beacom, A., and Koehler, C. (1977). Immune adherence in renal glomeruli. Complement receptor sites on glomerular capillary epithelial cells. *Am. J. Pathol.* **86**, 635–654.
70. Mannik, M. (1982). Pathophysiology of circulating immune complexes. *Arthritis Rheum.* **25**, 783–787.
71. Schifferli, J. A., Ng, Y. C., Paccaud, J. P., and Walport, M. J. (1989). The role of hypocomplementemia and low erythrocyte complement receptor type 1 numbers in determining abnormal immune complex clearance in humans. *Clin. Exp. Immunol.* **75**, 329–335.
72. Kimberly, R. P., and Ralph, R. (1983). Endocytosis by the mononuclear phagocyte system and autoimmune disease. *Am. J. Med.* **74**, 481–493.
73. Halma, C., Daha, M. R., and van Es, L. A. (1992). In vivo clearance by the mononuclear phagocyte system in

humans: An overview of methods and their interpretation. *Clin. Exp. Immunol.* **89**, 1–7.
74. Davies, K. A., Peters, A. M., Beynon, H. L. C., and Walport, M. J. (1992). Immune complex processing in patients with systemic lupus erythematosus. In vivo imaging and clearance sutdies. *J. Clin. Invest.* **90**, 2075–2083.
75. Davies, K. A., Erlendsson, K., Beynon, H. L. C., *et al*. (1993). Spenic uptake of immune complexes in man is complement dependent. *J Immunol*. **151**, 3866–3873.
76. Davies, K. A., Hird, V., Stewart, S., *et al*. (1990). A study of in vivo immune complex formation and clearance in man. *J. Immunol.* **144**, 4613–4620.
77. Mannik, M., and Arend, W. P. (1971). Fate of preformed immune complexes in rabbits and rhesus monkeys. *J. Exp. Med.* **137**, 19s–31s.
78. Halma, C., Breedveld, F. C., Daha, M. R., *et al*. (1991). Elimination of soluble 123I-labeled aggregates of IgG in patients with systemic lupus erythematosus. Effect of serum IgG and number of erythrocyte complement receptor type 1. *Arthritis Rheum.* **34**, 442–452.
79. Nash, J. T., Taylor, P. R., Botto, M., Norsworthy, P. J., Davies, K. A., and Walport, M. J. (2001). Immune complex processing in C1q-deficient mice. *Clin. Exp. Immunol.* **123**, 196–202.
80. Rifai, A., and Mannik, M. (1983). Clearance kinetics and fate of mouse IgA immune complexes prepared with monomeric or dimeric IgA. *J. Immunol.* **130**, 1826–1832.
81. Turner, M. W., and Hamvas, R. M. (2000). Mannose-binding lectin: Structure, function, genetics and disease associations. *Rev. Immunogenet.* **2**, 305–322.
82. Matsushita, M., and Fujita, T. (1992). Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J. Exp. Med*. **176**, 1497–1502.
83. Tenner, A., Robinson, S., and Ezekowitz, R. (1995). Mannose binding protein enhances mononuclear phagocytic function via a receptor that contains the 126,000 Mr component of the C1q receptor. *Immunity* **3**, 485–493.
84. Sullivan, K. E., Wooten, C., Goldman, D., and Petri, M. (1996). Mannose-binding protein genetic polymorphisms in black patients with systemic lupus erythematosus. *Arthritis Rheum.* **39**, 2046–2051.
85. Garred, P., Voss, A., Madsen, H. O., and Junker, P. (2001). Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. *Genes. Immun.* **2**, 442–450.
86. Villarreal, J., Crosdale, D., Ollier, W., *et al*. (2001). Mannose binding lectin and FcγRIIa (CD32) polymorphism in Spanish systemic lupus erythematosus patients. *Rheumatology (Oxford)*. **40**, 1009–1012.
87. Tsutsumi, A., Sasaki, K., Wakamiya, N., *et al*. (2001). Mannose-binding lectin gene: Polymorphisms in Japanese patients with systemic lupus erythematosus, rheumatoid arthritis and Sjogren's syndrome. *Genes Immun.* **2**, 99–104.
88. Harley, J. B., Moser, K. L., Gaffney, P. M., and Behrens, T. W. (1998). The genetics of human systemic lupus erythematosus. *Curr. Opin. Immunol.* **10**, 690–696.
89. Chen, J. A. P. (1992). Small nuclear ribonucleoprotein particles contain glycoproteins recognized by rheumatic disease-associated autoantibodies. *Lupus* **1**, 119–124.
90. Couser, W. G., and Salant, D. J. (1980). In situ immune complex formation and glomerular injury. *Kidney Int.* **17**, 1–13.
91. Haakenstad, A. O., Striker, G. E., and Mannik, M. (1982). The disappearance kinetics and glomerular deposition of small-latticed soluble immune complexes. *Immunology* **47**, 407–414.
92. Mannik, M., and Striker, G. E. (1980). Removal of glomerular deposits of immune complexes in mice by administration of excess antigen. *Lab. Invest.* **42**, 483–489.
93. Mannik, M., Agodoa, L. Y. C., and David, K. A. (1983). Rearrangement of immune complexes in glomeruli leads to persistence and development of electron dense deposits. *J. Exp. Med.* **157**, 1516–1528.
94. Gauthier, V. J., Mannik, M., and Striker, G. E. (1982). Effect of cationized antibodies in preformed immune complexes on deposition and persistence in renal glomeruli. *J. Exp. Med.* **156**, 766–777.
95. Gauthier, V. J., and Mannik, M. (1986). Only the initial binding of cationic immune complexes to glomerular anionic sites is mediated by charge-charge interactions. *J. Immunol.* **136**, 3266–3271.
96. Sheerin, N. S., Springall, T., Carroll, M., and Sacks, S. H. (1999). Altered distribution of intraglomerular immune complexes in C3-deficient mice. *Immunology* **97**, 393–399.
97. Fujigaki, Y., Batsford, S. R., Bitter-Suermann, D., and Vogt, A. (1995). Complement system promotes transfer of immune complex across glomerular filtration barrier. *Lab. Invest.* **72**, 25–33.
98. Gauthier, V. J., Striker, G. E., and Mannik. M. (1984). Glomerular localization of preformed immune complexes prepared with anionic antibodies or with cationic antigens. *Lab. Invest.* **50**, 636–644.
99. Radeke, H. H., Gessner, J. E., Uciechowski, P., Mägert, H. J., Schmidt, R. E., and Resh, K. (1994). Intrinsic human glomerular mesangial cells can express receptors for IgG complexes (hFc(RIII-A) and the associated Fc(RI (-chain. *J. Immunol.* **153**, 1281–1292.
100. Michael, A. F., Keane, W. F., Raij, L., Vernier, R. L., and Mauer, S. M. (1980). The glomerular mesangium. *Kidney Int.* **17**, 141–154.
101. Golbus, S. M., and Wilson, C. B. (1979). Experimental glomerulonephritis induced by in situ formation of immune complexes in glomerular capillary wall. *Kidney Int.* **16**, 148–157.
102. Couser, W. G., Steinmuller, D. R., Stilmant, M. M., Salant, D. J., and Lowenstein, L. H. (1978). Experimental glomerulonephritis in the isolated perfused rat kidney. *J. Clin. Invest.* **62**, 1275–1287.
103. Andres, G., Brentjens, J. R., Caldwell, P. R. B., Camussi, G., and Matsuo, S. (1986). Formation of immune deposits and disease. *Lab. Invest.* **55**, 510–520.

104. Fleuren, G., Groud, J., and Hoedemaeker, P. J. (1980). In situ formation of subepithelial glomerular immune complexes in passive serum sickness. *Kidney Int.* **17**, 631–637.
105. Oite, T., Batsford, S. R., Mihatsch, M. J., Takamiya, H., and Vogt, A. (1982). Quantitative studies of in situ immune complex glomerulonephritis in the rat induced by planted, cationized antigen. *J. Exp. Med.* **155**, 460–474.
106. Border, W. A., Ward, H. J., Kamil, E. S., and Cohen, A. H. (1982). Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen: Demonstration of a pathogenic role for electrical charge. *J. Clin. Invest.* **69**, 451–461.
107. Agodoa, L. Y. C., Gauthier, V. J., and Mannik, M. (1983). Precipitating antigen-antibody systems are required for the formation of subepithelial electron dense immune deposits in rat glomeruli. *J. Exp. Med*. **153**, 1259–1271.
108. Schmiedeke, T. M. J., Stöckl, F. W., Weber, R., Sugisaki, Y., Batsford, S. R., and Vogt, A. (1989). Histones have high affinity for the glomerular basement membrane: Effect of serum IgG and number of erythrocyte complement receptor type 1. *J. Exp. Med.* **169**, 1879–1894.
109. Stöckl, F. W., Muller, S., Batsford, S., *et al*. (1994). A role of histones and ubiquitin in lupus nephritis? *Clin. Nephrol*. **41**, 10–17.
110. Lefkowith, J. B., and Gilkeson, G. S. (1996). Nephritogenic autoantibodies in lupus. Current concepts and continuing controversies. *Arthritis Rheum.* **39**, 894–903.
111. Koffler, D., Agnello, V., Thoburn, R., and Kunkel, H. G. (1971). Systemic lupus erythematosus: Prototype of immune complex nephritis in man. *J. Exp. Med.* **134**, 169s–179s.
112. Maddison, P. J., and Reichlin, M. (1979). Deposition of antibodies to a soluble cytoplasmic antigen in the kidneys of patients with systemic lupus erythematosus. *Arthritis Rheum.* **22**, 858–863.
113. Wener, M. H., Mannik, M., Schwartz, M. M., and Lewis, E. J. (1987). Relationship between renal pathology and the size of circulating immune complexes in patients with systemic lupus erythematosus. *Medicine* **66**, 85–97.
114. Uwatoko, S., and Mannik, M. (1988). Low molecular weight C1q-binding IgG in patients with systemic lupus erythematosus consists of autoantibodies to the collagen-like region of C1q. *J. Clin. Invest.* **82**, 816–824.
115. Coremans, J. E. M., Spronk, P. E., Bootsma, H., *et al*. (1995). Changes in antibodies to C1q predict renal relapse in systemic lupus erythematosus. Am. *J. Kidney Dis.* **26**, 595–601.
116. Mannik, M., and Wener, M. (1997). H. Deposition of anitbodies to the collagen-like region of C1q in renal glomeruli of patients with proliferative lupus glomerulonephritis. *Arthritis Rheum.* **40**, 1504–1511.
117. Uwatoku, S., Gauthier, M. J., and Mannik, M. (1991). Autoantibodies to the collagen-like region of C1q deposit in glomeruli via C1q in immune deposits. *Clin. Immunol. Immunopathol* **61**, 268–273.
118. Uwatoku, S., and Mannik, M. (1990). The location of binding sites on C1q for DNA. *J. Immunol.* **144**, 3484–3488.
119. Mannik, M., Merrill, C. E., and Wener, M. H. (2003). Multiple autoantibodies form the glomerular immune deposits in patients with systemic lupus erythematosus. Submitted for publication.
120. Lee, L. A., Weston, W. L., Krueger, G. G., *et al*. (1986). An animal model of antibody binding in cutaneous lupus. *Arthritis Rheum.* **29**, 782–788.
121. Joselow, S. A., and Mannik, M. (1984). Localization of preformed, circulating immune complexes in murine skin. *J. Invest. Dermatol.* **82**, 335–340.
122. Joselow, S. A., Gown, A., and Mannik, M. (1985). Cutaneous deposition of immune complexes in chronic serum sickness of mice induced with cationized or unaltered antigen. *J. Invest. Dermatol.* **85**, 559–563.
123. Peress, N. S., Miller, F., and Palu, W. (1977). The choroid plexus in passive serum sickness. *J. Neuropathol. Exp. Neurol*. **36**, 561–566.
124. Huang, J. T., Mannik, M., and Gleisner, J. (1984). In situ formation of immune complexes in the choroid plexus of rats by sequential injection of a cationized antigen and unaltered antibodies. *J. Neuropathol. Exp. Neurol.* **43**, 489–499.
125. Cochrane, C. G. (1971). Mechanisms involved in the deposition of immune complexes in tissues. *J. Exp. Med.* **134**, 75S–89S.
126. Wener, M. H., Uwatoko, S., and Mannik, M. (1989). Antibodies to the collagen-like region of C1q in sera of patients with autoimmune rheumatic diseases. *Arthritis Rheum.* **32**, 544–551.
127. Kohro-Kawata, J., Wener, M. H., and Mannik, M. (2002). The effect of high salt concentration on detection of serum immune complexes and autoantibodies to C1q in patients with systemic lupus erythematosus. *J. Rheumatol.* **29**, 84–89.
128. Zubler, R. H., and Lambert, P. H. (1978). Detection of immune complexes in human diseases. *Prog. Allergy* **24**, 1–48.
129. Theofilopoulos, A. N., and Dixon, F. J. (1979). The biology and detection of immune complexes. *Adv. Immunol.* **28**, 89–220.
130. Wener, M. H. (1997). Tests for circulating immune complexes and autoantibodies to C1q. In: "Manual of Clinical Laboratory Immunology" (N. R. Rose, E. C. de Macario, J. D., Folds, H. C., Lane, and R. M. Nakamura, eds. 5th Ed. ASM Press, Washington, DC.
131. Neale, T. J., Theofilopoulos, A. N., and Wilson, C. B. (1979). Methods for the detection of soluble circulating immune complexes and their application. *Pathobiol. Annual.* **9**, 113–136.
132. Lambert, P. H., Dixon, F. J., Zubler, R. H., *et al*. (1978). A WHO collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. *J. Clin. Lab. Immunol.* **1**, 1–15.
133. Bentwich, Z., Bianco, N., Jager, L., *et al*. (1981). Use and abuse of laboratory tests in clnical immunology: Critical considerations of eight widely used diagnostic procedures. *Clin. Exp. Immunol.* **46**, 662–674.
134. Inman, R. D. (1982). Immune complexes in SLE. *Clin. Rheum. Dis.* **8**, 49–62.

135. Lloyd, W., and Schur, P. H. (1981). Immune complexes, complement, and anti-DNA in excerbations of systemic lupus erythematosus (SLE). *Medicine* **60**, 208–217.
136. Abrass, C. K., Nies, K. M., Louie, J. S., Border, W. A., and Glassock, R. J. (1980). Correlation and predictive accuracy of circulating immune complexes with disease activity in patient with systemic lupus erythematosus. *Arthritis Rheum.* **23**, 273–282.
137. Lewis, E. J., Hunsicker, L. G., Lan, S. P., Rohde, R. D., and Lachin, J. M. (1992). A controlled trial of plasmapheresis therapy in severe lupus nephritis: The Lupus Nephritis Collaborative Study Group. *N. Engl. J. Med.* **326**, 1373–1379.
138. Felson, D. T., LaValley, M. P., Baldassare, A. R., *et al*. (1994). The Prosorba column for treatment of refractory rheumatoid arthritis: A randomized, double-blind, sham-controlled trial. *Arthritis Rheum.* **42**, 2153–2159.
139. Sasso, E. H., Merrill, C., and Furst, T. E. (2001). Immunoglobulin binding properties of the Prosorba immunadsorption column in treatment of rheumatoid arthritis. *Ther Apher.* **5**, 84–91.
140. Berner, B., Scheel, A. K., Schettler, V., *et al*. (2001). Rapid improvement of SLE-specific cutaneous lesions by C1q immunoadsorption. *Ann Rheum Dis.* **60**, 898–899.
141. Pfueller, B., Wolbart, K., Bruns, A., Burmester, G. R., and Hiepe, F. (2001). Successful treatment of patients with systemic lupus erythematosus by immunoadsorption with a C1q column: A pilot study. *Arthritis Rheum.* **44**, 1962–1963.

15

ORIGINS OF ANTINUCLEAR ANTIBODIES

Westley H. Reeves
Minoru Satoh
Hanno B. Richards

The production of antinuclear antibodies (ANAs) is one of the defining features of systemic lupus erythematosus (SLE). Description of the LE cell phenomenon (phagocytosis of intact nuclear material by polymorphonuclear leukocytes) by Hargraves *et al*. in 1948 was the first evidence for the existence of these autoantibodies [1]. This was followed by the identification of antinuclear and anti-DNA antibodies in 1957 [2–4]. The landmark discovery of anti-Sm antibodies by Tan and Kunkel in 1966 provided definitive evidence that autoantibodies in lupus recognize nuclear structures other than nucleosomes [5], marking the beginning of a 30-year period during which the major autoantibody–autoantigen systems associated with systemic autoimmune diseases were identified and characterized. Despite significant progress in defining both autoantibodies and their molecular targets, an understanding of the ultimate causes of these abnormal antibodies remains elusive. This chapter reviews what has been learned about antinuclear antibodies, emphasizing recent progress in defining the mechanisms. Because certain antinuclear antibodies are highly specific markers for SLE, understanding their origins will be an important step toward determining the pathogenesis of lupus.

DIAGNOSTIC IMPORTANCE OF ANTINUCLEAR ANTIBODIES

The autoantibodies produced in SLE are directed primarily, although not exclusively, against nuclear antigens. This is the basis for the diagnostic utility of the fluorescent ANA assay, a highly sensitive screening test for SLE that is positive in >95% of patients [6, 7]. However, the specificity of a positive ANA is relatively low [8]. Early on, it was recognized that ANAs stained the nuclei of cells in a variety of different patterns (Fig. 1). It subsequently became clear that these patterns correspond to reactivity with different subsets of nuclear antigens. Some of the specificities are uniquely associated with SLE (reviewed in Tan [9, 10]).

The discovery that certain types of ANAs are specific for the diagnosis of SLE or for other systemic autoimmune diseases was a key advance in the immunodiagnosis of systemic autoimmunity. Autoantibodies specific for SLE, systemic sclerosis (SSc, scleroderma), and polymyositis/dermatomyositis (PM/DM) are of considerable use clinically (Table 1). Anti-dsDNA antibodies, for example, are found in ~70% of SLE patients at some point during their disease and are 95% specific for the diagnosis [7, 11]. Anti-Sm antibodies are found in ~10–25% of lupus patients' sera depending on ethnicity [12], and also are virtually pathognomonic of SLE [5, 13]. Antibodies to the ribosomal P0, P1, and P2 antigens [14] and proliferating cell nuclear antigen (PCNA) [15] are highly specific, but less sensitive, markers of the disease (Table 1). These "marker" autoantibodies are highly unusual in drug-induced lupus. In contrast, other types of autoantibodies, such as antisingle-stranded (ss) DNA, nRNP, Ku, and Su [12, 16], are associated with SLE, but are seen in other systemic autoimmune diseases as well. However, they usually are not found in healthy individuals. Anti-ssDNA and antihistone antibodies are associated with SLE as well as drug-induced

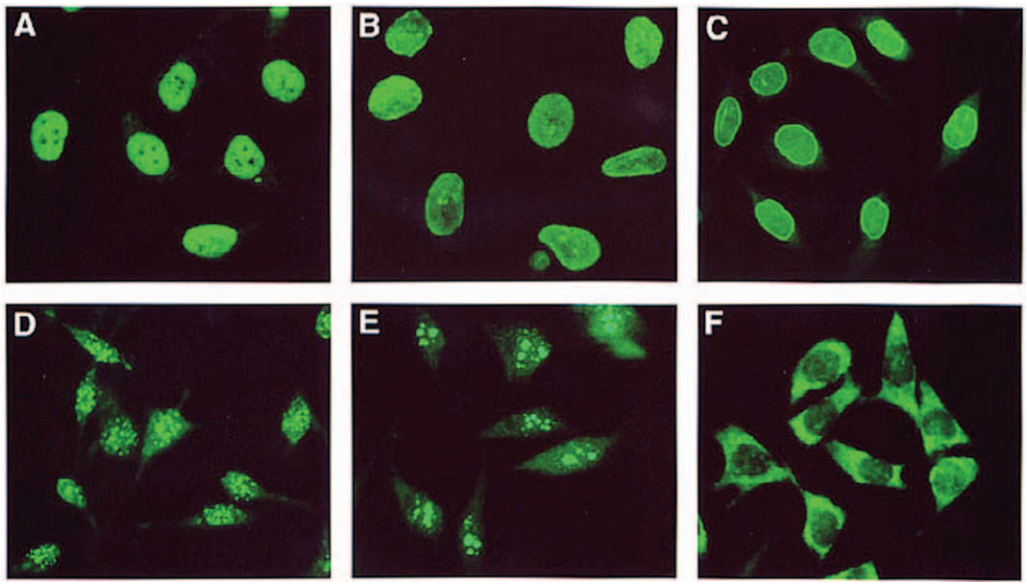


FIGURE 1 ***Prototype fluorescent antinuclear antibody patterns.*** HeLa cells (human cervical carcinoma cell line) were fixed with methanol and stained with human autoimmune serum at a dilution of 1:40. The binding of antinuclear antibodies to fixed and permeabilized cells was detected with fluorescein isothiocyanate (FITC)-conjugated goat antihuman IgG antibodies. (**A**) Fine speckled pattern sparing the nucleolus produced by anti-nRNP antibodies. (**B**) Diffuse (homogeneous) pattern produced by anti-dsDNA antibodies. (**C**) Rim (peripheral) pattern produced by antilamin B antibodies. (**D**) Centromere staining exhibited by serum from a patient with CREST syndrome producing anti-CENP-B autoantibodies. (**E**) Nucleolar pattern produced by antifibrillarin (U3 ribonucleoprotein) autoantibodies from a patient with scleroderma. (**F**) Cytoplasmic pattern produced by serum containing antiribosomal P autoantibodies.

lupus, and anti-Ro60 (SS-A), Ro52, and La are associated with primary Sjogren's syndrome, but also with sicca syndrome complicating SLE, scleroderma, or polymyositis (Table 1).

In view of the high specificity of anti-Sm and anti-dsDNA antibodies, it can be argued that serological criteria should be weighted more heavily in the diagnosis of SLE [6] than clinical features, which individually are less specific for SLE [7]. Remarkably, most of the same antibodies are associated with murine lupus: anti-dsDNA antibodies are produced by (NZB × NZW)F1 mice [17], anti-dsDNA, Sm, and ribosomal P by MRL mice [17, 18], and anti-Sm, dsDNA, and ribosomal P by mice with pristane-induced lupus [19–21] (Table 2).

There are several possible explanations for why certain autoantibodies [anti-dsDNA, Sm, proliferating cell nuclear antigen (PCNA), and ribosomal P] are specific for lupus, whereas others are specific for SSc (anti-topoisomerase I, fibrillarin, RNA polymerases I/III) or PM/DM (anti-tRNA synthetase). Autoantibodies could contribute directly to the disease process. For instance, the injection of certain anti-DNA antibodies causes glomerulonephritis [22–24]. Alternatively, the production of autoantibodies specific for SLE could reflect autoimmune mechanisms unique to the pathogenesis of SLE. For example, molecular mimicry between the U1 snRNP 70K protein (U1-70K) and a retroviral gag protein may lead to the production of anti-nRNP and Sm autoantibodies [25]. The question of whether disease marker autoantibodies, such as anti-Sm, cause disease or reflect a unique aspect of the disease process is key to understanding the pathogenesis of SLE. Intensive study of the antigens recognized by autoantibodies in SLE has provided some tantalizing clues.

AUTOANTIGENS ARE USUALLY MACROMOLECULAR COMPLEXES

Although the nuclei of cells contain thousands of proteins, only a small number (less than 50 or so) are autoantigens in systemic autoimmune diseases. Most of the antigens recognized by autoantibodies in SLE, PM/DM, SSc, and overlap syndromes are multiprotein or nucleoprotein complexes (Table 3). Many play key roles in cell regulation, including the organization of chromatin into transcriptionally active and inactive domains or RNA splicing [9, 10].

TABLE 1 Frequencies of Some Autoantibodies Associated with Human Systemic Autoimmune Disease (University of Florida Autoimmune Disease Center)[a]

		Frequency of autoantibody (%)		
Autoantibody	**Marker for**	**SLE (*n* = 317)**	**SSC (*n* = 46)**	**PM/DM (*n* = 25)**
dsDNA[b]	SLE	49	0	9
PCNA	SLE	0.6	0	0
Ribosomal P0, P1, P2	SLE	3	0	0
Sm	SLE	15[c]	0	0
nRNP	—	40[c]	4	4
Ku	—	6[c]	2	4
Ro60 (SS-A)	(Sicca)	36	7	40
La (SS-B)	(Sicca)	10	0	13
Scl-70 (topoisomerase I)	SSC	0.6[d]	17	0
RNA polymerase I and III	SSC	0.6[d]	20	0
Threonyl tRNA synthetase (PL7)	PM/DM	0	0	0[e]
Alanyl tRNA synthetase (PL12)	PM/DM	0	0	4
Glycyl tRNA synthetase (EJ)	PM/DM	0	0	4
Multienzyme complex (OJ)	PM/DM	0	0	4

[a] With the exception of anti-dsDNA, autoantibody specificities were determined by protein immunoprecipitation using sera from patients meeting ACR criteria for the classification of SLE [6] or scleroderma (SSC) [290] or with a primary clinical diagnosis of polymyositis or dermatomyositis (PM/DM). Demographics were as follows: SLE (91% female, 50% black, 43% white, 7% other); SSC (74% female; 32% black, 66% white, 2% other), and PM/DM (75% female, 46% black, 46% white, 8% other).

[b] *Crithidia luciliae* kinetoplast staining assay. Note that anti-dsDNA antibodies often are present only transiently, but data are from single serum samples. Estimates in the literature suggest that about 70% of SLE patients will produce anti-dsDNA at some time during their disease.

[c] There are marked racial/ethnic differences in frequency of certain autoantibodies (12).

[d] SSC criteria not available (*n* = 3, 2 topo I and 1 RNA polymerase I/III).

[e] Estimate from the literature is ~3%.

TABLE 2 Prevalence of Autoantibodies in Murine Lupus

	Spontaneous lupus (%)			Pristane-induced lupus (%)		
Autoantibody	**NZB/W (*n* = 70)**	**MRL/lpr (*n* = 16)**	**BXSB**	**BALB/c (*n* = 20)**	**B10.S (*n* = 21)**	**B6 (*n* = 45)**
dsDNA	100	100	100	38	n.d.	—
PCNA	—	—	—	—	—	—
Ribosomal P0, P1, P2	—	—	—	—	62	16
Sm or RNP	—	44	—	55	5	24
Ro60 (SS-A)	—	—	—	—	—	—
La (SS-B)	—	—	—	—	—	—
Scl-70 (topoisomerase I)	—	—	—	—	—	—
RNA polymerase I and III	—	—	—	—	—	—
Threonyl tRNA synthetase (PL7)	—	—	—	—	—	—
Alanyl tRNA synthetase (PL12)	—	—	—	—	—	—
Glycyl tRNA synthetase (EJ)	—	—	—	—	—	—
Multienzyme complex (OJ)	—	—	—	—	5	—

n, d, not done.

TABLE 3 Some Examples of RNA–Protein and DNA–Protein Autoantigens

Antigen	Autoantibodies	Protein component	Nucleic acid component
U1 snRNP	α-Sm, α-nRNP, α-U1RNA	A, B′/B, C, D1/2/3, E, F, G, 70K	U1 small nuclear RNA
Y5 RNP	α-Ro (SS-A), α-La (SS-B), α-Y5RNA	60K Ro, 45K La	Y5 small RNA
Nucleosome	α-DNA, α-histone	H2A/H2B, H3, H4	DNA
DNA-PK	α-Ku, α-DNAPK$_{cs}$	Ku70, Ku80, DNA-PK$_{cs}$	DNA

The nucleosome, which consists of DNA packaged in a complex of histones H2A/H2B, H3, and H4 [26], was the first DNA–protein autoantigen to be identified [27]. Other DNA–protein autoantigens include the transcription factor NOR-90 [28, 29], centromeres [30], PCNA, a component of DNA polymerase δ [31], and Ku antigen, a component of the DNA-dependent protein kinase (DNA-PK) [32].

RNA–protein complexes, especially small nuclear ribonucleoprotein particles (snRNPs), also are important autoantigens (reviewed in Tan [9, 10]). U snRNPs (U1, U2, U4–6, U5), which carry Sm and nRNP antigenic determinants, were the first small ribonucleoprotein autoantigens recognized [33]. Others include the Y1-5 small ribonucleoproteins recognized by anti-Ro/SS-A antibodies, RNA polymerase III precursor RNA particles recognized by anti-La/SS-B antibodies, the 7-2 and 8-2 small nucleolar ribonucleoproteins recognized by anti-To/Th antibodies, U3 small nucleolar ribonucleoproteins recognized by antifibrillarin antibodies, tRNA synthetases, and the signal recognition particle [9, 10].

It may be significant that the macromolecular complexes recognized by autoantibodies in different disease states tend to be localized in different subcellular compartments. Many of the major lupus autoantigens are nucleoplasmic structures, such as spliceosomes (macromolecular arrays containing U1, U2, U4–6, and U5 snRNP particles) or nucleosomes. In contrast, antigens recognized by scleroderma-specific autoantibodies tend to be nucleolar structures involved in the transcription of ribosomal RNA, whereas those recognized by myositis-specific autoantibodies are cytoplasmic complexes involved in protein synthesis. The disease-specific targeting of particular subcellular compartments remains unexplained.

Characterization of Autoimmune Responses to Multicomponent Antigens

Many techniques have been used to study autoantibody specificities, including gel diffusion techniques (double immunodiffusion and counterimmunoelectrophoresis), enzyme-linked immunosorbent assays (ELISA), and Western blot (immunoblot) analysis. However, none of these approaches is adequate for evaluating immune responses to multicomponent antigens. While gel diffusion and ELISA permit detection of autoantibodies to native antigenic determinants, the composition of the antigenic particles and fine specificities of the autoantibodies cannot be investigated. Conversely, autoantibody fine specificities can be discerned by immunoblot analysis, but not the interrelationships between various components of a particle because the test is performed after dissociating the antigen. In addition, the harsh detergents used for this assay destroy many determinants dependent on the tertiary or quaternary structure of the antigen. For these reasons, immunoprecipitation of radiolabeled cell extracts has become an important tool for identifying autoantibody specificities in the research setting.

Immunoprecipitation is performed as illustrated in Fig. 2A. Tissue culture cells are grown in the presence of a radioactive amino acid, usually [^{35}S]methionine, and an extract containing radiolabeled cell proteins is incubated with protein A–Sepharose beads to which human autoantibodies are attached via the Fc region (Fig. 2A). The beads are washed extensively to remove unbound material, and then the bound proteins are eluted using heat and the detergent sodium dodecyl sulfate (SDS) before SDS–polyacrylamide gel electrophoresis. The radioactive proteins are visualized by exposing the gel to X-ray film. Figure 2B shows a typical immunoprecipitation experiment. Antiribosomal P antibodies (lane 1) immunoprecipitate three proteins of relative mobilities ~38, 19, and 17 kDa (P0, P1, and P2), whereas anti-nRNP (lane 2) and anti-Sm (lane 3) antibodies immunoprecipitate the same set of eight proteins ranging from 31 to 9 kDa (A, B′/B, C, D, E/F, and G, see also later). Another protein of ~70 kDa is not visualized because it is methionine deficient. Anti-PCNA (lane 4), anti-Ki (lane 5), anti-Ro (SS-A, lane 6), anti-La (SS-B, lane 7), and anti-Ku (lane 8) autoantibodies are associated with distinctive patterns, easily distinguished on the autoradiograph. Different types of autoantibodies may immunoprecipitate the same set (e.g., anti-nRNP and Sm, lanes 2 and 3) or partially overlapping sets (e.g., anti-Ro and La, lanes 6 and 7) of proteins. The association of several proteins with one another in a particle is responsible for the overlapping patterns. Similar tech-

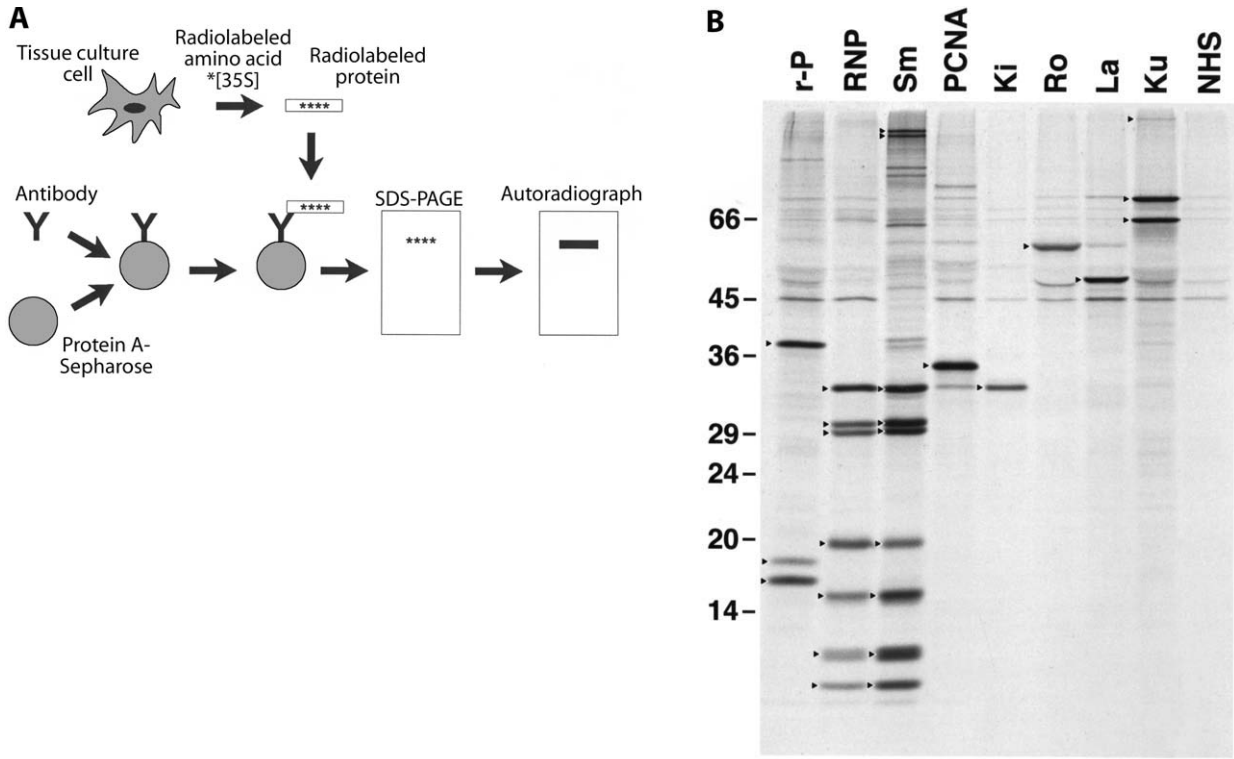


FIGURE 2 ***Immunoprecipitation assay.*** (**A**) Diagrammatic representation of technique. Tissue culture cells are labeled *in vitro* with a radioactive amino acid, usually [^{35}S]methionine. An extract containing the metabolically labeled proteins is added to protein A–Sepharose beads that have been preincubated with human autoimmune serum containing IgG autoantibodies. Autoantibodies attached to the beads via their Fc region recognize the radiolabeled proteins in the extract and bind them. The beads are washed, and the radioactive proteins are eluted from the beads by boiling in SDS sample buffer. The proteins can then be analyzed by SDS–polyacrylamide gel electrophoresis, which separates them on the basis of size. The gel is then exposed to X-ray film to visualize the bands on the gel. (**B**) Typical autoradiograph showing various autoantibody specificities. Immunoprecipitation was performed as diagrammed in A using prototype sera containing anti-ribosomal P (r-P), nRNP (RNP), Sm, proliferating cell nuclear antigen (PCNA), Ki, Ro (SS-A), La (SS-B), or Ku autoantibodies. Immunoprecipitation with normal human serum (NHS) is shown as a control. Positions of the protein bands characteristic of each specificity are indicated with arrowheads. Positions of molecular mass standards in kilodaltons are indicated on the left.

niques are used to analyze the nucleic acid components of small ribonucleoprotein particles [33, 34]. In this case, cells are labeled metabolically with [^{32}P]orthophosphate, and the immunoprecipitates are extracted with phenol–chloroform before analyzing the immunoprecipitated nucleic acids by gel electrophoresis.

Anti-Sm and nRNP Autoantibodies Recognize the U1 snRNP

The U1 snRNP, an RNA–protein autoantigen, is a typical multicomponent autoantigen illustrative of the general principles applying to many other autoantigen/autoantibody systems (Fig. 3). It is a macromolecular complex consisting of a group of proteins designated U1-70K, A, B′/B, C, D1/2/3, E, F, and G, which are associated with U1 small nuclear RNA [33]. The proteins B′/B, D, E, F, and G assemble into a stable 6S particle (the Sm core particle) reactive with anti-Sm, but not anti-nRNP, antibodies [35]. Autoantibodies to the Sm core particle are unique to SLE [5, 13]. In contrast, antibodies to the proteins A, C, and 70K, which carry nRNP antigenic determinants, may be found in SSc, PM/DM, and other subsets of systemic autoimmune disease, as well as SLE [13, 36]. High levels of anti-nRNP antibodies, without anti-Sm, are seen in mixed connective tissue disease (MCTD) [37]. The A and 70K proteins interact directly with U1 RNA via RNA recognition motifs [38], whereas the association of U1-C with the U1 snRNP, although RNA dependent, is mediated through interactions with the B′/B components of the Sm core particle [39]. In addition to the U1 snRNP,

TABLE 4 Protein and RNA Components of Abundant U snRNPs[a]

snRNP particle	RNA	Shared proteins	Unique proteins	Antibody binding
U1 snRNP	U1	B′/B,D,E,F,G[b]	70K, A, C	Sm, nRNP
U2 snRNP	U2	B′/B,D,E,F,G[b]	A′, B″	Sm, A′, B″
U5 snRNP	U5	B′/B,D,E,F,G[b]	8 proteins	Sm, 100, 102, 200K (doublet)
U4/U6 snRNP	U4 and U6	B′/B,D,E,F,G[b]	150K	Sm, 150K

[a] Data from Refs. 33,291–294.
[b] *Proteins B′/B, D, E, F, and G form the 6S Sm core particle, which is shared by all of the U snRNPs listed.*

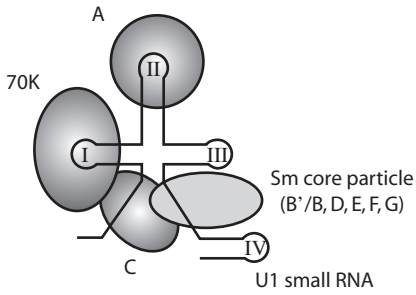


FIGURE 3 ***Structure of the U1 snRNP particle.*** The U1 snRNP is a complex of several proteins (designated 70K, A, B′/B, C, D, E, F, and G) bound to a single molecule of U1 small nuclear RNA. The 70K, A, and C proteins (dark shading) are recognized by anti-nRNP antibodies. The B′/B, D, E, F, and G proteins form a 6S particle termed the Sm core particle. This particle contains the major antigenic determinants recognized by anti-Sm antibodies. Base pairing within the U1 RNA molecule results in the formation of four stem-loop structures (I–IV). Two of these (stem-loops II and IV) are recognized by autoantibodies.

other small ribonucleoprotein complexes, each with a unique uridine-rich (U) RNA species, carry the Sm core particle. These include the U2, U4/U6, and U5 snRNPs, as well as a number of other less abundant U snRNPs [40]. U1, U2, U5, and U4/6 snRNPs are present at levels ranging from 10^6 copies (U1 and U2) to 2×10^5 copies (U5 and U4/U6) per mammalian cell [40]. The Sm core particle [35] is a component of each of these snRNP particles. In addition, each of these major snRNP particles carries unique protein components (Table 4).

Autoantibodies from systemic autoimmune disease patients' sera were instrumental in defining the function of the U1 and other U snRNPs carrying Sm antigenic determinants. In 1979, Lerner and Steitz first reported that the U series of small nuclear RNAs was immunoprecipitated by human sera containing anti-nRNP or anti-Sm activity [33]. This led to characterization of the small RNA species and the discovery that the U1, U2, and other Sm antigen-containing ribonucleoproteins are critical for splicing precursor heterogeneous nuclear RNAs into messenger RNA [41]. The importance of these particles in RNA splicing is underscored by the observation that splicing is inhibited *in vitro* in the presence of anti-nRNP or Sm autoantibodies [42]. During splicing, a series of U snRNP particles assembles into an even larger structure termed a spliceosome, which recognizes intron–exon junctions, cleaves intervening sequences, and rejoins the mature mRNA [41]. U1 small RNA recognizes a precursor RNA and base pairs with the 5′ splice site leading to cleavage and leaving an invariant guanosine residue at the 5′ end of the intron, which subsequently forms a lariat-like structure. Lariat formation requires binding of the U2 snRNP to the branch point [41]. After cleavage at the 3′ end of the intron, the U5 and U4/U6 snRNPs bind near the 3′ splice site, forming a spliceosome, and the exons are religated to produce mature mRNA. Spliceosomes are responsible for the fine speckled immunofluorescence pattern associated with anti-nRNP or anti-Sm autoantibody activity (Fig. 1A).

Understanding the structure of the U1 snRNP particle (Fig. 3) greatly simplifies understanding the immunoprecipitation patterns exhibited by sera from patients (Fig. 4). Sera containing either anti-Sm or anti-nRNP antibodies immunoprecipitate the U1 small RNA molecule, as shown by analysis of the ^{32}P-labeled RNAs attached to the immunoprecipitates (Fig. 4A). Sera containing anti-Sm immunoprecipitate the U2, U4, U5, and U6 small RNAs, but sera having only anti-nRNP activity do not. This is explained by the fact that the Sm core particle is a component of each of the snRNP particles, whereas the U1-70K, U1-A, and U1-C proteins recognized by anti-nRNP antibodies (Fig. 3) are unique to the U1 snRNP. Analysis of the proteins immunoprecipitated by anti-Sm versus anti-nRNP antibodies reveals a similar set of polypeptides (Fig. 2B and Fig. 4B, A–G) because the Sm core particle is associated with the anti-nRNP reactive proteins U1-A, and U1-C in the abundant U1 snRNP (the U1-70K protein is not visualized by standard protein immunoprecipitation techniques because it is methionine deficient and therefore labels inefficiently). The different fine specificities

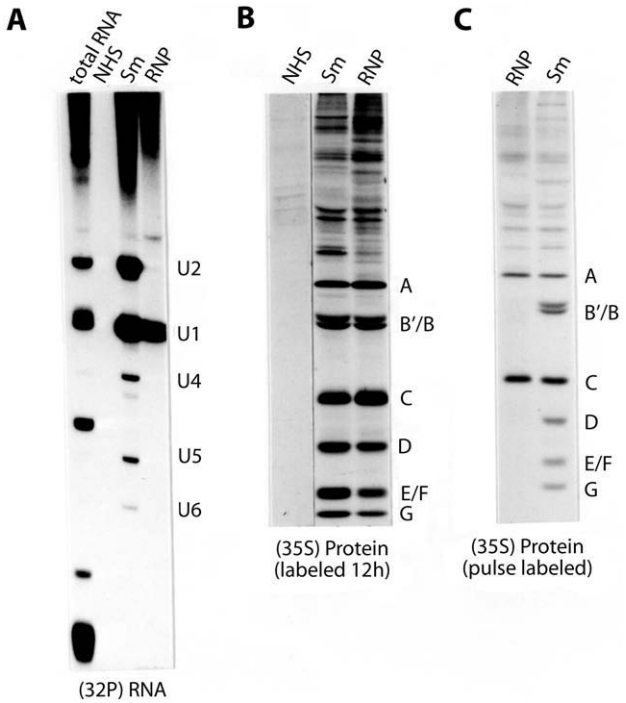


FIGURE 4 ***Immunoprecipitation of the U1 snRNP particle.*** (**A**) K562 (human erythroleukemia) cells were labeled with [^{32}P]orthophosphate, and an extract was immunoprecipitated with anti-Sm or anti-nRNP serum or with normal human serum (NHS). Immunoprecipitates were subjected to phenol extraction, and the radiolabeled RNA was analyzed by gel electrophoresis followed by autoradiography. Positions of U2, U1, U4, U5, and U6 small RNAs are indicated. From Satoh *et al*. [42a], with permission. (**B**) The same sera were used to immunoprecipitate extracts from cells labeled for 12 h with [^{35}S]methionine. Note that both anti-Sm and anti-nRNP immunoprecipitates exhibit the same pattern (bands A–G). (**C**) Sera were used to immunoprecipitate the extract from cells pulse labeled for 6 min with [^{35}S]methionine. This allows the autoantibodies to recognize newly synthesized components of the U1 snRNP before they are assembled completely. Note the difference between the pattern exhibited by anti-nRNP serum (bands A and C) vs anti-Sm serum (which also contains anti-nRNP antibodies, and immunoprecipitates bands A and C as well as B′/B, D, E/F, and G).

of anti-nRNP and anti-Sm are obvious when immunoprecipitation is carried out after dissociating the U1 snRNP into its individual components [43, 44] or before complete assembly of the particle (e.g., by pulse labeling) (Fig. 4C). In this case, anti-nRNP sera immunoprecipitate the U1-A and U1-C proteins, but not the components of the Sm core particle (B′/B, D, E, F, G). Anti-Sm sera nearly always contain anti-nRNP activity as well [36, 45] and thus immunoprecipitate all of the proteins under these conditions (Fig. 4C).

IMMUNE RESPONSE TO MACROMOLECULAR COMPLEXES

Autoantigen structure has implications for autoantibody specificity. Autoantibodies characteristic of SLE and other systemic autoimmune diseases occur as groups of interrelated specificities. Mattioli and Reichlin [36] first reported the strong association of anti-Sm antibodies with anti-nRNP. "Linked sets" of autoantibodies have been described in other systems as well [46]. Anti-Ro (SS-A) and La (SS-B) antibodies are associated with one another [47] and with antibodies to the Y5 small RNA molecule [48], with which both antigens associate [49]. Likewise, autoantibodies to DNA and histones (chromatin) are associated with one another [50, 51]. Thus, macromolecular complexes appear to be seen by the immune system as units.

Autoantibodies to Components of the U1 snRNP: A Prototype-Linked Set

Anti-Sm autoantibodies are strongly associated with anti-nRNP [36, 45]. In most cases, levels of anti-nRNP in human autoimmune sera greatly exceed the levels of anti-Sm [45]. Interestingly, MRL mice make primarily anti-Sm [43]. Analysis of the fine specificities of antibodies reactive with components of the U1 snRNP indicates that many sera contain autoantibodies to multiple polypeptides. In one study [52], only 1 of 29 sera containing anti-nRNP or Sm antibodies recognized a single protein component of the U1 snRNP, and the majority recognized three or more (Table 5).

Another class of autoantibodies recognizes antigenic sites found only on the native antigen: conformational epitopes dependent on the tertiary structure of individual proteins, such as U1-C [43] or epitopes related to the quaternary structure of the U1 snRNP. The latter include an epitope or epitopes formed by the association of the E, F, and G Sm polypeptides [53] and autoantibodies that stabilize the protein–protein interactions between the U1-C protein and the Sm core particle or between U1-A and the Sm core particle [44].

In addition to autoantibodies to the protein constituents of the U1 snRNP, the U1 small RNA can be recognized by autoantibodies [54, 55]. Autoantibodies to U1 RNA are detected in about 40% of anti-nRNP/Sm-positive sera [56, 57]. Interestingly, these antibodies are uniquely reactive with the stem of stem-loop II and in the loop of stem-loop IV of U1 RNA (Fig. 3) [55, 57]. With the exception of autoantibodies to the 5′ trimethylguanosine cap shared by U1-U5 small RNAs [58], autoantibodies specific for U2, U4, U5, or U6 small RNAs have not been reported. Autoanti-

TABLE 5 Autoantibody Recognition of Components of the U1 snRNP by Western Blot[a]

	Number of sera recognizing					
Serum type	**1 protein**	**2 proteins**	**3 proteins**	**4 proteins**	**5 proteins**	**6 proteins**
Anti-nRNP only	0	0	5	6	0	0
Anti-Sm ± nRNP	1	4	5	2	5	1

[a] *Data compiled from Western blot analyses of sera reactive with U1-70K, U1-A, U1-C, Sm-B′/B, Sm-D, and Sm-E proteins [52].*

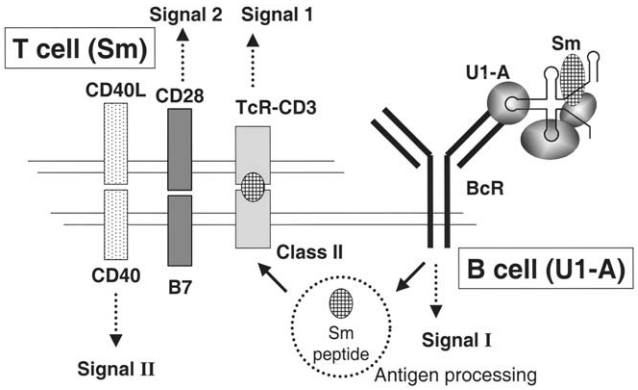


FIGURE 5 ***Pathogenesis of T- and B-cell responses to the U1 snRNP antigen.*** The mechanism of intermolecular–intrastructural help in the production of antibodies against the U1 snRNP is illustrated. An autoreactive B cell carrying surface immunoglobulin receptors for the U1-A protein takes up the U1 snRNP (signal I). The complex is degraded by endosomal proteases resulting in the presentation of a peptide derived from the Sm core particle to an activated autoreactive T cell in the context of MHC class II. The T cell expresses CD40 ligand (CD40L), which engages CD40 on the surface of the B cell, providing a costimulatory signal (signal II). T-cell activation also requires two signals: one delivered by engagement of the T-cell receptor (TcR)–CD3 complex and the other by the engagement of B7 on the activated B cell by the costimulatory molecule CD28. In this way, a T cell expressing a receptor for an Sm peptide can stimulate autoantibody production by B cells specific for other proteins, such as U1-A.

bodies to U1 RNA are linked strongly to the production of anti-nRNP antibodies and are absent in sera containing anti-Sm, but not nRNP, antibodies [56].

Intermolecular–Intrastructural Help

Immune responses to multicomponent autoantigens are in many respects analogous to responses against viral particles. In the latter case, T cells specific for one component of the virion provide help to B cells producing antibodies against other components (Fig. 5), a phenomenon termed "intermolecular–intrastructural help" [59]. This has been examined closely in humoral immune responses to influenza, hepatitis B, and vesicular stomatitis virus (VSV) [60–62]. In the case of influenza, T cells specific for the nucleoprotein help B cells specific for the hemagglutinin [60]. In the special case of a complex consisting of both foreign and self-antigens, T cells specific for nonself components may drive the production of autoantibodies to the self-components [62, 63]. For example, transgenic mice expressing the membrane-associated glycoprotein of vesicular stomatitis virus (VSV-G) do not produce autoantibodies to the "self" antigen VSV-G when immunized with that protein. However, autoantibodies are induced readily when transgenic mice are infected with wild-type VSV [62]. Activated B cells specific for self VSV-G process intact VSV particles and present nonself VSV peptides to T_H cells resulting in autoantibody production.

Intermolecular–intrastructural help may also play a role in the development of autoimmune responses to the U1 snRNP. Mice immunized with human U1-A (Fig. 3) plus affinity-purified mouse U1 snRNPs develop autoreactive T cells specific for mouse U1-A and autoantibodies to the self U1-A, U1-70K, and B′/B proteins, as well as U2-B″, which shares an antigenic determinant with U1-A [64]. Immunization with ribonuclease-treated snRNPs leads to the development of anti-U1-A and U2-B″ antibodies, but not anti-U1-70K or anti-B′/B, suggesting that the spreading of autoimmunity to other components requires intact U1 snRNPs.

Intermolecular–intrastructural help may also promote the progression of autoimmunity against Ro60 (SS-A) and La (SS-B). Autoantibodies to these proteins, which transiently associate with one another in the cell [49], frequently coexist in SLE patients' sera. Mice immunized with recombinant murine La (SS-A) develop anti-La autoantibodies, suggesting that immunological ignorance may govern nonresponsiveness to La [65]. Interestingly, these mice also develop anti-Ro (SS-A) antibodies. Conversely, immunization with Ro causes the production of anti-La as well as anti-Ro antibodies. A similar phenomenon occurs in rabbits immunized with Sm-B peptides [66]. The simplest explanation is that T cells specific for the immunizing antigen can provide help to B cells carrying receptors for other components of the Ro-La complex or U1 snRNP (Fig. 5). The same mechanism is likely to be involved in autoim-

mune responses to chromatin [51]. In mice with spontaneous lupus, αβ and γδ T-cell receptor-bearing T cells specific for nucleosomal peptides have been implicated in the production of anti-DNA antibodies [51, 67, 68]. T_H clones promote the production *in vitro* of anti-dsDNA and ssDNA, as well as histone autoantibodies [51], consistent with the model. The antigenic peptides appear to be generated only when intact nucleosomes are taken up by antigen-presenting cells (APC), suggesting that the interaction of DNA with the histones is a critical determinant of antigen processing [51].

B cells may be critical APC that promote the spread of autoimmunity to other components of a macromolecular complex. Although much less efficient at nonspecific phagocytosis of antigens than "professional" antigen-presenting cells, such as macrophages or dendritic cells, B cells bearing specific surface immunoglobulin receptors focus antigen on their surface, allowing them to take up the antigen recognized by their surface receptors 1000 to 10,000 more efficiently than macrophages [69]. The expression of MHC class II permits B cells to display peptide–MHC class II complexes that activate T-cell responses. B cells can be highly efficient antigen-presenting cells that can deliver a potent T-cell stimulatory signal if the costimulatory molecules B7-1 or B7-2 on their surface engage CD28 on the surface of the T cell (Fig. 5). However, they also can deliver a potent tolerogenic signal in the absence of B7 expression [70]. Unlike macrophages or dendritic cells, which express B7 constitutively, expression is inducible on B cells. The potential for activated autoreactive B cells to promote the diversification of autoimmunity is supported by studies of cytochrome c [71, 72]. Mice immunized with foreign cytochrome c in adjuvant produce a population of antibodies cross-reactive with self-cytochrome c. Adoptive transfer of foreign cytochrome c-primed B cells to naive mice causes T cells of the recipients to respond to murine cytochrome c, suggesting that the activated autoreactive B cells can prime autoreactive T cells. Similarly, the activation of B cells specific for the U1-A component of U1 snRNPs can prime autoreactive T cells, leading to diversification of the immune response to U1 snRNPs [73].

ROLE OF T-CELL DEFECTS IN AUTOANTIBODY PRODUCTION

T-Cell-Dependent vs -Independent Autoantibody Production

Like antibody production in general, autoantibody production can be T cell dependent or independent. The importance of T-cell help has been examined in both human SLE and animal models. Evidence for T-cell-dependent autoantibody production includes the predominance of IgG2a antinuclear antibodies in MRL mice [74], the lower levels of autoantibodies in MRL/*lpr* or NZB/W mice treated with anti-CD4 antibodies or CTLA4Ig [75–77], and the necessity for cognate T–B-cell interactions in autoantibody production [78]. The strongest evidence that autoantibodies are generated through a process of autoantigen-driven B-cell activation is the large number of somatic mutations in anti-DNA and anti-Sm autoantibody V regions [79, 80]. Many of the anti-DNA antibodies from MRL/*lpr* mice are members of the same expanded clones [79]. Moreover, the high frequency of replacement versus silent mutations and their nonrandom distribution argues that anti-DNA antibodies are selected on the basis of their receptor specificity. Thus, the analysis of autoantibody V regions strongly suggests that T cells are involved.

Autoreactive T cells that proliferate and/or secrete cytokines in response to lupus antigens, such as histones, have been identified in mouse models of lupus and have a helper phenotype. Autoreactive T cells also have been identified in human lupus, including T cells responsive to histones or nucleosomes [67], U1 snRNP antigens [81, 82], and the ribosomal P antigen [83]. As in the mouse, autoreactive T-cell clones bearing αβ as well as γδ T-cell receptors have been isolated. Although most of the autoantibodies characteristic of lupus (see earlier discussion) are T cell dependent, low-affinity, polyreactive anti-ssDNA autoantibodies produced by the B1 subset bear germline Ig variable region sequences and may be less dependent on T-cell help.

Self-tolerance in T Cells

The immune system has evolved to avoid reactivity with self through clonal deletion of autoreactive cells and peripheral inactivation (anergy). Self-antigens may also be sequestered or otherwise prevented from encountering the immune system [84], resulting in a state of immune ignorance. These control mechanisms appear to apply to B as well as T cells. The major mechanism for removing autoreactive T cells with high-affinity antigen receptors is clonal deletion in the thymus [85, 86]. In contrast, immunological tolerance to antigens not expressed in the thymus is maintained by peripheral T-cell inactivation [87, 88].

Ligation of the T-cell antigen receptor (Fig. 5, signal 1) is insufficient, by itself, to activate naïve T cells to proliferate or differentiate. Activation requires a costimulatory signal (signal 2) delivered by antigen-presenting cells [87, 89, 90]. In the absence of a costimulatory signal, T-cell receptor occupancy by antigen results in unresponsiveness (anergy) or apoptotic cell death. The best-characterized costimulatory molecules are B7-1 (CD80) and B7-2 (CD86), which are expressed on the surface of

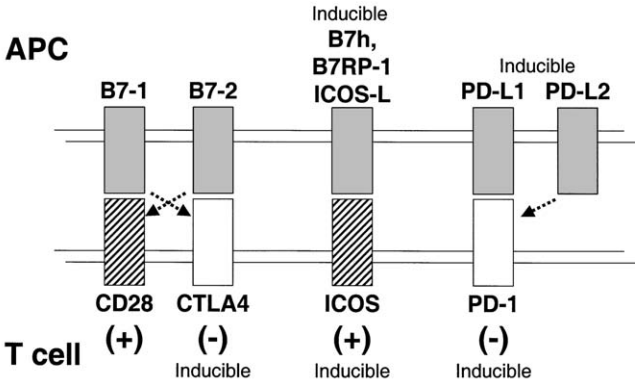


FIGURE 6 ***Diversity of T-cell costimulatory molecules.*** Receptor pairs involved in costimulating T-cell activation are illustrated. The B7-1 and B7-2 proteins (CD80 and CD86, respectively), which are expressed constitutively by professional antigen-presenting cells (APC), can costimulate T-cell activation through interactions with CD28 or can downmodulate activation by interacting with CTLA4. The B7h (B7RP-1, ICOS-L) protein is expressed inducibly by APCs and delivers a positive costimulatory signal to T cells expressing ICOS (also inducible). Two additional molecules expressed inducibly by APC, PD-L1 and PDL-2, can downregulate T-cell activation by interacting with the inducible T-cell surface marker PD-1. Thus, costimulatory molecules expressed by the APC can either positively (cross-hatched) or negatively (unshaded) regulate T-cell activation by interacting with proteins expressed either constitutively (CD28) or inducibly (CTLA4, ICOS, PD-1) by the T cell.

macrophages, dendritic cells, activated B cells, and other antigen-presenting cells [89, 91, 92]. Delivery of signal 2 along with signal 1 to a naïve T cell alters gene expression, increasing expression of the IL-2 receptor α chain, IL-2, and CD40 ligand (CD154) [90]. In naïve T cells, the constitutively expressed CD28 molecule is the only receptor for B7-1 and B7-2, but after cross-linking of CD28 by B7, expression of the higher affinity receptor CTLA-4 (CD152) is induced (Fig. 6). CTLA-4 is a negative regulator containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) that serves to inhibit T-cell activation when ligated along with the T-cell receptor [93, 94]. When B7 engages CTLA-4, the tyrosine phosphatase SHP-2 is recruited, resulting in the downregulation of T-cell receptor signaling. Due to its 20-fold higher affinity for B7, CTLA-4 engagement inhibits the production of IL-2, thus limiting the proliferation of activated T cells. It also plays a critical role in the induction of peripheral tolerance (anergy) [94]. Moreover, CTLA-4 is expressed on a subset of human regulatory T cells expressing CD4 and CD25, which has been implicated in the regulation of autoimmunity [95, 96].

More recently, additional CD28-like proteins have been identified (Fig. 6), including an inducible costimulator (ICOS) and PD-1. ICOS binds to a ligand expressed in an inducible manner by dendritic cells, monocytes B cells, and nonprofessional APC, which has been designated B7h or B7RP-1 (ICOS-L) [97, 98]. B7h (B7RP-1) differs from B7-1 and B7-2 in its inducible expression by nonprofessional antigen-presenting cells, such as fibroblasts, in response to tumor necrosis factor (TNF)-α or LPS. Expression of B7h (B7RP-1) may help augment T-cell activation in inflamed nonlymphoid tissues. A similar situation exists in the case of a counterreceptor PD-L1 for the CD28-like inhibitory molecule PD-1. PD-1 binds to two inducible ligands (PD-L1 and PD-L2) expressed by interferon (IFN)-γ-activated monocytes and dendritic cells [99, 100]. Interestingly, PD-L1 is also expressed in certain nonlymphoid parenchymal tissues, such as the heart and lung. PD-1 is a negative regulator of T-cell activation and, like CTLA-4, contains an ITIM [101]. It is expressed only at low levels in resting lymphocytes, but is strongly induced following activation, suggesting that its negative regulatory effect is manifested at a late stage of activation to restrict progression of the response rather than at the early activation stage [101]. Other B7-like molecules have been described as well, but their function is less well defined [102]. It is apparent that T-cell activation is tightly regulated. In view of the importance of costimulatory signals in determining tolerance vs activation, defects in these complex regulatory pathways have the potential to promote autoimmunity.

T-Cell Costimulation in Autoimmunity

The regulation of costimulatory activity can influence susceptibility to autoimmune diseases (Table 6). Treatment of murine lupus with CTLA-4-Ig, which blocks B7-1 and B7-2, results in decreased anti-DNA antibody production and decreases proteinuria [76]. Conversely, ectopic expression of B7-1 in the presence of inflammation (TNF-α) in the pancreas can induce type I diabetes in mice [103], and ectopic expression of B7-1 on T cells may contribute to the lupus-like manifestations of *lpr* and *gld* mice [104]. CD28-deficient mice exhibit abnormalities in the production the T-cell-dependent isotypes IgG1 and IgG2b, but not IgG2a [105]. Interestingly, they remain susceptible to autoimmune disease and still can produce IgG2a autoantibodies, although disease severity in an autoimmune myocarditis model was reduced [106]. However, polyclonal B-cell activation and anti-DNA antibodies in the graft-versus-host disease model of lupus are both CD28 dependent [107]. These data suggest that there are redundant costimulatory pathways and that other costimulatory molecules, such as ICOS, may compensate for the absence of CD28. ICOS-deficient mice have defective T-cell activation but unex-

TABLE 6 Defective Regulation of T-Cell Activation: Effects on Autoimmunity

Molecule	Defect	ANA	Other autoimmune manifestations	Ref.
B7-1	Ectopic expression on T cells	?	?	104
CD28	Deficiency	↓	↓ nephritis but still susceptible to autoimmune diabetes	107
CD28	Blockade with CTLA4-Ig	↓	↓ proteinuria	76
CTLA-4	Deficiency	N/A[a]	Lymphoproliferation, myocarditis, pancreatitis	295
ICOS	Deficiency	N/A	Increased susceptibility to EAE, decreased IL-4 and IL-13, increased IFN-γ production	108
PD-1	Deficiency	↑	Increased IgG3, nephritis, arthritis	101

[a] Not available.

pectedly are more susceptible to experimental autoimmune myelitis (EAE) [108, 109], suggesting that ICOS has a protective effect. Clearly, ICOS plays a major role in CD40-mediated class switching and is expressed in germinal centers [110]. CD40-CD40L interaction is critical for class switching, T-cell-dependent antibody responses, and the formation of germinal centers, the main site of T–B collaboration [111–113]. ICOS deficiency profoundly affects antibody responses to T-cell-dependent antigens, such as keyhole limpet hemocyanin (KLH), but has no effect on responses to T-cell-independent antigens [109]. In contrast to its enhancement of T-cell autoimmunity, ICOS deficiency should inhibit T-cell-dependent autoantibody production. This issue remains to be examined, however.

As illustrated by the induction of CTLA-4 expression subsequent to T-cell activation by TcR and CD28 ligation [90, 93], the balance between costimulatory (CD28 and ICOS) and inhibitory (CTLA-4 and PD-1) signals changes after the initiation of an immune response and may be a key regulator of T-cell-dependent immune responses. The relative levels of B7 (costimulatory) vs PL-L1 (inhibitory) may determine the extent of T-cell activation and therefore the threshold between tolerance and autoimmunity [99]. PD-1-deficient mice develop endocapillary proliferative glomerulonephritis resembling lupus nephritis, arthritis with pannus, and dilated cardiomyopathy [101]. Some mice produce IgG1 autoantibodies against a 33-kDa myocardial protein (T. Honjo, 7th International Workshop on Autoantibodies and Autoimmunity, Awajishima, Japan, 2001). As in MRL mice, the disease process is accelerated by the *lpr* mutation, but the effects of PD-1 deficiency on autoantibody formation are atypical for SLE. Anti-dsDNA antibodies and rheumatoid factor are not detected [101]. There is selective glomerular deposition of IgG3 instead of the IgG2a predominance seen in MRL and NZB/W lupus, a selective increase in serum IgG3, and augmented IgG3 antibodies against the T-cell-independent antigen TNP-Ficoll [114]. Because most murine IgG3 antibodies are cryoglobulins [115, 116], the autoimmune syndrome may more closely resemble mixed essential cryoglobulinemia than lupus.

Role of T-Cell Subsets in Autoantibody Formation

$CD4^+$ effector T cells are somewhat arbitrarily classified according to the cytokines they produce. T_H1 cells produce IFN-γ and interleukin (IL)-2, whereas T_H2 cells produce IL-4, IL-5, IL-10, and IL-13, and T_H3 cells produce transforming growth factor β (TGF-β) [117–119]. Although the T_H1-T_H2-T_H3 paradigm is more clear-cut in mice than in humans, it remains a useful concept in the human system. T_H1 and T_H2 cytokines may stimulate the production of different subsets of autoantibodies, and the differential activation of T_H1 vs T_H2 cells could influence autoantibody production. The role of T_H1 and T_H2 cytokines in autoantibody formation is discussed further later.

B7-1, B7-2, and ISOS-L (B7h, B7RP-1) differ in their ability to costimulate different helper T-cell subsets and in the kinetics of their induction [89, 92]. Moreover, T_H1 and T_H2 cells differ in their requirements for costimulation and, consequently, also in their susceptibility to the induction of peripheral tolerance [120, 121]. T_H2 responses are dependent initially on high doses of antigen and on CD28 costimulation, but their maintenance is less dependent on costimulation [122]. In contrast, T_H1 responses are initially less dependent on CD28 costimulation, but more dependent on a continued provision of costimulatory signals than T_H2 responses. T_H2 responses are also relatively more dependent on ICOS costimulation, and ICOS-deficient mice produce only low levels of IL-4 and IL-13 [108].

ROLE OF B-CELL DEFECTS IN AUTOANTIBODY PRODUCTION

The two major subsets of B cells, B-1 and B-2, are defined by their surface phenotypes and anatomical distribution [123]. The B-1 subset is enriched in the peritoneal and pleural cavities and has an IgM^{high}, IgD^{low}, $B220^{high}$, $CD23^{neg}$ phenotype. B-1a cells express moderate levels of CD5, whereas B1-b cells are $CD5^{neg}$. In contrast, the B-2 (conventional) subset is IgM^{low}, IgD^{high}, $B220^{high}$, $CD23^{high}$, $CD5^{neg}$. Most studies suggest that B-1 cells produce polyreactive antibodies, exhibit only limited somatic mutation, develop independently of T cells, and are prone to make low-affinity autoantibodies [123]. In contrast, B-2 cells produce high-affinity, somatically mutated antibodies that are generated in the presence of antigen-specific T cells. B-1 and B-2 subsets express different V_H gene repertoires, suggesting that surface immunoglobulin specificity influences the signals received by B cells during development, helping to determine whether they become B-1 or B-2 cells [124].

The protein tyrosine phosphatase SHP-1 regulates cell numbers in the B-1 compartment [125]. Deficiency of SHP-1 or proteins interacting with it (CD22 and Lyn) results in an enlarged pool of peritoneal B-1 cells and the presence of B-1 cells in the spleen and lymph nodes. SHP-1 is also recruited when surface IgG and FcγRIIb are cross-linked [126], but the effect of FcγRIIb deficiency on the B1 compartment is not known. B-1a cells express CD5, another protein that interacts with SHP-1. $CD5^{null}$ mice do not have increased numbers of B-1 cells, possibly reflecting a compensatory effect of CD22 [125]. Conversely, a deficiency of CD19, a positive regulator of B-2 cell development, decreases the number of peritoneal B-1a cells [127].

B-Cell Activation

Although B-cell responses to repetitive, multivalent antigens, such as pneumococcal polysaccharide, can be T cell independent, the activation of B cells recognizing low valency antigens generally requires two signals: one delivered by the antigen receptor (Fig. 5, signal I) and a costimulatory signal delivered when CD40 on the B cell engages CD40L on an activated T cell (signal II). The B-cell antigen receptor consists of membrane immunoglobulin and the associated molecules Igα (CD79α) and Igβ (CD79β) (Fig. 5). Antigen cross-linking activates the Src-like kinases Lyn, Fyn, and Blk and the tyrosine kinase Syk [125, 128]. CD22 and FcγRIIb negatively regulate antigen receptor signaling via SHP-1 (Fig. 7). Conversely, the CD19-CD21-CD81 (TAPA-1) complex positively regulates signaling and enhances antibody responses to T-cell-dependent antigens. Thus, the strength signal I is exquisitely regulated. Signal II is regulated primarily at the level of the antigen-specific T cell, which expresses CD40L only upon activation [129, 130]. B-cell, like T-cell, activation must be regulated closely in order to avoid autoimmunity.

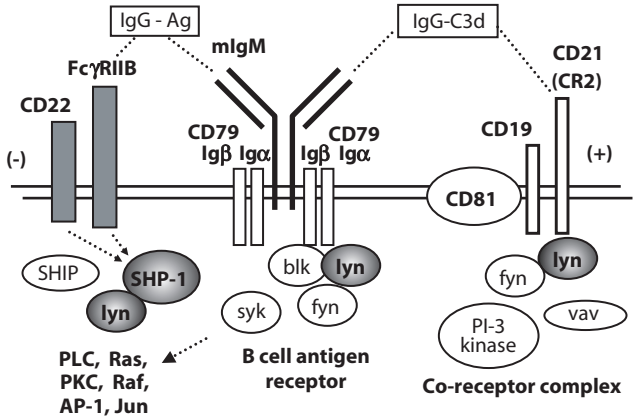


FIGURE 7 ***Regulation of B-cell activation.*** The B-cell antigen receptor (center) consists of membrane IgM (mIgM) and CD79 (Igα and Igβ chains). Signal transduction through the BCR is mediated, in part, by the kinases Lyn, Blk, Fyn, and Syk. BCR signaling is modulated positively by CD19, CD21 (CR2), and CD45, which signal through Lyn, Fyn, and other proteins. BCR signaling is modulated negatively by the inhibitory receptors CD22, pir-b, and FcγRIIb, which signal through SHP-1, Lyn, and other proteins. Deficiency of the negative regulators CD22, FcγRIIb, SHP-1 and Lyn (shaded) results in an autoimmune phenotype.

Self-tolerance in B Cells

Autoreactive B cells, like T cells, are censored by a variety of mechanisms that depend on the presence or absence of T-cell help, the form of the antigen, and the type of B cell. There are differences in the susceptibility of mature vs immature B cells as well as between B cells responding to T-cell-independent antigens (B-1 subset) vs T-cell-dependent antigens (B-2 subset). Deletion (apoptosis), anergy, and immunological ignorance all play a role in determining whether B cells become activated or are tolerized [131]. Peripheral ignorance to lupus autoantigens such as Sm may be maintained by the differentiation of autoreactive B cells into B-1 cells, which have a higher activation threshold than conventional B cells [132]. Abnormal activation-induced cell death, e.g., due to a defect in the apoptosis gene Fas, can promote the activation of B cells that are ignored rather than actively anergized, leading to autoantibody production [133].

TABLE 7 Defective Regulation of B-Cell Activation: Effects on Autoimmunity

Molecule	Defect	ANA	Other autoimmune manifestations	Ref.
Lyn	Deficiency	↑	Nephritis	143,144,296
CD22	Deficiency	↑ (DNA)	None	142,297
SHP-1	Deficiency	↑ (DNA)	Nephritis	145
FcγRIIB	Deficiency	↑ (DNA, chromatin)[a]	Nephritis, vasculitis	147

[a] Strain dependent.

In the case of active tolerance to multivalent antigens or membrane-bound proteins, such as MHC class I molecules, immature B cells expressing transgenes encoding the rearranged genes for both chains of an autoantibody specific for the antigen never develop and undergo apoptosis [131]. The same is true of B cells expressing receptors with high affinity for double-stranded DNA, which in nonautoimmune-prone mice are deleted in the bone marrow at the pre-B to immature B transitional stage [134, 135]. Like central deletion of T cells, central deletion of autoreactive B cells also appears relatively normal in autoimmune strains such as MRL [136, 137].

In contrast to anti-dsDNA antibodies, anti-ssDNA antibodies are not centrally deleted in nonautoimmune mice. However, upon exiting the bone marrow milieu, they are rendered anergic [134]. Peripheral B-cell tolerance appears abnormal in lupus mice. In contrast to nonautoimmune strains, MRL/*lpr* mice transgenic for an anti-DNA heavy chain (3H9) produce serum IgM anti-ssDNA antibodies, suggesting that peripheral B-cell tolerance is abnormal [138].

B-cell nonresponsiveness to many self-antigens that are present at low levels, probably including some nuclear antigens, appears to be controlled mainly by a lack of T-cell help [131, 139, 140]. In addition, Fas deficiency leads to an abnormal accumulation of $CD4^+$ T cells in the B-cell follicles. Developmental arrest and follicular exclusion of anti-dsDNA B cells are abnormally regulated in MRL/*lpr* mice [141], suggesting that follicular exclusion is an important tolerization mechanism for certain types of autoreactive B cells. As there are multiple mechanisms of B-cell tolerance, the use of broad terms such as "deletion" or "anergy" may be insufficient to describe the multiple checkpoints involved [131].

Abnormal B-Cell Activation Promotes Autoimmunity

As discussed earlier, CD22 and FcγRIIb negatively regulate B-cell antigen receptor signaling via SHP-1 (Fig. 7). Interestingly, mice with defective CD22, Lyn, or SHP-1 have a similar phenotype: increased numbers of B-1 cells and autoantibody production [125]. CD22-deficient mice develop increased numbers of B-1 cells (but not as dramatic as SHP-1-deficient mice) along with high titers of anti-DNA antibodies (Table 7), but it has not been shown conclusively whether they are directed against ssDNA or dsDNA [142]. These and other autoantibodies (e.g., IgG2a anticardiolipin, antimyeloperoxidase) were reported to have high affinities, but unlike Lyn- or SHP-1-deficient animals, CD22-deficient mice do not develop autoimmune disease.

In contrast to CD22-/- mice, Lyn-deficient mice (Table 7) develop splenomegaly, autoantibodies, and renal disease along with increased numbers of B-1b cells and high levels of serum IgM [143]. However, IgG levels are normal, and immune responses to both T-cell-dependent and -independent antigens are abnormal with poorly formed germinal centers [143]. Fas-mediated apoptosis is impaired in Lyn -/- mice and may act synergistically with the increased level of B-cell activation in Lyn -/- mice to promote autoimmunity [144]. The autoimmune disease in Lyn -/- mice remains incompletely characterized: although glomerular immune complexes are present, it is not known if proteinuria ensues, nor is it known whether these mice produce high-affinity autoantibodies typical of SLE, such as IgG anti-dsDNA or anti-Sm.

SHP-1 inhibits B cells from accumulating in the B-1a compartment [125], and not surprisingly, SHP-1-deficient (motheaten viable) mice have a massive expansion of $CD5^+$ B cells and selective IgM hypergammaglobulinemia. In addition to autoantibodies and glomerular immune complex deposition, motheaten mice have a variety of hematological disorders, including the accumulation of macrophages and granulocytes in the lungs, resulting in their demise at ~9 weeks of age [145, 146]. The autoimmune syndrome differs significantly from SLE (Table 7). Although anti-DNA antibodies reactive on solid-phase assays are produced, they do not give a nuclear staining pattern on immunofluorescence [145]. Their cytoplasmic staining pattern suggests that they differ in key respects from the typical anti-dsDNA antibodies of SLE.

Deficiency of FcγRII also leads to autoimmune disease (glomerulonephritis and vasculitis) and the

production of antichromatin and anti-DNA autoantibodies in B6 mice, but not in BALB/c [147]. However, other lupus autoantibodies, such as anti-Sm, are not produced, and it is not known whether true anti-dsDNA autoantibodies are produced. These mice develop prominent proteinuria and kidney disease in a strain-specific manner, closely mimicking SLE.

Strength of the B-cell costimulatory signal (signal II, Fig. 5) also contributes to autoimmunity. CD40L expression is abnormally regulated in both human and murine lupus [148, 149]. Although CD40L transgenic mice do not develop spontaneous autoimmunity [150], CD40–CD40L interactions are important for generating high-affinity autoantibodies. Neutralizing antibodies to CD40L have been used either by themselves or in combination with CTLA-4Ig to treat murine lupus, resulting in the amelioration of renal disease and decreased autoantibody production [151–153]. These antibodies may have a beneficial effect when administered subsequent to the onset of autoimmunity, suggesting that CD40–CD40L interactions are required to maintain autoantibody production. Unfortunately, trials in human autoimmune disease have been stopped due to thrombotic complications [154].

ROLE OF ANTIGEN-PRESENTING CELLS

T and B lymphocytes are the primary mediators of adaptive immunity and consequently are critical players in the formation of autoantibodies. It is important to recognize, however, that the activation of antigen-specific T and B lymphocytes is tightly controlled by antigen-presenting cells and innate immunity.

Innate Immunity

The innate immune system consists of various types of APC, natural killer (NK) cells, complement, and interferons α and β [155, 156]. Dendritic cells (DC) in particular are a key link between innate and adaptive immunity through their ability to initiate and modulate immune responses [155, 157]. Antigens from apoptotic cells are picked up by peripheral DC and transported to the lymph nodes [155, 158, 159]. The presentation of self-antigens to T cells by immature DC is an important mechanism of tolerance induction [160, 161]. In contrast, when DC are confronted with a "dangerous" foreign antigen, which may be associated with self-antigens in an infected cell, they undergo cytokine-mediated maturation leading to the expression of increased surface CD86 (B7-2), MHC class II, and MLR-stimulating activity, with concomitant downregulation of phagocytic capacity [155]. In the presence of IFN-γ, DC mature to a T_H1-polarizing phenotype characterized by high IL-12 production (DC1 effector cells), whereas DC matured in the presence of PGE_2 (DC2 effector cells) are deficient in IL-12 production and have a T_H2 polarizing phenotype [162–164]. Mature DC exert powerful T-cell stimulatory effects, but because of the prior inactivation of self-reactive T cells by immature DC, they generally do not stimulate autoimmunity. $CD11c^+$ myeloid DC and, to a lesser degree, $CD4^+$ DC are reduced greatly in the peripheral blood of SLE patients [165]. Although this may be a consequence of the defective development of DC precursors or more rapid turnover, an alternative explanation is that they are sequestered at sites of inflammation.

Innate Immunity and Autoantibody Formation

The induction of lupus by pristane and other hydrocarbons may be an instance in which disordered innate immunity leads to autoimmunity. Intraperitoneal injection of pristane in nonautoimmune mice induces anti-Sm, anti-nRNP, anti-dsDNA, anti-ribosomal P, and other autoantibodies characteristic of SLE, as well as immune complex-mediated glomerulonephritis and arthritis [19, 20]. Peritoneal macrophages phagocytose the oil and produce exuberant amounts of IL-6, IL-12, and TNF-α. The production of autoantibodies is dependent on these proinflammatory cytokines as well as on intact Fas-Fas ligand signaling, as *lpr* or *gld* strains are resistant [166–168]. Pristane exposure enhances APC survival and results in the acquisition of DC-specific surface markers (M. Satoh, unpublished data). The activation of APC by pristane may be analogous to the stimulation of innate immunity by microbial substances that bind "pattern receptors" [169].

Central Role of IFN-α in Linking Innate and Adaptive Immunity

Three subsets of DC precursors can be identified in peripheral blood: $CD14^+$ monocytes, $CD14c^-$ precursor DC, and $CD11c^+$ precursor DC [156]. The $CD11c^-$ subset, also called "plasmacytoid monocytes" or "plasmacytoid T cells," is identical to a rare cell type that produces large amounts of interferons α and β. These cells, known as interferon-producing cells, have a plasma cell-like morphology with an unusual surface phenotype ($CD4^+$, $CD31^+$, $CD36^+$, $CD68^+$, $CD62L^+$, $CXCR3^+$, and $CD11c^-$, $CD3^-$, $CD19^-$, $CD14^-$, $CD16^-$) and are the major IFN-α-producing cells [170–172]. They migrate to inflamed lymph nodes at high endothelial venules and produce large amounts of IFN-α on

exposure to inactivated herpes simplex virus, influenza virus, or following CD40 ligation [171]. IFN-α promotes survival of antigen-activated T cells [173] and can promote autoantibody formation.

IFN-α is used to treat hepatitis C infection, malignant carcinoid syndrome, and chronic myelogenous leukemia. Therapeutic use has been associated with autoimmune phenomena, including sarcoidosis [174], autoimmune thyroiditis, and autoimmune hepatitis [175]. The induction of antinuclear antibodies and anti-dsDNA antibodies, as well as overt lupus, has been reported as well [176–178]. Moreover, the serum level of IFN-α correlates with anti-dsDNA antibody levels and disease activity in SLE [179–181].

In addition to its effects on T-cell development and autoantibody formation, IFN-α promotes the differentiation of monocytes into DC-like cells [182]. These cells can capture antigens from dying cells and present them to $CD4^+$ T cells [182]. Enhanced expression of superantigens encoded by human endogenous retroviruses (HERVs) with stimulation of T cells expressing particular Vβ chains is another effect of IFN-α that could promote autoimmune disease [183].

Autoimmunity with Abnormal Clearance of Apoptotic Cells

Abnormal clearance of apoptotic material, normally a noninflammatory process mediated by several phagocyte receptors [184–186], may promote autoantibody production (Table 8). A classic example is the acceleration of autoantibody production by Fas or Fas ligand mutations [187]. The mechanisms remain incompletely understood: the prolonged survival of autoreactive lymphocytes, which normally are removed by Fas-mediated apoptosis, could play a role. Alternatively, the inability to remove cells by this pathway could lead to disposal by more inflammatory pathways, such as necrosis. It is interesting that defects in the clearance of apoptotic cells can also promote autoimmunity.

The development of antinuclear antibodies and nephritis (Table 8) by mice deficient in the serum amyloid P component (SAP) [188] is consistent with the idea that abnormal clearance of apoptotic cells promotes autoantibody formation. SAP coats apoptotic cells and blebs containing chromatin fragments and other autoantigens and helps solubilize native chromatin by displacing H1 histone, promoting its clearance by phagocytes. Likewise, C1q-deficient mice clear apoptotic cells abnormally and develop autoantibodies and lupus-like disease [189]. However, an autoimmune-prone background seems to be necessary along with C1q deficiency. The notion that delayed clearance of apoptotic material promotes autoantibody formation is further supported by the development of lupus-like disease and autoantibodies by mice deficient in Mer, a member of the Axl/Mer/Tyro3 receptor tyrosine kinase family [190]. Mer-deficient macrophages show a selective defect in the phagocytosis of apoptotic cells.

TABLE 8 Defective APC Function: Effects on Autoimmunity

Molecule	Defect	ANA	Other autoimmune manifestations	Ref.
C1q	Deficiency	↑	Nephritis	189
SAP	Deficiency	↑	Nephritis	188
Mer	Deficiency	↑	N/A[a]	190

[a] Not available.

CYTOKINE DEPENDENCE OF AUTOANTIBODY FORMATION

The importance of APCs in generating autoantibodies is underscored by the critical role of the cytokines they produce. Tumor necrosis factor-α is of particular relevance, although IL-1, IL-6, IL-12, and possibly IL-10 also promote autoantibody formation.

TNF-α and Related Molecules

The induction of antinuclear and anti-dsDNA antibodies and the occasional development of overt SLE are important side effects of TNF-α blockade [176, 191]. In rheumatoid arthritis patients, the incidence of antinuclear antibodies increased from 29 to 53% in infliximab-treated patients and anti-dsDNA antibodies were detected in 14% [192]. However, only rare patients (1 of 156 in this study) developed clinical manifestations of SLE. Thus, TNF-α may be protective against lupus-like disease, as also suggested by mouse studies. TNF-α appears to have a physiological role in suppressing the emergence of autoreactive lymphocytes in the NZB/W model, and deficiency enhances the production of anti-dsDNA antibodies [193, 194]. Low TNF-α responsiveness is characteristic of macrophages from the NZW parent [193], and more recent studies have suggested that a polymorphism of the 3′-untranslated region of the NZW TNF gene may reduce translational efficiency [195]. Similar TNF dependency has been noted in the MRL model [196]. However, other reports suggest that an increased production of TNF-α [197] or an increased signal transduction via TNFRII (p75 TNF receptor) [198] are associated with SLE, although not with autoantibody formation.

BLyS (BAFF, zTNF4, TALL-1, THANK) is a member of the TNF ligand family that binds to a receptor (TACI) expressed on B cells [199, 200]. It is a potent B-cell coactivator, and transgenic mice expressing high levels of BLyS develop polyclonal hypergammaglobulinemia and expansion of B-1a cells. NZB/W and MRL mice have high levels of circulating soluble BLyS, as do lupus patients [199, 201]. The effects of BLyS on IgM levels and on polyclonal hypergammaglobulinemia are more profound than is the enhancement of IgG autoantibodies, suggesting that it may not be a key regulator of autoantibody formation in SLE. Indeed, studies of TACI mutant mice suggest that TACI, through its interactions with BLyS and APRIL, may be more important as a costimulator of T-cell-independent (TI-2) antibody responses against polysaccharides such as pneumococcal polysaccharide [202].

Other Cytokines Produced by APC

IL-10, which is produced by APC and certain T cells (see later), affects polyclonal hypergammaglobulinemia and enhances autoantibody production in mice [203, 204]. IL-6 enhances autoantibody formation in both humans and mice. In pristane-induced lupus, IL-6 is required for the production of anti-DNA and antichromatin autoantibodies, but not for anti-Sm, RNP, or Su [167]. IL-6 accelerates nephritis both in pristane-induced lupus and in spontaneous models [167, 205]. In humans, the overproduction of IL-6 in patients with cardiac myxomas [206] and Castleman's disease [207] is associated with antinuclear and other autoantibodies.

IL-1β also may enhance polyclonal IgG production in mice, but the effect on the production of anti-DNA antibodies is small [208, 209]. There may be a greater effect on nephritis [210]. In addition, IL-1RA-deficient mice develop a chronic inflammatory condition associated with severe arthritis resembling rheumatoid arthritis along with anti-dsDNA autoantibodies [211]. Finally, IL-12 and IL-18 are APC-derived cytokines that induce IFN-γ production and accelerate lupus-like disease and autoantibody production in murine lupus [212, 213]. These cytokines promote the differentiation of helper T-cell subsets that have substantial effects on autoantibody formation.

Cytokines Produced by T Cells

Both T_H1 (IFN-γ) and T_H2 (IL-4, IL-5, and IL-10) cytokines have been implicated in murine lupus. T_H2 cytokines may promote autoantibody production in some situations [204, 214], including the lupus-like disease in mice with chronic graft-versus-host disease [215]. In NZB/W mice, IL-4, IL-5, and IL-6 are involved in anti-DNA antibody production and renal disease [216], and treatment with anti-IL-10 antibodies decreases anti-DNA antibody production, delays the onset of nephritis, and prolongs survival [204]. However, although IL-4 transgenic B6 mice develop spontaneous immune complex nephritis [217], IL-4 transgenic NZW/C57Bl/6Yaa mice are protected [218]. At present, it appears that in most spontaneous and inducible models of lupus in mice, IFN-γ plays a much more important role than IL-4 and IL-10.

Indirect evidence that IFN-γ plays a role in autoantibody formation comes from the observation that anti-Sm and chromatin autoantibodies in MRL/*lpr* mice are predominantly IgG2a, an IFN-γ-dependent isotype [219]. More direct evidence comes from the observation that the expression of IFN-γ in the skin of transgenic mice leads to the production of anti-DNA and -Sm autoantibodies [220]. In pristane-induced lupus the production of anti-DNA and antichromatin autoantibodies and the development of renal disease are abrogated in IFN-γ-deficient mice, and the frequency of anti-nRNP, anti-Sm, and anti-Su autoantibody production is decreased greatly [168]. In contrast, IL-4 deficiency has little effect [168]. Anti-DNA levels are also very low in IFN-γ-deficient MRL/*lpr* [221–223] and NZB/W mice [224], suggesting that the IFN-γ dependence of this specificity is not limited to inducible lupus. Consistent with these observations, neutralizing monoclonal antibodies against IFN-γ or soluble IFN-γ receptors ameliorate lupus in mice [225, 226], whereas IFN-γ treatment accelerates disease progression [225, 227].

The evidence implicating IFN-γ in humans is less compelling. The predominance of IgG1 and IgG3 autoantibodies in SLE [228] is consistent with a role, because these isotypes, like IgG2a in the mouse, are IFN-γ dependent [229]. Also, a polymorphism of the IFN-γ receptor appears to be associated with susceptibility to SLE [230]. The increased urinary excretion of neopterin, a marker of IFN-γ-activated macrophages in active vs inactive lupus is consistent with the possibility that IFN-γ is involved [231, 232]. Moreover, lupus patients exhibit higher levels of serum IL-18 than healthy controls [213]. However, in contrast to mice, it has been suggested that both disease activity and hypergammaglobulinemia correlate directly with IL-10 production and inversely with IFN-γ [203, 233, 234]. Deficient production of IL-12 has also been reported [234]. However, these studies of serum cytokine levels [235] and *in vitro* production of cytokines by peripheral blood mononuclear cells (PBMC) [234, 236] are inconclusive. Isolation of PBMC on Ficoll gradients causes artifactual IL-10 production [237], and the production of cytokines in peripheral blood may not reflect cytokine production in involved tissues, such as the

skin, joints, or kidney. Case reports suggest that treatment of rheumatoid arthritis patients with IFN-γ can induce or exacerbate lupus-like clinical disease as well as the production of antinuclear, anti-dsDNA, and anti-Sm autoantibody production [176, 238–240]. Further studies are needed to determine whether IFN-γ dependence distinguishes murine from human lupus. In view of the demonstration that IL-10 signaling can be uncoupled at the level of transcriptional activation, it is possible that the high levels of IL-10 are accompanied by ineffectual signal transduction [236].

THE BASIS OF ANTIGEN SELECTIVITY

A major gap in our understanding of the pathogenesis of autoimmune responses is how antigen specificity is conferred. It is difficult to explain the exquisite selectivity of the autoantibody response in SLE for a relatively small number of mostly intracellular (nuclear and sometimes cytoplasmic) self-antigens by a global defect in tolerance or the interactions between T and B cells or between APC and T cells. An understanding of antigen selectivity is key to understanding lupus and other autoimmune diseases. Of the thousands of intracellular proteins, why are only a few prominent as autoantigens in lupus? Current thinking focuses on several possibilities: (1) abnormal processing generates autoantigens, (2) autoantigens are selected because they are sequestered into apoptotic blebs, (3) immune responses against foreign antigens can lead to the production of B or T cells cross-reactive with self-antigens, and (4) autoantigens may be encoded by a subset of genes carrying mutational hotspots that lead to the appearance of somatically mutated, and consequently antigenic, self-proteins.

Altered Antigen Processing

The expressed T-cell repertoire responsive to an exogenous antigen is focused onto a limited number of major T-cell-inducing determinants rather than being directed broadly against all portions of the antigen [241]. MHC-linked genes control this through a determinant selection mechanism in which immunodominant peptides bind to MHC molecules that can present the peptide, but not to MHC molecules that do not present the peptide [241, 242]. Another factor influencing T-cell repertoire expression is the existence of hindering structures on naturally processed fragments that differentially affect presentation by different MHC molecules [241, 243]. The effects of these hindering structures can be overcome by "artificial processing" of an antigen, e.g., by cyanogen bromide cleavage. Hindering structures that affect presentation by MHC molecules can include structural constraints imposed by the tertiary structure of a protein [244] and intermolecular contacts between subunits of an oligomeric antigen [245, 246]. For example, recognition of the human chorionic gonadotropin (hCG) α/β dimer by α-specific T-cell hybridomas is reduced dramatically compared to free hCG-α or heat-dissociated dimers, suggesting that the quaternary structure of hCG affects the presentation of antigenic determinants of the monomers by interfering with proteolytic degradation into peptides capable of binding MHC molecules [246].

The amount of a determinant bound to a MHC molecule with high affinity is a major factor influencing T-cell responsiveness [247]. A major determinant is dominant because it is processed efficiently and binds efficiently to MHC. If this determinant is presented to a T cell in the presence of a costimulatory signal, T-cell activation results. Conversely, if costimulation is absent and/or it is presented by immature DC, the same peptide may be an efficient tolerogen [248]. In the case of minor determinants, the level of a peptide may be insufficient to trigger T-cell activation efficiently, and induction of a tolerogenic signal may be equally inefficient. Thus, tolerance is restricted primarily to the major T-cell-inducing determinants [249, 250]. Although T-cell responses to minor determinants are not stimulated under normal circumstances, they might be induced by cross-reactive microbial antigens or by altered self. Once a response to a minor determinant is initiated, it can be perpetuated by self-antigen because the conditions for memory T-cell responses are less stringent than those required for activating naïve T cells [249].

"Cryptic" self-peptides not normally seen by the immune system at sufficient levels to induce tolerance may be presented in several circumstances: (1) when a foreign antigen binds to self [251], (2) when the tertiary structure is altered by the binding of a hapten [252], and (3) when the protein structure is altered by somatic mutation (Fig. 8). This "altered self" model may have important implications for the pathogenesis of autoimmunity.

Induction of Autoimmunity by Complexes of Self and Nonself Proteins

One mechanism of generating altered self is through the binding of a foreign antigen to a self-protein (Fig. 8). Interaction of the p53 tumor suppressor protein with the SV40 large T antigen (SVT) is an example. SVT transforms cells by binding and inactivating p53 [253], increasing the half-life and cellular levels of p53 markedly [254, 255]. Rodents with SV40-induced

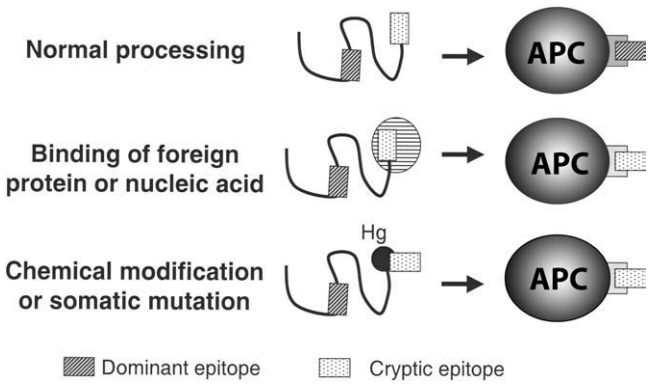


FIGURE 8 ***Generation of cryptic self-cell epitopes.*** The normal processing pathway for a self-protein with major (cross-hatched) and minor (stippled) epitopes by an antigen-processing cell (APC) is shown (top). Only the major epitope is presented at sufficient levels to trigger immune tolerance. (Middle) The binding of a foreign antigen to the self protects the minor epitope from degradation, leading to presentation of the minor epitope. Because tolerance to this peptide has not been established by processing of self, an immune response can be initiated. (Bottom) The antigen has been modified by chemical reaction of mercury with a cysteine residue, altering the conformation or the protein and increasing presentation of the minor epitope. This again leads to the development of immunity. A somatic mutation (not shown) could enhance presentation of the minor epitope similarly.

tumors develop autoantibodies against p53 [256, 257]. Moreover, immunization of mice with low doses of murine p53 does not induce an immune response to the self-antigen, whereas immunization with the same dose of p53 in the form of a complex with SVT results in the production of high levels of autoantibodies to p53 [251]. Once primed with the complex, immunization with murine p53 alone can boost the immune response, possibly because of the lower activation threshold of primed T cells (Fig. 8).

Many other viral infections can precipitate autoimmunity. Epstein–Barr virus infection has been associated with antinuclear antibodies [258], BK virus with antihistone antibodies [63], coxsackievirus infection with autoimmune myocarditis [259], avian leukosis virus with thyroiditis [260], and reovirus infection with polyendocrinopathy [261], to name only a few examples. In reovirus infection, autoimmunity targets only those tissues where the virus actually multiplies [261]. Thus, at least in this example of virally induced autoimmunity, close proximity of the virus and specific autoantigens is a critical factor in the induction of autoantibodies.

Induction of Autoantibodies by Haptens

Chemical alteration of self-proteins is another mechanism for generating altered self (Fig. 8). Mercuric chloride elicits a H-2^s-restricted immune response to the nucleolar antigen fibrillarin [252, 262, 263]. In humans, these autoantibodies are specific for scleroderma [264]. The Hg(II) ion is highly reactive with sulfhydryl groups and more weakly with hydroxyl, carbonyl, and phosphoryl groups [265]. Binding of Hg(II) to cysteines may form metal–protein complexes so stable that they alter conformation and unfolding of proteins inside endosomes, leading to an enhanced presentation of minor cryptic epitopes (Fig. 8).

Apoptosis

A third mechanism for generating altered self is the abnormal degradation of self-proteins (Fig. 8). A number of lupus autoantigens are present in apoptotic blebs and are degraded by caspases and other proteolytic enzymes, such as granzyme B, which may generate immunogenic peptides [266]. These cleavages could define certain proteins as autoantigens [267]. Although apoptosis is a normal cellular process, abnormalities of the usual apoptotic degradation pathways could generate cryptic self-peptides. For instance, the scleroderma-specific autoantigens topoisomerase I (Scl-70), RNA polymerase II, and human upstream binding factor (hUBF, NOR-90) are uniquely fragmented by reactive oxygen species in a metal (iron or copper)-dependent manner, raising the possibility that metal-catalyzed oxidation reactions might target a subset of antigens for autoimmunity [268].

Somatic Mutation of Autoantigens

Mutations arise spontaneously within the coding regions of genes and may be translated into abnormal proteins. A mutation limited to a single cell is unlikely to trigger an immune response due to the low level of altered protein. However, somatic mutations in proliferating cells can induce autoimmunity, probably by altering protein conformation, generating cryptic self-peptides that induce T-cell immunity followed by autoantibody formation [269, 270]. The high frequency of autoantibodies against somatically mutated oncoproteins such as c-myb [271], c-myc [272], and HER-2/neu [273] illustrates this principle. Similarly, autoantibodies are produced against the chronic myeloid leukemia-specific *bcr-abl* joining region formed by the reciprocal translocation between chromosomes 22 and 9 [274]. Autoantibodies to p53 occur at a frequency of ~15% in breast cancer and other tumors associated with p53

FIGURE 9 ***Molecular mimicry.*** Amino acid sequence homology between U1-70K and Moloney murine leukemia virus (MuLV) $p30^{gag}$ proteins is illustrated. Two amino acid sequences from the U1 snRNP 70K protein are aligned with a sequence from MuLV $p30^{gag}$. Homologous sequences are shaded. Immunological cross-reactivity of the homologous sequences can also be shown. Data from Query and Keene [25].

mutations [275–277]. Autoantibodies are present only in sera from patients with missense mutations compatible with the expression of detectable levels of cellular p53 [277, 278]. Moreover, autoantibodies are found only in sera of those patients whose mutant p53 forms a complex with heat shock protein 70 (hsp70), which prolongs the half-life of p53 [277]. No anti-p53 antibodies are found in sera from patients with stop, splice/stop, splice, or frameshift mutations. The autoantibodies recognize wild-type p53, suggesting that they are not specific for the mutant antigens [276, 278].

Molecular Mimicry

Immunological cross-reactivity of one antigen with another, termed "molecular mimicry" [279], may also play a role in autoimmunity. Cross-reactivity of a viral antigen with self can lead to autoantibody production. Cross-reactions between herpes simplex virus and intermediate filaments [280], the Epstein–Barr virus EBNA protein and several cellular proteins [281], and a retroviral $p30^{gag}$ protein and the U1 snRNP 70K protein [25] have been reported. Immunization of mice with the murine leukemia virus $p30^{gag}$ protein induces autoantibodies to a portion of the U1-70K protein exhibiting sequence similarity with the viral protein (Fig. 9), suggesting that retroviral infection can induce autoimmunity to U1-70K.

Autoantibody production may begin as a response to a single cross-reactive epitope, and receptor-mediated uptake of autoantigen by autoreactive B cells could diversify the autoantibody response by generating autoreactive T cells [25]. For example, immunization of mice with foreign cytochrome c induces a population of activated B cells bearing receptors that recognize self-cytochrome c [71, 72]. Adoptive transfer of B cells from these mice to naive recipients can prime autoreactive, cytochrome c-specific T cells. Thus, although molecular mimicry may be limited initially to a single cross-reactive epitope, autoantigen-specific B cells can diversify the response by acting as APC.

Molecular mimicry can also involve groups of self-antigens that share epitopes. For example, all Sm proteins share two regions of sequence homology, Sm motifs 1 and 2 [282]. Consequently, cross-reactive antibodies are prominent in the immune response to U1 snRNPs [66, 283]. Monoclonal antibody Y12, derived from an MRL mouse, recognizes an epitope shared by the highly homologous Sm-B and B′ proteins, as well as a conformational epitope of Sm-D [284] and a conformation-dependent epitope formed by the complex of Sm-E, F, and G [53]. Cross-reactivity of human anti-Sm antibodies with B′/B and D has been reported as well. Other human autoantibodies recognize two epitopes of U1-A cross-reactive with B′/B and C or with U2-B″ [283]. Certain cross-reactive epitopes may not be exposed in the native U1 snRNP particle. Thus, while the majority of anti-nRNP sera exhibit binding to the partially denatured Sm B′ and B proteins on Western blots, they do not recognize the B′/B proteins when incorporated into intact snRNPs. Cross-reactions can also arise due to reactivity of an autoantibody with a common posttranslational modification, such as poly(ADP) ribose [285].

FUTURE DIRECTIONS

Great strides have been made in understanding the pathogenesis of autoantibody production as pathways of lymphocyte activation have become better defined. Studies of genetically altered mice have led to the discovery that many defects are capable of promoting autoimmunity. Progress has also been made in understanding the role of the environment in autoimmunity. However, the basis for antigen selectivity remains poorly understood. Future research will need to focus on the latter question more closely. Also, it will be important to examine which of the genetic defects associated with lupus-like manifestations are relevant to human SLE, as underscored by studies of the Fas^{lpr} mutation. In mice, *lpr* was once regarded as a "lupus gene" because it greatly accelerates lupus-like disease in MRL mice and induces antinuclear antibodies on a B6 background. Human Fas mutations (Canale–Smith syndrome) promote the development of lymphadenopathy, as in mice, but the autoimmune manifestations are not characteristic of SLE [286, 287]. These individuals most frequently develop hematological autoimmunity, including autoimmune thrombocytopenic purpura and autoimmune hemolytic anemia [287]. However, lupus is unusual, both in Fas-deficient patients and in patients with defects of other compo-

nents of this pathway, such as Fas ligand and caspase 10 [288, 289]. Other murine lupus susceptibility genes identified by gene-mapping approaches will need to be scrutinized similarly for their relevance to the human disease. It may be useful to rigorously define pathological manifestations and serological abnormalities that uniquely define the lupus syndrome. Clearly, not all antinuclear antibodies can be equated with the lupus syndrome. Low-affinity anti-DNA or anti-Sm antibodies can be detected in a variety of conditions other than SLE, and their production in mutant mice should not be taken as evidence that the abnormal gene product(s) confers lupus susceptibility. It will be equally important to define more thoroughly the characteristic pathological lesions of lupus. Glomerular immune complex deposition, by itself, is probably not sufficiently specific for the lupus syndrome to be of much value in establishing whether a mutant mouse has lupus. Further complicating the ability to relate the information gained in mouse models to the human situation is the likelihood that SLE is not a single disease. If a loose definition of lupus is applied, mouse data indicate that there are numerous forms of the disease, each with a different clinical syndrome. A stricter definition might make it possible to define lupus as a disease caused by defects in a limited number of immunological pathways. With the rapid pace of discovery in mouse models, it may be timely to reappraise how we define SLE (serologically and pathologically) and to apply similar criteria to mice.

Acknowledgments

Supported by research grants AR40391 and AR44731 from NIH/NIAMS and General Clinical Research Center grant MOIR00082.

References

1. Hargraves, M. M., Richmond, H., and Morton, R. (1948). Presentation of two bone-marrow elements: The Tart cell and LE cell. *Proc. Staff Meet. Mayo Clin.* **23**, 25–28.
2. Holborow, E. J., Weir, D. M., and Johnson, G. D. (1957). A serum factor in lupus erythematosus with affinity for tissue nuclei. *Br. Med. J.* **2**, 732–734.
3. Ceppellini, R., Poli, E., and Celada, F. (1957). A DNA-reacting factor in serum of a patient with lupus erythematosus diffusus. *Proc. Soc. Exp. Biol. Med.* **96**, 572–574.
4. Robbins, W. C., Holman, H. R., Deicher, H., and Kunkel, H. G. (1957). Complement fixation with cell nuclei and DNA in lupus erythematosus. *Proc. Soc. Exp. Biol. Med.* **96**, 575–579.
5. Tan, E. M., and Kunkel, H. G. (1966). Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J. Immunol.* **96**, 464–471.
6. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N., and Winchester, R. J. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271–1277.
7. Edworthy, S. M., Zatarain, E., McShane, D. J., and Bloch, D. A. (1988). Analysis of the 1982 ARA lupus criteria data set by recursive partitioning methodology: New insights into the relative merit of individual criteria. *J. Rheumatol.* **15**, 1493–1498.
8. Shiel, W. C., and Jason, M. (1989). The diagnostic associations of patients with antinuclear antibodies referred to a community rheumatologist. *J. Rheumatol.* **16**, 782–785.
9. Tan, E. M. (1982). Autoantibodies to nuclear antigens (ANA): Their biology and medicine. *Adv. Immunol.* **33**, 167–240.
10. Tan, E. M. (1989). Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* **44**, 93–151.
11. Weinstein, A., Bordwell, B., Stone, B., Tibbetts, C., and Rothfield, N. F. (1983). Antibodies to native DNA and serum complement (C3) levels: Application to diagnosis and classification of systemic lupus erythematosus. *Am. J. Med.* **74**, 206–216.
12. Wang, J., Satoh, M., Kabir, F., Shaw, M., Domingo, M. A., Mansoor, R., Behney, K., Dong, X., Lahita, R. G., Richards, H. B., *et al*. (2001). Myositis and serositis with autoantibodies to Ku antigen in African-Americans with systemic lupus erythematosus. *Arthritis Rheum.* **44**, 2367–2370.
13. Kurata, N., and Tan, E. M. (1976). Identification of antibodies to nuclear acidic antigens by counterimmunoelectrophoresis. *Arthritis Rheum.* **19**, 574–580.
14. Elkon, K. B., Bonfa, E., and Brot, N. (1992). Antiribosomal antibodies in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **18**, 377–390.
15. Takasaki, Y., Fishwild, D., and Tan, E. M. (1984). Chraracterization of proliferating cell nuclear antigen recognized by autoantibodies in lupus sera. *J. Exp. Med.* **159**, 981–992.
16. Satoh, M., Langdon, J. J., Chou, C. H., McCauliffe, D. P., Treadwell, E. L., Ogasawara, T., Hirakata, M., Suwa, A., Cohen, P. L., Eisenberg, R. A., *et al*. (1994). Characterization of the Su antigen, a macromolecular complex of 100/102 and 200 kDa proteins recognized by autoantibodies in systemic rheumatic diseases. *Clin. Immunol. Immunopathol.* **73**, 132–141.
17. Theofilopoulos, A. N., and Dixon, F. J. (1985). Murine models of systemic lupus erythematosus. *Adv. Immunol.* **37**, 269–390.
18. Elkon, K. B., Bonfa, E., Llovet, R., and Eisenberg, R. A. (1989). Association betweeen anti-Sm and antiribosomal P protein autoantibodies in human systemic lupus erythematosus and MRL/lpr mice. *J. Immunol.* **143**, 1549–1554.
19. Satoh, M., and Reeves, W. H. (1994). Induction of lupus-associated autoantibodies in BALB/c mice by intraperitoneal injection of pristane. *J. Exp. Med.* **180**, 2341–2346.

20. Satoh, M., Kumar, A., Kanwar, Y. S., and Reeves, W. H. (1995). Antinuclear antibody production and immune complex glomerulonephritis in BALB/c mice treated with pristane. *Proc. Natl. Acad. Sci. USA* **92**, 10934–10938.
21. Satoh, M., Hamilton, K. J., Ajmani, A. K., Dong, X., Wang, J., Kanwar, Y. S., and Reeves, W. H. (1996). Autoantibodies to ribosomal P antigens with immune complex glomerulonephritis in SJL mice treated with pristane. *J. Immunol.* **157**, 3200–3206.
22. Ohnishi, K., Ebling, F. M., Mitchell, B., Singh, R. R., Hahn, B. H., and Tsao, B. P. (1994). Comparison of pathogenic and non-pathogenic murine antibodies to DNA: Antigen binding and structural characteristics. *Int. Immunol.* **6**, 817–830.
23. Vlahakos, D. V., Foster, M. H., Adams, S., Katz, M., Ucci, A. A., Barrett, K. J., Datta, S. K., and Madaio, M. P. (1992). Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. *Kidney Int.* **41**, 1690–1700.
24. Lefkowith, J. B., and Gilkeson, G. S. (1996). Nephritogenic autoantibodies in lupus: Current concepts and continuing controversies. *Arthritis Rheum.* **39**, 894–903.
25. Query, C. C., and Keene, J. D. (1987). A human autoimmune protein associated with U1 RNA contains a region of homology that is cross-reactive with retroviral p30gag antigen. *Cell* **51**, 211–220.
26. Monestier, M., and Kotzin, B. L. (1992). Antibodies to histones in systemic lupus erythematosus and drug-induced lupus syndromes. *Rheum. Dis. Clin. North Am.* **18**, 415–436.
27. Friou, G. J. (1958). Identification of the nuclear component of the interaction of lupus erythematosus globulin with nuclei. *J. Immunol.* **80**, 476–481.
28. Rodriguez-Sanchez, J., Gelpi, C., Juarez, C., and Hardin, J. A. (1987). A new autoantibody in scleroderma that recognizes a 90-kDa component of the nucleolus organizing region of chromatin. *J. Immunol.* **139**, 2579–2584.
29. Chan, E. K. L., Imai, H., Hamel, J. C., and Tan, E. M. (1991). Human autoantibody to RNA polymerase I transcription factor hUBF: Molecular identity of nucleolus organizer region autoantigen NOR-90 and ribosomal RNA transcription upstream binding factor. *J. Exp. Med.* **174**, 1239–1244.
30. Moroi, Y., Peebles, C., Fritzler, M. J., Steigerwald, J., and Tan, E. M. (1980). Autoantibody to centromere (kinetochore) in scleroderma sera. *Proc. Natl. Acad. Sci. USA* **77**, 1627–1631.
31. Miyachi, K., Fritzler, M. J., and Tan, E. M. (1978). Autoantibody to a nuclear antigen in proliferating cells. *J. Immunol.* **121**, 2228–2234.
32. Reeves, W. H., Wang, J., Ajmani, A. K., Stojanov, L., and Satoh, M. (1997). The Ku autoantigen. *In* "The Antibodies" (M. Zanetti and J. D. Capra, eds.), pp. 33–84. Harwood Academic, P Amsterdam.
33. Lerner, M. R., and Steitz, J. A. (1979). Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **76**, 5495–5499.
34. Lerner, M. R., Boyle, J. A., Hardin, J. A., and Steitz, J. A. (1981). Two novel classes of small ribonucleoproteins detected by autoantibodies associated with lupus erythematosus. *Science* **211**, 400–402.
35. Fisher, D. E., Conner, G. E., Reeves, W. H., Wisniewolski, R., and Blobel, G. (1985). Small nuclear ribonucleoprotein particle assembly in vivo: Demonstration of a 6S RNA-free core precursor and posttranslational modification. *Cell* **42**, 751–758.
36. Mattioli, M., and Reichlin, M. (1973). Physical association of two nuclear antigens and mutual occurrence of their antibodies: the relationship of the Sm and RNA protein (Mo) systems in SLE sera. *J. Immunol.* **110**, 1318–1324.
37. Sharp, G. C., Irvin, W. S., Tan, E. M., Gould, R. G., and Holman, H. R. (1972). Mixed connective tissue disease—an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med.* **52**, 148–159.
38. Query, C. C., Bentley, R. C., and Keene, J. D. (1989). A common RNA recognition motif identified within a defined U1 RNA binding domain of the 70K U1 snRNP protein. *Cell* **57**, 89–101.
39. Nelissen, R. L. H., Will, C. L., van Venrooij, W. J., and Luhrmann, R. (1994). The association of the U1-specific 70K and C proteins with U1 snRNPs is mediated in part by common U snRNP proteins. *EMBO J.* **13**, 4113–4125.
40. Craft, J. (1992). Antibodies to snRNPs in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **18**, 311–335.
41. Padgett, R. A., Grabowski, P. J., Konarska, M. M., Seiler, S., and Sharp, P. A. (1986). Splicing of messenger RNA precursors. *Annu. Rev. Biochem.* **55**, 1119–1150.
42. Padgett, R. A., Mount, S. M., Steitz, J. A., and Sharp, P. A. (1983). Splicing of messenger RNA precursors is inhibited by antisera to small nuclear ribonucleoproteins. *Cell* **35**, 101–107.
42a. Satoh, M., Miyazaki, K., Mimori, T., Akizuki, M., Ichikawa, Y., Homma, M., Ajmani, A. K., and Reeves, W. H. (1995). Changing autoantibody profiles with variable clinical manifestations in a patient with systemic lupus erythematosus and polymyositis. *Br. J. Rheumatol.* **34**, 915–919.
43. Satoh, M., Langdon, J. J., Hamilton, K. J., Richards, H. B., Panka, D., Eisenberg, R. A., and Reeves, W. H. (1996). Distinctive immune response patterns of human and murine autoimmune sera to U1 small nuclear ribonucleoprotein C protein. *J. Clin. Invest.* **97**, 2619–2626.
44. Satoh, M., Richards, H. B., Hamilton, K. J., and Reeves, W. H. (1997). Human anti-nRNP autoimmune sera contain a novel subset of autoantibodies that stabilizes the molecular interaction of U1RNP-C protein with the Sm core proteins. *J. Immunol.* **158**, 5017–5025.
45. Reeves, W. H., Fisher, D. E., Lahita, R. G., and Kunkel, H. G. (1985). Autoimmune sera reactive with Sm antigen contain high levels of RNP-like antibodies. *J. Clin. Invest.* **75**, 580–587.
46. Hardin, J. A. (1986). The lupus autoantigens and the pathogenesis of SLE. *Arthritis Rheum.* **29**, 457–460.

47. Mattioli, M., and Reichlin, M. (1974). Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus: Description of a cytoplasmic nonribosomal antigen. *Arthritis Rheum.* **17**, 421–429.
48. Boulanger, C., Chabot, B., Menard, H., and Boire, G. (1995). Autoantibodies in human anti-Ro sera specifically recognize deproteinized hY5 Ro RNA. *Clin. Exp. Immunol.* **99**, 29–36.
49. Boire, G., and Craft, J. (1990). Human Ro ribonucleoprotein particles: Characterization of native structure and stable association with the La polypeptide. *J. Clin. Invest.* **85**, 1182–1190.
50. Burlingame, R. W., Boey, M. L., Starkebaum, G., and Rubin, R. L. (1994). The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J. Clin. Invest.* **94**, 184–192.
51. Mohan, C., Adams, S., Stanik, V., and Datta, S. K. (1993). Nucleosome: A major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J. Exp. Med.* **177**, 1367–1381.
52. Pettersson, I. M., Hinterberger, M., Mimori, T., Gottlieb, E., and Steitz, J. A. (1984). The structure of mammalian small nuclear ribonucleoproteins: Identification of multiple protein components reactive with anti-(U1)RNP and anti-Sm antibodies. *J. Biol. Chem.* **259**, 5907–5914.
53. Brahms, H., Raker, V. A., van Venrooij, W. J., and Luhrmann, R. (1997). A major, novel systemic lupus erythematosus autoantibody class recognizes the E, F, and G Sm snRNP proteins as an E-F-G complex but not in their denatured states. *Arthritis Rheum.* **40**, 672–682.
54. Wilusz, J., and Keene, J. D. (1986). Autoantibodies specific for U1 RNA and initiator methionine tRNA. *J. Biol. Chem.* **261**, 5467–5472.
55. Deutscher, S. L., and Keene, J. D. (1988). A sequence-specific conformational epitope on U1 RNA is recognized by a unique autoantibody. *Proc. Natl. Acad. Sci. USA* **85**, 3299–3303.
56. van Venrooij, W. J., Hoet, R., Hageman, B., Mattaj, I. W., and Van de Putte, L. B. (1990). Anti-(U1) small nuclear RNA antibodies in anti-small nuclear ribonculeoprotein sera from patients with connective tissue diseases. *J. Clin. Invest.* **86**, 2154–2160.
57. Hoet, R. M., de Weerd, P., Gunnewiek, J. K., Koornneef, I., and van Venrooij, W. J. (1992). Epitope regions of U1 small nuclear RNA recognized by anti-U1RNA-specific autoantibodies. *J. Clin. Invest.* **90**, 1753–1762.
58. Okano, Y., and Medsger, T. A. (1992). Novel human autoantibodies reactive with 5′-terminal trimethylguanosine cap structures of U small nuclear RNA. *J. Immunol.* **149**, 1093–1098.
59. Lake, P., and Mitchison, N. A. (1976). Regulatory mechanisms in the immune response to cell-surface antigens. *Cold Spring Harbor Symp. Quant. Biol.* **41**, 589–595.
60. Russell, S. M., and Liew, F. Y. (1979). T cells primed by influenza virion internal components can cooperate in antibody response to haemagglutinin. *Nature (London)* **280**, 147–148.
61. Milich, D. R., McLachlan, A., Thornton, G. B., and Hughes, J. L. (1987). Antibody production to the nucleocapsid and envelope of the hepatitis B virus primed by a single synthetic T cell site. *Nature (London)* **329**, 547–549.
62. Zinkernagel, R. M., Cooper, S., Chambers, J., Lazzarini, R. A., Hengartner, H., and Arnheiter, H. (1990). Virus-induced autoantibody response to a transgenic viral antigen. *Nature (London)* **345**, 68–71.
63. Flaegstad, T., Fredriksen, K., Dahl, B., Traavik, T., and Rekvig, O. P. (1988). Inoculation with BK virus may break immunological tolerance to histone and DNA antigens. *Proc. Natl. Acad. Sci. USA* **85**, 8171–8175.
64. Fatenejad, S., Mamula, M. J., and Craft, J. (1993). Role of intermolecular/intrastructural B- and T-cell determinants in the diversification of autoantibodies to ribonucleoprotein particles. *Proc. Natl. Acad. Sci. USA* **90**, 12010–12014.
65. Topfer, F., Gordon, T., and McCluskey, J. (1995). Intra- and intermolecular spreading of autoimmunity involving the nuclear self-antigens La (SS-B) and Ro (SS-A). *Proc. Natl. Acad. Sci. USA* **92**, 875–879.
66. James, J. A., Gross, T., Scofield, R. H., and Harley, J. B. (1995). Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B′-derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. *J. Exp. Med.* **181**, 453–461.
67. Rajagopalan, S., Zordan, T., Tsokos, G. C., and Datta, S. K. (1990). Pathogenic anti-DNA autoantibody-inducing T helper cell lines from patients with active lupus nephritis: Isolation of CD4–8- T helper cell lines that express the gamma delta T-cell antigen receptor. *Proc. Natl. Acad. Sci. USA* **87**, 7020–7024.
68. Shivakumar, S., Tsokos, G. C., and Datta, S. K. (1989). T cell receptor alpha/beta expressing double-negative (CD4-/CD8-) and CD4+ T helper cells in humans augment the production of pathogenic anti-DNA autoantibodies associated with lupus nephritis. *J. Immunol.* **143**, 103–112.
69. Lanzavecchia, A. (1985). Antigen-specific interaction between T and B cells. *Nature (London)* **314**, 537–539.
70. Jenkins, M. K., Burrell, E., and Ashwell, J. D. (1990). Antigen presentation by resting B cells: Effectiveness at inducing T cell proliferation is determined by costimulatory signals, not T cell receptor occupancy. *J. Immunol.* **144**, 1585–1590.
71. Lin, R. H., Mamula, M. J., Hardin, J. A., and Janeway, C. A. (1991). Induction of autoreactive B cells allows priming of autoreactive T cells. *J. Exp. Med.* **173**, 1433–1439.
72. Mamula, M. J., and Janeway, C. A. (1993). Do B cells drive the diversification of immune responses? *Immunol. Today* **14**, 151–152.
73. Mamula, M. J., Fatenejad, S., and Craft, J. (1994). B cells process and present lupus autoantigens that initiate autoimmune T cell responses. *J. Immunol.* **152**, 1453–1461.
74. Eisenberg, R. A., Craven, S. Y., and Cohen, P. L. (1987). Isotype progression and clonality of anti-Sm autoantibodies in MRL/Mp-lpr/lpr mice. *J. Immunol.* **139**, 728–733.

75. Wofsy, D., and Seaman, W. E. (1985). Successful treatment of autoimmunity in NZB/NZW F1 mice with monoclonal antibody to L3T4. *J. Exp. Med.* **161**, 378–391.
76. Finck, B. K., Linsley, P. S., and Wofsy, D. (1994). Treatment of murine lupus with CTLA4Ig. *Science* **265**, 1225–1227.
77. Gilkeson, G. S., Spurney, R., Coffman, T. M., Kurlander, R., Ruiz, P., and Pisetsky, D. S. (1992). Effect of anti-CD4 antibody treatment on inflammatory arthritis in MRL-lpr/lpr mice. *Clin. Immunol. Immunopathol.* **64**, 166–172.
78. Sobel, E. S., Kakkanaiah, V. N., Kakkanaiah, M., Cheek, R. L., Cohen, P. L., and Eisenberg, R. A. (1994). T-B collaboration for autoantibody production in lpr mice is cognate and MHC-restricted. *J. Immunol.* **152**, 6011–6016.
79. Shlomchik, M., Mascelli, M., Shan, H., Radic, M. Z., Pisetsky, D., Marshak-Rothstein, A., and Weigert, M. (1990). Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. Exp. Med.* **171**, 265–298.
80. Bloom, D. D., Davignon, J. L., Retter, M. W., Shlomchik, M. J., Pisetsky, D. S., Cohen, P. L., Eisenberg, R. A., and Clarke, S. H. (1993). V region gene analysis of anti-Sm hybridomas from MRL/Mp-lpr/lpr mice. *J. Immunol.* **150**, 1591–1610.
81. Hoffman, R. W., Takeda, Y., Sharp, G. C., Lee, D. R., Hill, D. L., Kaneoka, H., and Caldwell, C. W. (1993). Human T cell clones reactive against U-small nuclear ribonucleoprotein autoantigens from connective tissue disease patients and healthy individuals. *J. Immunol.* **151**, 6460–6469.
82. Okubo, M., Yamamoto, K., Kato, T., Matsuura, N., Nishimaki, T., Kasukawa, R., Ito, K., Mizushima, Y., and Nishioka, K. (1993). Detection and epitope analysis of autoantigen-reactive T cells to the U1-small nuclear ribonucleoprotein A protein in autoimmune disease patients. *J. Immunol.* **151**, 1108–1115.
83. Crow, M. K., DelGuidice-Asch, G., Zehetbauer, J. B., Lawson, J. L., Brot, N., Weissbach, H., and Elkon, K. B. (1994). Autoantigen-specific T cell proliferation induced by the ribosomal P2 protein in patients with systemic lupus erythematosus. *J. Clin. Invest.* **94**, 345–352.
84. Theofilopoulos, A. N. (1995). The basis of autoimmunity. I. Mechanisms of aberrant self-recognition. *Immunol. Today* **16**, 90–98.
85. Von Boehmer, H. (1990). Developmental biology of T cells in T cell receptor transgenic mice. *Annu. Rev. Immunol.* **8**, 531–556.
86. Miller, J. F. A. P., and Flavell, R. A. (1994). T-cell tolerance and autoimmunity in transgenic models of central and peripheral tolerance. *Curr. Opin. Immunol.* **6**, 892–899.
87. Harding, F. A., McArthur, J. G., Gross, J. A., Raulet, D. H., and Allison, J. P. (1992). CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature (London)* **356**, 607–609.
88. Ashton Rickardt, P. G., Bandeira, A., Delaney, J. R., Van Kaer, L., Pircher, H. P., Zinkernagel, R. M., and Tonegawa, S. (1994). Evidence for a differential avidity model of T cell selection in the thymus. *Cell* **76**, 651–663.
89. Thompson, C. B. (1995). Distinct roles for the costimulatory ligands B7-1 and B7-2 in T helper cell differentiation. *Cell* **81**, 979–982.
90. McAdam, A. J., Schweitzer, A. N., and Sharpe, A. H. (1998). The role of B7 co-stimulation in activation and differentiation of CD4+ and CD8+ T cells. *Immunol. Rev.* **165**, 231–247.
91. Linsley, P. S., Brady, W., Grosmaire, L., Aruffo, A., Damle, N. K., and Ledbetter, J. A. (1991). Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J. Exp. Med.* **173**, 721–730.
92. Kuchroo, V. K., Das, M. P., Brown, J. A., Ranger, A. M., Zamvil, S. S., Sobel, R. A., Weiner, H. L., Nabavi, N., and Glimcher, L. H. (1995). B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: Application to autoimmune disease therapy. *Cell* **80**, 707–718.
93. Bluestone, J. A. (1997). Is CTLA-4 a master switch for peripheral T cell tolerance? *J. Immunol.* **158**, 1989–1993.
94. Greenwald, R. J., Boussiotis, V. A., Lorsbach, R. B., Abbas, A. K., and Sharpe, A. H. (2001). CTLA-4 regulates induction of anergy in vivo. *Immunity* **14**, 145–155.
95. Read, S., Malmstrom, V., and Powrie, F. (2000). Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J. Exp. Med.* **192**, 295–302.
96. Takahashi, T., Tagami, T., Yamazaki, S., Uede, T., Shimizu, J., Sakaguchi, N., Mak, T. W., and Sakaguchi, S. (2000). Immunologic self-tolerance maintained by CD25(+) CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J. Exp. Med.* **192**, 303–310.
97. Swallow, M. M., Wallin, J. J., and Sha, W. C. (1999). B7h, a novel costimulatory homolog of B7.1 and B7.2, is induced by TNFalpha. *Immunity* **11**, 423–432.
98. Yoshinaga, S. K., Whoriskey, J. S., Khare, S. D., Sarmiento, U., Guo, J., Horan, T., Shih, G., Zhang, M., Coccia, M. A., Kohno, T., *et al*. (1999). T-cell co-stimulation through B7RP-1 and ICOS. *Nature (London)* **402**, 827–832.
99. Freeman, G. J., Long, A. J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., Fitz, L. J., Malenkovich, N., Okazaki, T., Byrne, M. C., *et al*. (2000). Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* **192**, 1027–1034.
100. Latchman, Y., Wood, C. R., Chernova, T., Chaudhary, D., Borde, M., Chernova, I., Iwai, Y., Long, A. J., Brown, J. A., Nunes, R., *et al*. (2001). (PD-L2 is a second ligand for PD-I and inhibits T cell activation. *Nature Immunol.* **2**, 261–268.
101. Nishimura, H., Nose, M., Hiai, H., Minato, N., and Honjo, T. (1999). Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* **11**, 141–151.

102. Chapoval, A. I., Ni, J., Lau, J. S., Wilcox, R. A., Flies, D. B., Liu, D., Dong, H., Sica, G. L., Zhu, G., Tamada, K., *et al*. (2001). B7-H3: A costimulatory molecule for T cell activation and IFN-gamma production. *Nature Immunol.* **2**, 269–274.
103. Guerder, S., Picarella, D. E., Linsley, P. S., and Flavell, R. A. (1994). Costimulator B7-1 confers antigen-presenting-cell function to parenchymal tissue and in conjunction with tumor necrosis factor α leads to autoimmunity in transgenic mice. *Proc. Natl. Acad. Sci. USA* **91**, 5138–5142.
104. Weintraub, J. P., and Cohen, P. L. (1999). Ectopic expression of B7-1 (CD80) on T lymphocytes in autoimmune lpr and gld mice. *Clin. Immunol.* **91**, 302–309.
105. Shahinian, A., Pfeffer, K., Lee, K. P., Kundig, T. M., Kishihara, K., Wakeham, A., Kawai, K., Ohashi, P. S., Thompson, C. B., and Mak, T. W. (1993). Differential T cell costimulatory requirements in CD28-deficient mice. *Science* **261**, 609–612.
106. Bachmaier, K., Pummerer, C., Shahinian, A., Ionescu, J., Neu, N., Mak, T. W., and Penninger, J. M. (1996). Induction of autoimmunity in the absence of CD28 costimulation. *J. Immunol.* **157**, 1752–1757.
107. Ogawa, S., Nitta, K., Hara, Y., Horita, S., Nihei, H., and Abe, R. (2000). CD28 knockout mice as a useful clue to examine the pathogenesis of chronic graft-versus-host reaction. *Kidney Int.* **58**, 2215–2220.
108. Dong, C., Juedes, A. E., Temann, U. A., Shresta, S., Allison, J. P., Ruddle, N. H., and Flavell, R. A. (2001). ICOS co-stimulatory receptor is essential for T-cell activation and function. *Nature* (*London*) **409**, 97–101.
109. Tafuri, A., Shahinian, A., Bladt, F., Yoshinaga, S. K., Jordana, M., Wakeham, A., Boucher, L. M., Bouchard, D., Chan, V. S., Duncan, G., *et al*. (2001). ICOS is essential for effective T-helper-cell responses. *Nature (London)* **409**, 105–109.
110. McAdam, A. J., Greenwald, R. J., Levin, M. A., Chernova, T., Malenkovich, N., Ling, V., Freeman, G. J., and Sharpe, A. H. (2001). ICOS is critical for CD40-mediated antibody class switching. *Nature (London)* **409**, 102–105.
111. Kawabe, T., Naka, T., Yoshida, K., Tanaka, T., Fujiwara, H., Suematsu, S., Yoshida, N., Kishimoto, T., and Kikutani, H. (1994). The immune responses in CD40-deficient mice: Impaired immunoglobulin class switching and germinal center formation. *Immunity* **1**, 167–178.
112. Han, S., Hathcock, K., Zheng, B., Kepler, T. B., Hodes, R., and Kelsoe, G. (1995). Cellular interaction in germinal centers: Roles of CD40 ligand and B7-2 in established germinal centers. *J. Immunol.* **155**, 556–567.
113. Foy, T. M., Laman, J. D., Ledbetter, J. A., Aruffo, A., Claassen, E., and Noelle, R. J. (1994). gp39-CD40 interactions are essential for germinal center formation and the development of B cell memory. *J. Exp. Med.* **180**, 157–163.
114. Nishimura, H., Minato, N., Nakano, T., and Honjo, T. (1998). Immunological studies on PD-1 deficient mice: Implication of PD-1 as a negative regulator for B cell responses. *Int. Immunol.* **10**, 1563–1572.
115. Abdelmoula, M., Spertini, F., Shibata, T., Gyotoku, Y., Luzuy, S., Lambert, P. H., and Izui, S. (1989). IgG3 is the major source of cryoglobulins in mice. *J. Immunol.* **143**, 526–532.
116. Fulpius, T., Berney, T., Lemoine, R., Pastore, Y., Reininger, L., Brighouse, G., and Izui, S. (1994). Glomerulopathy induced by IgG3 anti-trinitrophenyl monoclonal cryoglobulins derived from nonautoimmune mice. *Kidney Int.* **45**, 962–971.
117. Mosmann, T. R., Cherwinski, H., Bond, M. W., Geidlin, M. A., and Coffman, R. L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**, 2348–2357.
118. Mosmann, T. R., and Sad, S. (1996). The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol. Today* **17**, 138–146.
119. Wahl, S. M. (1994). Transforming growth factor beta: The good, the bad, and the ugly. *J. Exp. Med.* **180**, 1587–1590.
120. Gilbert, K. M., Hoang, K. D., and Weigle, W. O. (1990). Th1 and Th2 clones differ in their response to a tolerogenic signal. *J. Immunol.* **144**, 2063–2071.
121. Sloan-Lancaster, J., Evavold, B. D., and Allen, P. M. (1994). Th2 cell clonal anergy as a consequence of partial activation. *J. Exp. Med.* **180**, 1195–1205.
122. McKnight, A. J., Perez, V. L., Shea, C. M., Gray, G. S., and Abbas, A. K. (1994). Costimulator dependence of lymphokine secretion by naive and activated CD4+ T lymphocytes from TCR transgenic mice. *J. Immunol.* **152**, 5220–5225.
123. Hayakawa, K., and Hardy, R. R. (2000). Development and function of B-1 cells. *Curr. Opin. Immunol.* **12**, 346–353.
124. Lam, K. P., and Rajewsky, K. (1999). B cell antigen receptor specificity and surface density together determine B-1 versus B-2 cell development. *J. Exp. Med.* **190**, 471–477.
125. Cyster, J. G., and Goodnow, C. C. (1997). Tuning antigen receptor signaling by CD22: Integrating clues from antigens and the microenvironment. *Immunity* **6**, 509–517.
126. D'Ambrosio, D., Hippen, K. L., Minskoff, S. A., Mellman, I., Pani, G., Siminovitch, K. A., and Cambier, J. C. (1995). Recruitment and activation of PTP1C in negative regulation of antigen receptor signaling by Fc gamma RIIB1. *Science* **268**, 293–297.
127. Rickert, R. C., Rajewsky, K., and Roes, J. (1995). Impairment of T-cell-dependent B-cell responses in B-1 cell development in CD19-deficient mice. *Nature (London)* **376**, 352–355.
128. Doody, G. M., Dempsey, P. W., and Fearon, D. T. (1996). Activation of B lymphocytes: Integrating signals from CD19, CD22 and Fc gamma RIIb1. *Curr. Opin. Immunol.* **8**, 378–382.
129. Banchereau, J., Bazan, F., Blanchard, D., Briere, F., Galizzi, J. P., van Kooten, C., Liu, Y. J., Rousset, F., and Saeland, S. (1994). The CD40 antigen and its ligand. *Annu. Rev. Immunol.* **12**, 881–922.

130. Crow, M. K., and Kirou, K. A. (2001). Regulation of CD40 ligand expression in systemic lupus erythematosus. *Curr. Opin. Rheumatol.* **13**, 361–369.
131. Goodnow, C. C., Cyster, J. G., Hartley, S. B., Bell, S. E., Cooke, M. P., Healy, J. I., Akkaraju, S., Rathmell, J. C., Pogue, S. L., and Shokat, K. P. (1995). Self-tolerance checkpoints in B lymphocyte development. *Adv. Immunol.* **59**, 279–368.
132. Qian, Y., Santiago, C., Borrero, M., Tedder, T. F., and Clarke, S. H. (2001). Lupus-specific antiribonucleoprotein B cell tolerance in nonautoimmune mice is maintained by differentiation to B-1 and governed by B cell receptor signaling thresholds. *J. Immunol.* **166**, 2412–2419.
133. Wang, H., and Shlomchik, M. J. (1999). Autoantigen-specific B cell activation in Fas-deficient rheumatoid factor immunoglobulin transgenic mice. *J. Exp. Med.* **190**, 639–649.
134. Erikson, J., Radic, M. Z., Camper, S. A., Hardy, R. R., Carmack, C., and Weigert, M. (1991). Expression of anti-DNA immunoglobulin transgenes in non-autoimmune mice. *Nature (London)* **349**, 331–334.
135. Chen, C., Nagy, Z., Radic, M. Z., Hardy, R. R., Huszar, D., Camper, S. A., and Weigert, M. (1995). The site and stage of anti-DNA B-cell deletion. *Nature (London)* **373**, 252–255.
136. Rathmell, J. C., and Goodnow, C. C. (1994). Effects of the lpr mutation on elimination and inactivation of self-reactive B cells. *J. Immunol.* **153**, 2831–2842.
137. Rubio, C. F., Kench, J., Russell, D. M., Yawger, R., and Nemazee, D. (1996). Analysis of central B cell tolerance in autoimmune-prone MRL/lpr mice bearing autoantibody transgenes. *J. Immunol.* **157**, 65–71.
138. Roark, J. H., Kuntz, C. L., Nguyen, K., Caton, A. J., and Erikson, J. (1995). Breakdown of B cell tolerance in a mouse model of systemic lupus erythematosus. *J. Exp. Med.* **181**, 1156–1167.
139. Murakami, M., Tsubata, T., Okamoto, M., Shimizu, A., Kumagai, S., Imura, H., and Honjo, T. (1992). Antigen-induced apoptotic death of Ly-1 B cells responsible for autoimmune disease in transgenic mice. *Nature (London)* **357**, 77–80.
140. Okamoto, M., Murakami, M., Shimizu, A., Ozaki, S., Tsubata, T., Kumagai, S., and Honjo, T. (1992). A transgenic model of autoimmune hemolytic anemia. *J. Exp. Med.* **175**, 71–79.
141. Mandik-Nayak, L., Seo, S., Sokol, C., Potts, K. M., Bui, A., and Erikson, J. (1999). MRL-*lpr*/*lpr* mice exhibit a defect in maintaining developmental arrest and follicular exclusion of anti-double-stranded DNA B cells. *J. Exp. Med.* **189**, 1799–1814.
142. O'Keefe, T. L., Williams, G. T., Batista, F. D., and Neuberger, M. S. (1999). Deficiency in CD22, a B cell-specific inhibitory receptor, is sufficient to predispose to development of high affinity autoantibodies. *J. Exp. Med.* **189**, 1307–1313.
143. Hibbs, M. L., Tarlinton, D. M., Armes, J., Grail, D., Hodgson, G., Maglitto, R., Stacker, S. A., and Dunn, A. R. (1995). Multiple defects in the immune system of *lyn*-deficient mice, culminating in autoimmune disease. *Cell* **83**, 301–311.
144. Wang, J., Koizumi, T., and Watanabe, T. (1996). Altered antigen receptor signaling and impaired Fas-mediated apoptosis of B cells in *Lyn*-deficient mice. *J. Exp. Med.* **184**, 831–838.
145. Westhoff, C. M., Whittier, A., Kathol, S., McHugh, J., Zajicek, C., Shultz, L. D., and Wylie, D. E. (1997). DNA-binding antibodies from viable motheaten mutant mice: Implications for B cell tolerance. *J. Immunol.* **159**, 3024–3033.
146. Shultz, L. D. (1988). Pleiotropic effects of deleterious alleles at the "motheaten" locus. *Curr. Top. Microbiol. Immunol.* **137**, 216–222.
147. Bolland, S., and Ravetch, J. V. (2000). Spontaneous autoimmune disease in Fc(gamma)RIIB-deficient mice results from strain-specific epistasis. *Immunity* **13**, 277–285.
148. Koshy, M., Berger, D., and Crow, M. K. (1996). Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J. Clin. Invest.* **98**, 826–837.
149. Desai-Mehta, A., Lu, L., Ramsey-Goldman, R., and Datta, S. K. (1996). Hyperexpression of CD40 ligand by B and T cells in human lupus and its role in pathogenic autoantibody production. *J. Clin. Invest.* **97**, 2063–2073.
150. Perez-Melgosa, M., Hollenbaugh, D., and Wilson, C. B. (1999). CD40 ligand is a limiting factor in the humoral response to T cell-dependent antigens. *J. Immunol.* **163**, 1123–1127.
151. Early, G. S., Zhao, W., and Burns, C. M. (1996). Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand black x New Zealand white mice. *J. Immunol.* **157**, 3159–3164.
152. Kalled, S. L., Cutler, A. H., Datta, S. K., and Thomas, D. W. (1998). Anti-CD40 ligand antibody treatment of SNF_1 mice with established nephritis: preservation of kidney function. *J. Immunol.* **160**, 2158–2165.
153. Daikh, D. I., Finck, B. K., Linsley, P. S., Hollenbaugh, D., and Wofsy, D. (1997). Long-term inhibition of murine lupus by brief simultaneous blockade of the B7/CD28 and CD40/gp39 costimulation pathways. *J. Immunol.* **159**, 3104–3108.
154. Kawai, T., Andrews, D., Colvin, R. B., Sachs, D. H., and Cosimi, A. B. (2000). Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nature Med.* **6**, 114.
155. Banchereau, J., and Steinman, R. M. (1998). Dendritic cells and the control of immunity. *Nature (London)* **392**, 245–252.
156. Palucka, K., and Banchereau, J. (1999). Linking innate and adaptive immunity. *Nature Med.* **5**, 868–870.
157. Cyster, J. G. (1999). Chemokines and the homing of dendritic cells to the T cell areas of lymphoid organs. *J. Exp. Med.* **189**, 447–450.
158. Inaba, K., Turley, S., Yamaide, F., Iyoda, T., Mahnke, K., Inaba, M., Pack, M., Subklewe, M., Sauter, B., Sheff, D., *et al*. (1998). Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex

class II products of dendritic cells. *J. Exp. Med.* **188**, 2163–2173.
159. Reis e Sousa, C., Hieny, S., Scharton-Kersten, T., Jankovic, D., Charest, H., Germain, R. N., and Sher, A. (1997). *In vivo* microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J. Exp. Med.* **186**, 18189–11829.
160. Steinman, R. M., Turley, S., Mellman, I., and Inaba, K. (2000). The induction of tolerance by dendritic cells that have captured apoptotic cells. *J. Exp. Med.* **191**, 411–416.
161. Hawiger, D., Inaba, K., Dorsett, Y., Guo, M., Mahnke, K., Rivera, M., Ravetch, J. V., Steinman, R. M., and Nussenzweig, M. C. (2001). Dendritic cells induce peripheral T cell unresponsiveness under steady state conditions *in vivo*. *J. Exp. Med.* **194**, 769–779.
162. Rissoan, M. C., Soumelis, V., Kadowaki, N., Grouard, G., Briere, F., de Waal, M. R., and Liu, Y. J. (1999). Reciprocal control of T helper cell and dendritic cell differentiation. *Science* **283**, 1183–1186.
163. Vieira, P. L., de Jong, E. C., Wierenga, E. A., Kapsenberg, M. L., and Kalinski, P. (2000). Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J. Immunol.* **164**, 4507–4512.
164. Pulendran, B., Smith, J. L., Caspary, G., Brasel, K., Pettit, D., Maraskovsky, E., and Maliszewski, C. R. (1999). Distinct dendritic cell subsets differentially regulate the class of immune response *in vivo*. *Proc. Natl. Acad. Sci. USA* **96**, 1036–1041.
165. Scheinecker, C., Zwolfer, B., Koller, M., Manner, G., and Smolen, J. S. (2001). Alterations of dendritic cells in systemic lupus erythematosus: phenotypic and functional deficiencies. *Arthritis Rheum.* **44**, 856–865.
166. Satoh, M., Weintraub, J. P., Yoshida, H., Shaheen, V. M., Richards, H. B., Shaw, M., and Reeves, W. H. (2000). Fas and Fas ligand mutations inhibit autoantibody production in pristane-induced lupus. *J. Immunol.* **165**, 1036–1043.
167. Richards, H. B., Satoh, M., Shaw, M., Libert, C., Poli, V., and Reeves, W. H. (1998). IL-6 dependence of anti-DNA antibody production: Evidence for two pathways of autoantibody formation in pristane-induced lupus. *J. Exp. Med.* **188**, 985–990.
168. Richards, H. B., Satoh, M., Jennette, J. C., Croker, B. P., and Reeves, W. H. (2001). Interferon-gamma promotes lupus nephritis in mice treated with the hydrocarbon oil pristane. *Kidney Int.* **60**, 2173–2180.
169. Medzhitov, R., and Janeway, C. A. (1997). Innate immunity: The virtues of a nonclonal system of recognition. *Cell* **91**, 295–298.
170. Grouard, G., Rissoan, M. C., Filgueira, L., Durand, I., Banchereau, J., and Liu, Y. J. (1997). The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J. Exp. Med.* **185**, 1101–1111.
171. Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A., and Colonna, M. (1999). Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nature Med.* **5**, 919–923.
172. Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., Antonenko, S., and Liu, Y. J. (1999). The nature of the principal type 1 interferon-producing cells in human blood. *Science* **284**, 1835–1837.
173. Marrack, P., Kappler, J., and Mitchell, T. (1999). Type I interferons keep activated T cells alive. *J. Exp. Med.* **189**, 521–529.
174. Fiorani, C., Sacchi, S., Bonacorsi, G., and Cosenza, M. (2000). Systemic sarcoidosis associated with interferon-alpha treatment for chronic myelogenous leukemia. *Haematologica* **85**, 1006–1007.
175. Dumoulin, F. L., Leifeld, L., Sauerbruch, T., and Spengler, U. (1999). Autoimmunity induced by interferon-alpha therapy for chronic viral hepatitis. *Biomed. Pharmacother.* **53**, 242–254.
176. Ioannou, Y., and Isenberg, D. A. (2000). Current evidence for the induction of autoimmune rheumatic manifestations by cytokine therapy. *Arthritis Rheum.* **43**, 1431–1442.
177. Kalkner, K. M., Ronnblom, L., Karlsson Parra, A. K., Bengtsson, M., Olsson, Y., and Oberg, K. (1998). Antibodies against double-stranded DNA and development of polymyositis during treatment with interferon. *Q. J. Med.* **91**, 393–399.
178. Ronnblom, L. E., Alm, G. V., and Oberg, K. E. (1991). Autoimmunity after alpha-interferon therapy for malignant carcinoid tumors. *Ann. Intern. Med.* **115**, 178–183.
179. Kim, T., Kanayama, Y., Negoro, N., Okamura, M., Takeda, T., and Inoue, T. (1987). Serum levels of interferons in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **70**, 562–569.
180. Hooks, J. J., Moutsopoulos, H. M., Geis, S. A., Stahl, N. I., Decker, J. L., and Notkins, A. L. (1979). Immune interferon in the circulation of patients with autoimmune disease. *N. Engl. J. Med.* **301**, 5–8.
181. Preble, O. T., Black, R. J., Friedman, R. M., Klippel, J. H., and Vilcek, J. (1982). Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. *Science* **216**, 429–431.
182. Blanco, P., Palucka, A. K., Gill, M., Pascual, V., and Banchereau, J. (2001). Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* **294**, 1540–1543.
183. Stauffer, Y., Marguerat, S., Meylan, F., Ucla, C., Sutkowski, N., Huber, B., Pelet, T., and Conrad, B. (2001). Interferon-alpha-induced endogenous superantigen: A model linking environment and autoimmunity. *Immunity* **15**, 591–601.
184. Savill, J., Fadok, V., Henson, P., and Haslett, C. (1993). Phagocyte recognition of cells undergoing apoptosis. *Immunol. Today* **14**, 131–136.
185. Henson, P. M., Bratton, D. L., and Fadok, V. A. (2001). The phosphatidylserine receptor: A crucial molecular switch? *Nature Rev. Mol. Cell Biol.* **2**, 627–633.
186. Fadok, V. A., Bratton, D. L., and Henson, P. M. (2001). Phagocyte receptors for apoptotic cells: recognition, uptake, and consequences. *J. Clin. Invest* **108**, 957–962.

187. Cohen, P. L., and Eisenberg, R. A. (1991). *Lpr* and *gld*: Single gene models of systemic autoimmunity and lymphoproliferative disease. *Annu. Rev. Immunol.* **9**, 243–269.
188. Bickerstaff, M. C., Botto, M., Hutchinson, W. L., Herbert, J., Tennent, G. A., Bybee, A., Mitchell, D. A., Cook, H. T., Butler, P. J., Walport, M. J., *et al*. (1999). Serum amyloid P component controls chromatin degradation and prevents antinuclear autoimmunity. *Nature Med.* **5**, 694–697.
189. Botto, M., Dell'Agnola, C., Bygrave, A. E., Thompson, E. M., Cook, H. T., Petry, F., Loos, M., Pandolfi, P. P., and Walport, M. J. (1998). Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature Gene.* **19**, 56–59.
190. Scott, R. S., McMahon, E. J., Pop, S. M., Reap, E. A., Caricchio, R., Cohen, P. L., Earp, H. S., and Matsushima, G. K. (2001). Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature (London)* **411**, 207–211.
191. Pisetsky, D. S. (2000). Tumor necrosis factor alpha blockers and the induction of anti-DNA autoantibodies. *Arthritis Rheum.* **43**, 2381–2382.
192. Charles, P. J., Smeenk, R. J., de Jong, J., Feldmann, M., and Maini, R. N. (2000). Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: Findings in open-label and randomized placebo-controlled trials. *Arthritis Rheum.* **43**, 2383–2390.
193. Jacob, C. O., and McDevitt, H. O. (1988). Tumour necrosis factor alpha in murine autoimmune 'lupus' nephritis. *Nature (London)* **331**, 356–358.
194. Kontoyiannis, D., and Kollias, G. (2000). Accelerated autoimmunity and lupus nephritis in NZB mice with an engineered heterozygous deficiency in tumor necrosis factor. *Eur. J. Immunol.* **30**, 2038–2047.
195. Jacob, C. O., Lee, S. K., and Strassmann, G. (1996). Mutational analysis of TNF-alpha gene reveals a regulatory role for the 3′-untranslated region in the genetic predisposition to lupus-like autoimmune disease. *J. Immunol.* **156**, 3043–3050.
196. Zhou, T., Edwards, C. K., Yang, P., Wang, Z., Bluethmann, H., and Mountz, J. D. (1996). Greatly accelerated lymphadenopathy and autoimmune disease in *lpr* mice lacking tumor necrosis factor receptor I. *J. Immunol.* **156**, 2661–2665.
197. Sullivan, K. E., Wooten, C., Schmeckpeper, B. J., Goldman, D., and Petri, M. A. (1997). A promoter polymorphism of tumor necrosis factor alpha associated with systemic lupus erythematosus in African-Americans. *Arthritis Rheum.* **40**, 2207–2211.
198. Morita, C., Horiuchi, T., Tsukamoto, H., Hatta, N., Kikuchi, Y., Arinobu, Y., Otsuka, T., Sawabe, T., Harashima, S., Nagasawa, K., *et al*. (2001). Association of tumor necrosis factor receptor type II polymorphism 196R with systemic lupus erythematosus in the Japanese: Molecular and functional analysis. *Arthritis Rheum.* **44**, 2819–2827.
199. Gross, J. A., Johnston, J., Mudri, S., Enselman, R., Dillon, S. R., Madden, K., Xu, W., Parrish-Novak, J., Foster, D., Lofton-Day, C., *et al*. (2000). TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature (London)* **404**, 995–999.
200. Wu, Y., Bressette, D., Carrell, J. A., Kaufman, T., Feng, P., Taylor, K., Gan, Y., Cho, Y. H., Garcia, A. D., Gollatz, E., *et al*. (2000). Tumor necrosis factor (TNF) receptor superfamily member TACI is a high affinity receptor for TNF family members APRIL and BLyS. *J. Biol. Chem.* **275**, 35478–35485.
201. Zhang, J., Roschke, V., Baker, K. P., Wang, Z., Alarcon, G. S., Fessler, B. J., Bastian, H., Kimberly, R. P., and Zhou, T. (2001). Cutting edge: A role for B lymphocyte stimulator in systemic lupus erythematosus. *J. Immunol.* **166**, 6–10.
202. von Bulow, G. U., van Deursen, J. M., and Bram, R. J. (2001). Regulation of the T-independent humoral response by TACI. *Immunity* **14**, 573–582.
203. Llorentae, L., Zou, W., Levy, Y., Richaud-Patin, Y., Wijdenes, J., Alcocer-Varela, J., Morel-Fourrier, B., Brouet, J., Alarcon-Segovia, D., Galanaud, P., *et al*. (1995). Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med.* **181**, 839–844.
204. Ishida, H., Muchamuel, T., Sakaguchi, S., Andrade, S., Menon, S., and Howard, M. (1994). Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* **179**, 305–310.
205. Mihara, M., and Ohsugi, Y. (1990). Possible role of IL-6 in pathogenesis of immune complex-mediated glomerulonephritis in NZB/W F1 mice: Induction of IgG class anti- DNA autoantibody production. *Int. Arch. Allergy Appl. Immunol.* **93**, 89–92.
206. Jourdan, M., Bataille, R., Seguin, J., Zhang, X. G., Chaptal, P. A., and Klein, B. (1990). Constitutive production of interleukin-6 and immunologic features in cardiac myxomas. *Arthritis Rheum.* **33**, 398–402.
207. Nanki, T., Tomiyama, J., and Arai, S. (1994). Mixed connective tissue disease associated with multicentric Castleman's disease. *Scand. J. Rheumatol.* **23**, 215–217.
208. Lebedeva, T. V., and Singh, A. K. (1995). Increased responsiveness of B cells in the murine MRL/Ipr model of lupus nephritis to interleukin-1β. *J. Am. Soc. Nephrol.* **5**, 1530–1534.
209. Singh, A. K., Mao, C., and Lebedeva, T. V. (1994). *In vitro* role of IL-1 in heightened IgG, anti-DNA, and nephritogenic idiotype production by B cells derived from the murine MRL/lpr lupus model. *Clin. Immunol. Immunopathol.* **72**, 410–415.
210. Brennan, D. C., Yui, M. A., Wuthrich, R. P., and Kelley, V. E. (1989). Tumor necrosis factor and IL-1 in New Zealand black/white mice: Enhanced gene expression and acceleration of renal injury. *J. Immunol.* **143**, 3470–3475.
211. Horai, R., Saijo, S., Tanioka, H., Nakae, S., Sudo, K., Okahara, A., Ikuse, T., Asano, M., and Iwakura, Y. (2000). Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J. Exp. Med.* **191**, 313–320.

212. Huang, F. P., Feng, G. J., Lindop, G., Stott, D. I., and Liew, F. Y. (1996). The role of interleukin 12 and nitric oxide in the development of spontaneous autoimmune disease in MRL/MP-lpr/lpr mice. *J. Exp. Med.* **183**, 1447–1459.
213. Esfandiari, E., McInnes, I. B., Lindop, G., Huang, F. P., Field, M., Komai-Koma, M., Wei, X., and Liew, F. Y. (2001). A proinflammatory role of IL-18 in the development of spontaneous autoimmune disease. *J. Immunol.* **167**, 5338–5347.
214. Romagnani, S. (1994). Lymphokine production by human T cells in disease states. *Annu. Rev. Immunol.* **12**, 227–257.
215. Goldman, M., Druet, P., and Gleichmann, E. (1991). TH2 cells in systemic autoimmunity: Insights from allogeneic diseases and chemically-induced autoimmunity. *Immunol. Today* **12**, 223–227.
216. Herron, L. R., Coffman, R. L., Bond, M. W., and Kotzin, B. L. (1988). Increased autoantibody production by NZB/NZW B cells in response to IL-5. *J. Immunol.* **141**, 842–848.
217. Erb, K. J., Ruger, B., von Brevern, M., Ryffel, B., Schimpl, A., and Rivett, K. (1997). Constitutive expression of interleukin (IL)-4 *in vivo* causes autoimmune-type disorders in mice. *J. Exp. Med.* **185**, 329–339.
218. Santiago, M. L., Fossati, L., Jacquet, C., Muller, W., Izui, S., and Reininger, L. (1997). Interleukin-4 protects against a genetically linked lupus-like autoimmune syndrome. *J. Exp. Med.* **185**, 65–70.
219. Eisenberg, R. A., Winfield, J. B., and Cohen, P. L. (1982). Subclass restriction of anti-Sm antibodies in MRL mice. *J. Immunol.* **129**, 2146–2149.
220. Seery, J. P., Carroll, J. M., Cattell, V., and Watt, F. M. (1997). Antinuclear autoantibodies and lupus nephritis in transgenic mice expressing interferon gamma in the epidermis. *J. Exp. Med.* **186**, 1451–1459.
221. Peng, S. L., Moslehi, J., and Craft, J. (1997). Roles of interferon-gamma and interleukin-4 in murine lupus. *J. Clin. Invest.* **99**, 1936–1946.
222. Haas, C., and Le Hir, M. (1997). IFN-gamma is essential for the development of autoimmune glomerulonephritis in MRL/lpr mice. *J. Immunol.* **158**, 5484–5491.
223. Balomenos, D., Rumold, R., and Theofilopoulos, A. N. (1998). Interferon-gamma is required for lupus-like disease and lymphoaccumulation in MRL-lpr mice. *J. Clin. Invest.* **101**, 364–371.
224. Haas, C., Ryffel, B., and Le Hir, M. (1998). IFN-gamma receptor deletion prevents autoantibody production and glomerulonephritis in lupus-prone (NZB X NZW)F1 mice. *J. Immunol.* **160**, 3713–3718.
225. Jacob, C. O., van der Meide, P. H., and McDevitt, H. O. (1987). *In vivo* treatment of (NZB X NZW)F1 lupus-like nephritis with monoclonal antibody to gamma interferon. *J. Exp. Med.* **166**, 798–803.
226. Ozmen, L., Roman, D., Fountoulakis, M., Schmid, G., Ryffel, B., and Garotta, G. (1994). Soluble interferon-gamma receptor: A therapeutically useful drug for systemic lupus erythematosus. *J. Interferon Res.* **14**, 283–284.
227. Heremans, H., Billan, A., Colombatti, A., Hilders, J., and DeSomer, P. (1978). Interferon treatment of NZB mice: accelerated progression of autoimmune disease. *Infect. Immun.* **21**, 925.
228. Pearce, D. C., Yount, W. J., and Eisenberg, R. A. (1986). Subclass restriction of anti-SS-B (La) autoantibodies. *Clin. Immunol. Immunopathol.* **38**, 111–119.
229. Stavnezer, J. (1996). Antibody class switching. *Adv. Immunol.* **61**, 79–146.
230. Nakashima, H., Inoue, H., Akahoshi, M., Tanaka, Y., Yamaoka, K., Ogami, E., Nagano, S., Arinobu, Y., Niiro, H., Otsuka, T., *et al*. (1999). The combination of polymorphisms within interferon-gamma receptor 1 and receptor 2 associated with the risk of systemic lupus erythematosus. *FEBS Lett.* **453**, 187–190.
231. Leohirun, L., Thuvasethakul, P., Sumethkul, V., Pholcharoen, T., and Boonpucknavig, V. (1991). Urinary neopterin in patients with systemic lupus erythematosus. *Clin. Chem.* **37**, 47–50.
232. Lim, K. L., Muir, K., and Powell, R. J. (1994). Urine neopterin: A new parameter for serial monitoring of disease activity in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **53**, 743–748.
233. Hagiwara, E., Gourley, M. F., Lee, S., and Klinman, D. K. (1996). Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10: Interferon-gamma-secreting cells in the peripheral blood. *Arthritis Rheum.* **39**, 379–385.
234. Horwitz, D. A., Gray, J. D., Behrendsen, S. C., Kubin, M., Rengaraju, M., Ohtsuka, K., and Trinchieri, G. (1998). Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupus erythematosus. *Arthritis Rheum.* **41**, 838–844.
235. Houssiau, F. A., Lefebvre, C., Vanden Berghe, M., Lambert, M., Devogelaer, J. P., and Renauld, J. C. (1995). Serum interleukin 10 titers in systemic lupus erythematosus reflect disease activity. *Lupus* **4**, 393–395.
236. Mongan, A. E., Ramdahin, S., and Warrington, R. J. (2000). Interleukin-10 response abnormalities in systemic lupus erythematosus. *Scand. J. Immunol.* **46**, 406–412.
237. Wilson, B. M. G., Severn, A., Rapson, N. T., Chana, J., and Hopkins, P. (1991). A convenient human whole blood culture system for studying the regulation of tumour necrosis factor release by bacterial lipopolysaccharide. *J. Immunol. Methods* **139**, 233–240.
238. Seitz, M., Franke, M., and Kirchner, H. (1988). Induction of antinuclear antibodies in patients with rheumatoid arthritis receiving treatment with human recombinant interferon gamma. *Ann. Rheum. Dis.* **47**, 642–644.
239. Graninger, W. B., Hassfeld, W., Pesau, B. B., Machold, K. P., Zielinski, C. C., and Smolen, J. S. (1991). Induction of systemic lupus erythematosus by interferon-gamma in a patient with rheumatoid arthritis. *J. Rheumatol.* **18**, 1621–1622.
240. Machold, K. P., and Smolen, J. S. (1990). Interferon-gamma induced exacerbation of systemic lupus erythematosus. *J. Rheumatol.* **17**, 831–832.

241. Brett, S. J., Cease, K. B., and Berzofsky, J. A. (1988). Influences of antigen processing on the expression of the T cell repertoire: Evidence for MHC-specific hindering structures on the products of processing. *J. Exp. Med.* **168**, 357–373.
242. Buus, S., Sette, A., Colon, S. M., Miles, C., and Grey, H. M. (1987). The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. *Science* **235**, 1353–1358.
243. Shivakumar, S., Sercarz, E. E., and Krzych, U. (1989). The molecular context of determinants within the priming antigen establishes a hierarchy of T cell induction: T cell specificities induced by peptides of beta-galactosidase vs. the whole antigen. *Eur. J. Immunol.* **19**, 681–687.
244. Glimcher, L. H., Schroer, J. A., Chan, C., and Shevach, E. M. (1983). Fine specificity of cloned insulin-specific T cell hybridomas: Evidence supporting a role for tertiary conformation. *J. Immunol.* **131**, 2868–2874.
245. Atassi, M. Z., Yoshioka, M., and Bixler, G. S. (1989). T cells specific for alpha-beta interface regions of hemoglobin recognize the isolated subunit but not the tetramer and indicate presentation without processing. *Proc. Natl. Acad. Sci. USA* **86**, 6729–6733.
246. Rouas, N., Christophe, S., Housseau, F., Bellet, D., Guillet, J. G., and Bidart, J. M. (1993). Influence of protein-quaternary structure on antigen processing. *J. Immunol.* **150**, 782–792.
247. Adorini, L., Appella, E., Doria, G., and Nagy, Z. A. (1988). Mechanisms influencing the immunodominance of T cell determinants. *J. Exp. Med.* **168**, 2091–2104.
248. Mueller, D. L., Jenkins, M. K., and Schwartz, R. H. (1989). Clonal expansion versus functional clonal inactivation: A costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. *Annu. Rev. Immunol.* **7**, 445–480.
249. Gammon, G., and Sercarz, E. (1989). How some T cells escape tolerance induction. *Nature (London)* **342**, 183–185.
250. Cibotti, R., Kanellopoulos, J. M., Cabaniols, J. P., Halle-Panenko, O., Kosmatopoulos, K., Sercarz, E., and Kourilsky, P. (1992). Tolerance to a self protein involves its immunodominant but does not involve its subdominant determinants. *Proc. Natl. Acad. Sci. USA* **89**, 416–420.
251. Dong, X., Hamilton, K. J., Satoh, M., Wang, J., and Reeves, W. H. (1994). Initiation of autoimmunity to the p53 tumor suppressor protein by complexes of p53 and SV40 large T antigen. *J. Exp. Med.* **179**, 1243–1252.
252. Griem, P., and Gleichmann, E. (1995). Metal ion induced autoimmunity. *Curr. Opin. Immunol.* **7**, 831–838.
253. Bargonetti, J., Reynisdottir, I., Friedman, P. N., and Prives, C. (1992). Site-specific binding of wild-type p53 to cellular DNA is inhibited by SV40 T antigen and mutant p53. *Genes Dev.* **6**, 1886–1898.
254. Oren, M., Maltzman, W., and Levine, A. J. (1981). Post-translational regulation of the 54K cellular tumor antigen in normal and transformed cells. *Mol. Cell. Biol.* **1**, 101–110.
255. Reich, N. C., Oren, M., and Levine, A. J. (1983). Two distinct mechanisms regulate the levels of a cellular tumor antigen, p53. *Mol. Cell. Biol.* **3**, 2143–2150.
256. Lane, D. P., and Crawford, L. V. (1979). T antigen bound to a host protein in SV40-transformed cells. *Nature (London)* **278**, 261–263.
257. Linzer, D. I. H., and Levine, A. J. (1979). Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* **17**, 43–52.
258. Kaplan, M. E., and Tan, E. M. (1968). Antinuclear antibodies in infectious mononucleosis. *Lancet* **1**, 561–563.
259. Wolfgram, L. J., Beisel, K. W., and Rose, N. R. (1985). Heart-specific autoantibodies following murine coxsackievirus B3 myocarditis. *J. Exp. Med.* **161**, 1112–1121.
260. Ziemiecki, A., Kromer, G., Mueller, R. G., Hala, K., and Wick, G. (1988). ev22, a new endogenous avian leukosis virus locus found in chickens with spontaneous autoimmune thyroiditis. *Arch. Virol.* **100**, 267–271.
261. Onodera, T., Toniolo, A., Ray, U. R., Jenson, A. B., Knazek, R. A., and Notkins, A. L. (1981). Virus-induced diabetes mellitus. XX. Polyendocrinopathy and autoimmunity. *J. Exp. Med.* **153**, 1457–1473.
262. Hultman, P., Bell, L. J., Enestrom, S., and Pollard, K. M. (1992). Murine susceptibility to mercury. I. Autoantibody profiles and systemic immune deposits in inbred, congenic, and intra-H-2 recombinant strains. *Clin. Immunol. Immunopathol.* **65**, 98–109.
263. Reuter, R., Tessars, G., Vohr, H. W., Gleichmann, E., and Luhrmann, R. (1989). Mercuric chloride induces autoantibodies agains U3 small nuclear ribonucleoprotein in susceptible mice. *Proc. Natl. Acad. Sci. USA* **86**, 237–241.
264. Okano, Y., Steen, V. D., and Medsger, T. A. J. (1992). Autoantibody to U3 nucleolar ribonucleoprotein (fibrillarin) in patients with systemic sclerosis. *Arthritis Rheum.* **35**, 95–100.
265. Pollard, K. M., Lee, D. K., Casiano, C. A., Bluthner, M., Johnston, M. M., and Tan, E. M. (1997). The autoimmunity-inducing xenobiotic mercury interacts with the autoantigen fibrillarin and modifies its molecular and antigenic properties. *J. Immunol.* **158**, 3521–3528.
266. Andrade, F., Roy, S., Nicholson, D., Thornberry, N., Rosen, A., and Casciola-Rosen, L. (1998). Granzyme B directly and efficiently cleaves several downstream caspase substrates: Implications for CTL-induced apoptosis. *Immunity* **8**, 451–460.
267. Casciola-Rosen, L. A., Anhalt, G. J., and Rosen, A. (1995). DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J. Exp. Med.* **182**, 1625–1634.
268. Casciola-Rosen, L., Wigley, F., and Rosen, A. (1997). Scleroderma autoantigens are uniquely fragmented by metal-catalyzed oxidation reactions: Implications for pathogenesis. *J. Exp. Med.* **185**, 71–79.

269. Peace, D. J., Chen, W., Nelson, H., and Cheever, M. A. (1991). T cell recognition of transforming proteins encoded by mutated ras proto-oncogenes. *J. Immunol.* **146**, 2059–2065.
270. Reeves, W. H. (2001). Tumor immunity and autoimmunity: A case of Dr. Jekyll and Mr. Hyde. *Clin. Immunol.* **100**, 129–133.
271. Sorokine, I., Ben, M. K., Bracone, A., Thierry, D., Ishii, S., Imamoto, F., and Kohiyama, M. (1991). Presence of circulating c-myb oncogene product antibodies in human sera. *Int. J. Cancer* **47**, 665–669.
272. Ben-Mahrez, K., Sorokine, I., Thierry, D., Kawasumi, T., Ishir, S. R. S., and Kohiyama, M. (1990). Circulating antibodies against c-myc oncogene product in sera of colorectal cancer patients. *Int. J. Cancer* **46**, 35–38.
273. Disis, M. L., Calenoff, E., McLaughlin, G., Murphy, A. E., Chen, W., Groner, B., Jeschke, M., Lydon, N., McGlynn, E., Livingston, R. B., *et al*. (1994). Existent T-cell and antibody immunity to HER-2/neu protein in patients with breast cancer. *Cancer Res.* **54**, 16–20.
274. van Denderen, J., Hermans, A., Meeuwsen, T., Troelstra, C., Zegers, N., Boersma, W., Grosveld, G., and van Ewijk, W. (1989). Antibody recognition of the tumor-specific bcr-abl joining region in chronic myeloid leukemia. *J. Exp. Med.* **169**, 87–98.
275. DeLeo, A. B., Jay, G., Appella, E., DuBois, G. C., Law, L. W., and Old, L. J. (1979). Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc. Natl. Acad. Sci. USA* **76**, 2420–2424.
276. Schlichtholz, B., Legros, Y., Gillet, D., Gaillard, C., Marty, M., Lane, D., Calvo, F., and Soussi, T. (1992). The immune response to p53 in breast cancer patients is directed against immunodominant epitopes unrelated to the mutational hot spot. *Cancer Res.* **52**, 6380–6384.
277. Davidoff, A. M., Iglehart, J. D., and Marks, J. R. (1992). Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers. *Proc. Natl. Acad. Sci. USA* **89**, 3439–3442.
278. Winter, S. F., Minna, J. D., Johnson, B. E., Takahashi, T., Gazdar, A. F., and Carbone, D. P. (1992). Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. *Cancer Res.* **52**, 4168–4174.
279. Oldstone, M. B. A. (1987). Molecular mimicry and autoimmune disease. *Cell* **50**, 819–820.
280. Fujinami, R. S., Oldstone, M. B. A., Wroblewska, Z., Frankel, M. E., and Koprowski, H. (1983). Molecular mimicry in virus infection: Crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. *Proc. Natl. Acad. Sci. USA* **80**, 2346–2350.
281. Rhodes, G., Rumpold, H., Kurki, P., Patrick, K. M., Carson, D. A., and Vaughan, J. H. (1987). Autoantibodies in infectious mononucleosis have specificity for the glycine-alanine repeating region of the Epstein-Barr virus nuclear antigen. *J. Exp. Med.* **165**, 1026–1040.
282. Hermann, H., Fabrizio, P., Raker, V. A., Foulaki, K., Hornig, H., Brahms, H., and Luhrmann, R. (1995). snRNP Sm proteins share two evolutionarily conserved sequence motifs which are involved in Sm protein–protein interactions. *EMBO J.* **14**, 2076–2088.
283. Habets, W. J., Sillekens, P. T. G., Hoet, M. H., McAllister, G., Lerner, M. R., and van Venrooij, W. J. (1989). Small nuclear RNA-associated proteins are immunologically related as revealed by mapping of autoimmune reactive B-cell epitopes. *Proc. Natl. Acad. Sci. USA* **86**, 4674–4678.
284. Hirakata, M., Craft, J., and Hardin, J. A. (1993). Autoantigenic epitopes of the B and D polypeptides of the U1 snRNP: Analysis of domains recognized by the Y12 monoclonal anti-Sm antibody and by patient sera. *J. Immunol.* **150**, 3592–3601.
285. Kanai, Y., Kawaminami, Y., Miwa, M., Matsushima, T., Sugimura, T., Moroi, Y., and Yokohari, R. (1977). Naturally occurring antibodies to poly(ADP-ribose) in patients with systemic lupus erythematosus. *Nature (London)* **265**, 175–177.
286. Straus, S. E., Lenardo, M., and Puck, J. M. (1997). The Canale-Smith syndrome. *N. Engl. J. Med.* **336**, 1457–1458.
287. Straus, S. E., Sneller, M., Lenardo, M. J., Puck, J. M., and Strober, W. (1999). An inherited disorder of lymphocyte apoptosis: The autoimmune lymphoproliferative syndrome. *Ann. Intern. Med.* **130**, 591–601.
288. Wang, J., Zheng, L., Lobito, A., Chan, F. K., Dale, J., Sneller, M., Yao, X., Puck, J. M., Straus, S. E., and Lenardo, M. J. (1999). Inherited human caspase 10 mutations underlie defective lymphocyte and dendritic cell apoptosis in autoimmune lymphoproliferative syndrome type II. *Cell* **98**, 47–58.
289. Nagata, S. (1998). Human autoimmune lymphoproliferative syndrome, a defect in the apoptosis-inducing Fas receptor: A lesson from the mouse model. *J. Hum. Genet.* **43**, 2–8.
290. Masi, A. T., Rodnan, G. P., Medsger, T. A., Altman, R. D., D'Angelo, W. A., and Fries, J. F. (1980). Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee: Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum.* **23**, 581–590.
291. Hinterberger, M., Pettersson, I., and Steitz, J. A. (1983). Isolation of small nuclear ribonucleoproteins containing U1, U2, U4, U5, and U6 RNAs. *J. Biol. Chem.* **258**, 2604–2613.
292. Kinlaw, C. S., Robberson, B. L., and Berget, S. M. (1983). Fractionation and characterization of human small nuclear ribonucleoproteins containing U1 and U2 RNAs. *J. Biol. Chem.* **258**, 7181–7189.
293. Okano, Y., Targoff, I. N., Oddis, C. V., Fujii, T., Kuwana, M., Tsuzaka, K., Hirakata, M., Mimori, T., Craft, J., and Medsger, T. A. J. (1996). Anti-U5 small nuclear ribonucleoprotein (snRNP) antibodies: A rare anti-U snRNP specificity. *Clin. Immunol. Immunopathol.* **81**, 41–47.

294. Okano, Y., and Medsger, T. A. (1991). Newly identified U4/U6 snRNP-binding proteins by serum autoantibodies from a patient with systemic sclerosis. *J. Immunol.* **146**, 535–542.
295. Tivol, E. A., Borriello, F., Schweitzer, A. N., Lynch, W. P., Bluestone, J. A., and Sharpe, A. H. (1995). Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* **3**, 541–547.
296. Nishizumi, H., Taniuchi, I., Yamanashi, Y., Kitamura, D., Ilic, D., Mori, S., Watanabe, T., and Yamamoto, T. (1995). Impaired proliferation of peripheral B cells and indication of autoimmune disease in *lyn*-deficient mice. *Immunity* **3**, 549–560.
297. Smith, K. G. C., Tarlinton, D. M., Doody, G. M., Hibbs, M. L., and Fearon, D. T. (1998). Inhibition of the B cell by CD22: A requirement for Lyn. *J. Exp. Med.* **187**, 807–811.

16

THE CLINICAL PRESENTATION OF SYSTEMIC LUPUS ERYTHEMATOSUS

Robert G. Lahita

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects many organ systems, is more prevalent in females, has no known etiology, and has a distinct association with several immune response genes.

Some of the systems commonly involved include the muscle and joints, brain and peripheral nervous system, lungs, heart, kidneys, skin, serous membranes, and components of the blood. While other systems can be affected, they are affected with lesser frequency. This disease is complex and often affects one organ system to the exclusion of others. Moreover, the clinical manifestations are protean, overlap with other illnesses, and are often subtle. The pathogenetic factors causing lupus remain enigmatic but are likely related and modified by multiple systems, not just the immune system. This explains the convergence of seemingly unrelated abnormalities and the distinctive nature of the specific pathology from patient to patient in this very interesting illness.

The predilection of this disease for females after puberty cannot be adequately explained; however, suspected pathogenetic factors, such as sex steroid hormones or gonadotrophins such as prolactin, may play a role in the severity of the disease and the different clinical presentations. Environmental factors such as drugs, diet, and toxins have also been implicated in the pathogenesis of SLE.

Although there is an association with a constitutive gene on chromosome I in some families, lupus is largely associated with specific immune response genes in the MHC class II or class III regions. Certain genetic MHC II alleles such as HLA-DR2 and DR-3 are also found more commonly with certain autoantibody groups. Patients with inherited complement (MHC class III) deficiencies also develop a form of lupus with specific clinical characteristics (Chapter 5).

Although there are a few nonimmunologic laboratory characteristics of SLE, these are not specific. Immunological tests, which are specific to most autoimmune diseases such as lupus, include the presence of specific cytotoxic lymphocytes and a variety of autoantibodies, including a persistently positive antinuclear antibody (ANA). The following sections present overviews of data presented in other parts of the book.

GENERAL SYMPTOMS

One major problem in the patient with lupus is the nature of the general symptoms associated with the disease. The most problematic include fatigue, weight loss, and fever [1, 2]. These symptoms are often the initial complaint and are attributed to causes other than lupus, forming the basis for misdiagnosis. Fatigue is well recognized and is the most common and often the most debilitating symptom of SLE, as it is commonly overlooked, cannot be quantified except perhaps by exercise tolerance testing, and is often the only symptom to remain after therapy of an acute flare [2, 3]. Patients liken the fatigue of SLE to a chronic influenza. A

curious pattern of fatigue is described in SLE when compared to patients with other connective tissue diseases [4]. In SLE, fatigue decreased in the morning and increased in the evening in contrast to other conditions such as scleroderma where the opposite is true. Weight loss is common in patients with lupus and worsened when there is malabsorption due to overlapping illness such as CREST syndrome or MCTD. Although anorexia is common in severely debilitated patients with associated organ disease such as renal failure, it is not an isolated finding in SLE. The fever of lupus is usually low grade and rarely exceeds 102°F. A temperature greater than 102°F should warrant a search for infection [2]. Patients who are taking immunosuppressive drugs and have fever must be handled differently from the untreated patient, as high fevers may be masked or lowered by such agents and infections in immunosuppressed patients are more likely.

CRITERIA FOR LUPUS

The diagnosis of SLE should be made principally on clinical grounds with the support of laboratory tests [5]. While diagnostic criteria have been proposed for the classification of SLE [5], they are not universally applied in practice. Eleven criteria have been designated by the American College of Rheumatology (ACR) (Table 1) [6] for classification. The presence of 4 or more criteria is mandatory for the appropriate classification of SLE. When used, these have been of value in clinical practice and are 96% sensitive and specific [5]. In some studies of patients with cutaneous lupus, ACR criteria showed a sensitivity of 88%, a specificity of 79%, a positive predictive value of 56%, and a negative predictive value of 96% [7]. Despite this fact, the diagnosis of SLE is given routinely to patients in practice who fail to meet any criteria [8]. Patients who fail to meet 4 of the 11 criteria may have a lupus-like syndrome or a related autoimmune disease. These revised criteria have been reviewed and applied to a number of cohorts [9]. The criteria have also been criticized from a number of groups because of their failure to apply to every lupus-like condition [10]. In 2001, Wilson *et al.* published the criteria for antiphospholipid syndrome. These criteria, which may need revision over time, are very helpful to this important variant of SLE [11].

GENERAL CONSIDERATIONS REGARDING LUPUS

The initial diagnosis of lupus depends on the manner of clinical presentation. Table 2 [12, 13]. In addition, these are often ignored. As a result, one study shows the mean length of time from onset of symptoms to diagnosis to be 5 years, a lapse that could seriously affect prognosis. However, lupus is not always apparent at the initial visit of the patient and continued follow-up with interval histories, clinical examinations, and serological evaluations may be necessary in order to finalize the diagnosis. It is not uncommon for patients to present with isolated arthralgias, Raynaud's phenomenon, hypercoagulable states, fever of unknown origin, or respiratory symptoms such as dyspnea and pleural effusions. In short, the protean clinical manifestations of this illness can make the diagnosis difficult. Patients are often misdiagnosed with rheumatoid arthritis [14], fever of unknown origin [15], fibromyalgia [16], or asked to seek psychiatric help for a functional disorder [17]. Laboratory data in the form of an isolated false-positive syphilis test, a low platelet count, elevated PTT, or leukopenia may suggest many other diseases that are often considered before SLE. SLE is really a clinical diagnosis that relies heavily on a careful history and physical examination because autoantibodies are so frequently associated with nondisease; they alone are supportive but not diagnostic. A history of drug ingestion is particularly important, as reversible drug-induced lupus is responsible for some 10% of cases [18] and is often seen in older patients, particularly males. Lupus can be related to other illnesses that can occur in the patient's family [19]. These include rheumatoid arthritis, multiple sclerosis, idiopathic thrombocytopenic purpura (ITP), rheumatic fever, overlap syndromes such as Sjogren's, scleroderma, and the inflammatory diseases of muscle. There are HLA-D associations to these diseases as well. Fibromyalgia is an extremely important condition that often occurs with SLE. A positive ANA and muscle pain must be differentiated from SLE-induced arthropathy and myopathy [20]. Because of its importance to lupus, a separate chapter (Chapter 25) is included in this version of the book [21].

Many lupus patients are also known to have increased allergy by some early reports [22] to a variety of agents, especially sulfur. Sulfur-containing drugs should be avoided where possible in patients with SLE.

The frequencies of some common clinical presentations are given in Table 3. Simple laboratory tests can be helpful adjuncts in the diagnosis of SLE but should not be used by themselves as the sole criterion. Common laboratory assays can aid the physician in establishing the diagnosis. Most useful are the white blood cell (WBC) count which often shows leukopenia and lymphopenia [12, 23]. Anemia of chronic disease or, in rare instances, autoimmune hemolytic anemia can be differentiated by the examination of red cell indices, a reticulocyte count, a peripheral smear, and a positive

TABLE 1 Revised Criteria for the Diagnosis of Systemic Lupus Erythematosus[a]

Criterion	Definition
1. Malar rash	Fixed erythema, flat or raised, over the malar eminence, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions
3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
5. Arthritis	Nonerosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	a. Pleuritis: convincing history of pleuritic pain or rub heard by physician or evidence of pleural effusion b. Pericarditis: documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	a. Persistent proteinuria greater than 0.5 g per day or greater than 3 + if quantitation not performed b. Cellular casts: may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	a. Seizures: in the absence of offending drugs or known metabolic derangement; e.g., uremia, ketoacidosis, or electrolyte imbalance b. Psychosis: in the absence of offending drugs or known metabolic derangement; e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	a. Hemolytic anemia: with reticulocytosis b. Leukopenia: less than 4000/mm^3 total on two or more occasions c. Lymphopenia: less than 1500/mm^3 on two or more occasions d. Thrombocytopenia: less than 100,000/mm^3 in the absence of offending drugs
10. Immunologic disorder[b]	a. Anti-DNA: antibody to native DNA in abnormal titer b. Anti-SM: presence of antibody to SM nuclear antigen c. Positive finding of antiphospholipid antibodies based on (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test result for lupus anticoagulant using a standard method, or (3) a false-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. ANA	An abnormal titer of ANA by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced" lupus syndrome

[a] This classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person must have SLE if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.
[b] The modifications to criterion number 11 were made in 1996.

Coombs test. Thrombocytopenia coupled with elevated coagulation tests such as the PTT might suggest the presence of antiphospholipid antibodies and the lupus anticoagulant. A false-positive syphilis test along with an abnormal PTT can also suggest phospholipid antibodies. An abnormal urinalysis with the appearance of WBCs, red blood cells, granular casts, and proteinuria can also be helpful and raise a suspicion of lupus nephritis. While blood urea nitrogen (BUN) and creatinine levels are not usually elevated at the outset of the disease, they can be useful rarely as baseline values in a patient who progresses to azotemia. A chest X-ray and electrocardiogram should be obtained initially to rule out pulmonary pathology, to explain an enlarged cardiac silhouette, and differentiate EKG signs of pericarditis, enlarged cardiac chambers, or signs of ischemia.

More complex immunologic laboratory tests may both solidify the diagnosis when suspected clinically or contribute to the erroneous diagnosis of SLE in the absence of clinical signs (Table 4). The widespread availability of such testing, the lack of standardization, and inappropriate application of such tests contribute to this confusion. The most useful tests for SLE are the fluorescent antinuclear antibody test, antinative DNA assay, and total hemolytic complement (CH50). All of these tests are subject to wide variation, the ANA because of substrate variability, the anti-DNA because of single-stranded DNA contamination and cross-reactivity with phospholipid, and finally the CH50 because of the temperature lability of complement [24].

Many rheumatologists believe that ANA-negative lupus exists [25], but this is probably an artifact of

TABLE 2 Initial Manifestations of SLE[a]

Manifestation	Data from Estes and Christian [12]	Data from Dubois and McGehee [13]
rthritis or arthralgia maneous	53[a]	46
Discoid	9	11
Malar rash	9	8
Other skin manifestations	1	
Nephritis	6	4
Over	5	3
Other	17	16
Serositis (pleurisy, pericarditis)	5	
Seizures	3	
Raynaud's phenomenon	3	
Anemia	2	
Thrombocytopenia	2	
False-positive syphilis test	1	
Jaundice	1	

[a] Expressed as percentage.

TABLE 3 Frequency (%) of Some Common Clinical Manifestations of SLE

Manifestation	Data from Estes *et al.* [12]	Data from Hocherg *et al.* [6]
Musculoskeletal	95	83
Arthritis	95	76
Aseptic necrosis	7	24
Myositis	5	5
Cutaneous	88	81
Malar rash	39	61
Alopecia	37	45
Photosensitivity	n.a.[a]	45
Dermal vasculitis	21	27
Raynaud's phenomenon	21	44
Discoid lesions	14	15
Rheumatoid nodules	11	12
Oral ulcers	7	23
Fever	77	n.a.
Serositis	n.a.	63
Pleurisy	40	57
Pericarditis	19	23
Peritonitis	n.a.	8
Neuropsychiatric	59	55
Psychosis	37	16
Neurosis	5	n.a.
Grand mal seizures	13	26
Peripheral neuropathy	7	21
Cranial nerve palsies	n.a.	5
Hemiparesis	n.a.	5
Renal (nephritis)	53	31
Nephrotic syndrome	26	13
Hypertension	46	n.a.
Pulmonary	n.a.	n.a.
Lupus pneumonia	9	n.a.
Fibrosis	6	n.a.
Cardiac	n.a.	n.a.
Myocarditis	8	n.a.
Sinus tachycardia	13	n.a.
Heart failure	11	n.a.

[a] Not available.

substrate. Occasionally, other reactive antibodies, such as anti-Ro, anti-La, and other ribonucleoprotein antibodies, might be found in the absence of an ANA. Conversely, ANAs can be found in a wide variety of nonlupus individuals, such as the elderly, pregnant females, those ingesting certain medications, and in the setting of certain viral syndromes [26]. Antibodies to native DNA [27] and antibodies to Sm antigen, a nuclear glycoprotein, are also quite specific for SLE [28]. Relevant autoantibodies and their associations are given in Table 5 [12, 23].

MUSCULOSKELETAL SYSTEM

One of the most common presenting symptoms of lupus is arthritis. This is usually a nonerosive, nondeforming, and symmetric arthropathy. Multiple joints are involved, and 80–95% of patients with SLE have tender, swollen, and effusive joints. The joints are the most frequently involved organs in SLE. Most frequently involved joints are the proximal interphalangeal, metacarpal phalangeal, knees, and wrists. The deforming arthritis of Jaccoud can occur in the lupus patient. In such patients, swan neck deformity and/or profound ulnar deviation can usually be reduced and is rarely with erosive changes. The most frequent musculoskeletal X-ray changes are soft tissue swelling, acral sclerosis, and periarticular demineralization. Rare erosions of bone can occur and are another form of overlap syndrome [29]. Joints such as the temporomandibular and the sacroiliac may also be involved (the latter particularly in males) in the SLE patient [30]. Involvement of these joints suggests other diseases such as RA [30], mixed connective tissue disease (MCTD), or ankylosing spondylitis. Overlap syndromes such as MCTD resemble RA or scleroderma more than SLE, and erosions are more likely to be found on X-ray. Rheumatoid nodules can occur in SLE with the presence of a high rheumatoid factor [12] but this is rare. These exceptional joint changes contribute to the confusion in making a diagnosis of SLE, particularly in the elderly patient where SLE is distinctly uncommon [31].

Aseptic necrosis, a particular cause of joint pain in SLE patients, should be a part of every differential diagnosis. It is a feature found in patients who are ingesting corticosteroids and those with phospholipid antibodies [32]. Arthritis and use of cytotoxic agents are independent risk factors for the development of osteonecrosis. Avascular necrosis (AVN) is commonly found in the hips, carpal bones of the wrist, and heads of the humeri.

TABLE 4 Frequency (%) of Common Laboratory Manifestations of SLE[a]

Manifestation	Data from Estes et al. and Christian [12]	Data from Hochberg *et al.* [13]
Hematologic		
Anemia[a]	73	57
Leukopenia[b]	66	41
Thrombocytopenia	19	45
Positive direct Coombs'	27	27
Immunologic		
Antinuclear antibodies	87	94
Hypocomplementemia[d]	n.a.[f]	59
Rheumatoid factor	21	34
Hyperglobulinemia[e]	77	30
False-positive syphilis test	24	26
LE cells	78	71
Anti-dsDNA	n.a.	28
Anti-Sm	n.a.	17
Anti-RNP	n.a.	34

[a] Defined as hemoglobin <11 g/dl (Estes and Christian [12]) or hematocrit <35% (Hochberg *et al.* [21])
[b] Defined as white blood cells <4500/mm^3 (Estes and Christian [12]) or <4000/mm^3 (Hochberg *et al.* [23])
[c] Defined as platelets <100,000/mm^3 (Estes Christian [12]) or <150,000/mm^3 (Hochberg *et al.* [23])
[d] Defined as CH50 < 26 units
[e] Defined as globulins > 1.5 g/dl (Estes and Christian [12]) or >4 g/dl (Hochberg *et al.* [22])
[f] Not available.

Less commonly, the shafts of the long bones can be affected. Anywhere from 5 to 10% of patients with SLE can have AVN [33]; these findings are not always associated with steroid use. In many cases, AVN can be asymptomatic and detected by routine X-ray evaluation [34]. In decreasing order of sensitivity, magnetic resonance imaging (MRI), bone scan [Tc99], and radiograph are useful in detecting AVN. MRI is often positive when all other diagnostic modalities are negative.

Septic arthritis must also be considered as a cause of SLE joint pain [35], particularly when there is swelling and warmth of a joint coupled with peripheral leukocytosis. An aspiration of the swollen SLE joint with subsequent culture is mandatory and can be lifesaving.

Overt myositis can present in 3–5% of SLE patients [35], but clinical features can be found in as many as 50% of patients. Although the creative phosphokinase (CPK) is rarely elevated, the electromyogram (EMG) can be very abnormal. Biopsy of the muscle is rarely required for a definitive diagnosis, but if the CPK is exceptionally high or there is diagnostic confusion, one may wish to consider alternative diagnoses. The lymphocytic, monocytic, and plasma cell infiltration found in primary immune myopathies can be observed in varying degrees with SLE patients. A vacuolar myopathy is rare but may be found in untreated patients [36]. Muscle disease in SLE can also be secondary to corticosteroid therapy [37], and a form of muscle disease indistinguishable from polymyositis can be found in patients ingesting antimalarials [35].

CLINICAL INVOLVEMENT OF THE RENAL SYSTEM

Clinical evidence of kidney involvement is found in one-half to two-thirds of patients with SLE [12, 13, 35, 38]. Renal biopsy evidence of immune complex deposi-

TABLE 5 Some Common Autoantibodies in SLE and Drug-Induced Lupus

Condition	Autoantibody	% positive	Comment
SLE	Anti-dsDNA	30–70	Associated with nephritis, marker for SLE
	Anti-Sm	20–40	Marker for SLE
	Anti-RNP	40–60	Also seen in MCTD and PSS
	Anti-Ro/SS-A	10–15	Associated with sicca syndrome, also seen in Sjögren's syndrome
	Anti-PCNA	5–10	
	Anti-Ku	30–40	Also seen in overlap syndromes
	Antilarnin B	5–10	Also seen in autoimmune liver disease
	Antiribosomal P	5–10	Associated with psychosis
	Antihistone	30	Seen in many disorders
	Anti-ssDNA		Seen in many disorders
Drug-induced lupus	Antihistone	95–100	Seen in many disorders
	Anti-ssDNA		Seen in many disorders

TABLE 6 World Health Organization Classification of Lupus Nephritis[a]

Class	Pattern	Site of immune complex deposition
I	Normal	None
II	Mesangial	Mesangial only
III	Focal and segmental proliferative	Mesangial, subendothelial, ± subepithelial
IV	Diffuse proliferative	Mesangial, subendothelial, ± subepithelial
V	Membranous	Mesangial, subepithelial

[a] Adapted from Appel *et al.* [38], with permission.

tion is found in the kidneys of all patients with SLE [39, 40], regardless of urine sediment. Table 6 [12, 23] shows the various forms of glomerular disease as determined by the World Health Organization (WHO). Both diffuse proliferative glomerulonephritis and progressive forms of focal proliferative nephritis have poorer prognoses than membranous and mesangial forms of the disease [41, 42]. A major clinical point is that each renal lesion has activity. The greater the activity, the more important the need to treat the patient aggressively with large-dose steroids or cytotoxic agents. Those patients with inactive lesions (i.e., membranous nephropathy, sclerotic glomeruli, fibrous crescents, tubular atrophy, or interstitial fibrosis) do not require aggressive therapy. Because this pathology can best be observed with a renal biopsy, these are the principal reasons to obtain such a biopsy. Renal biopsies from SLE patients must always include two components: light microscopy and immunofluorescence. Serial renal biopsy has prognostic value and is recommended for the regulation of therapy [43, 44]. A biopsy without immunofluorescence analysis is not a good manner with which to gauge lupus renal activity. If it is feasible, electron microscopy should also be done.

A renal biopsy done under fluoroscopy or by CT guidance is best for the patient with SLE. An adequate number of glomeruli should be obtained for verifiable diagnosis. Hypothetical problems encountered with the renal biopsy include (1) that the biopsy is static or reflective of one point in time; (2) that the efficacy of the activity/chronicity index is questionable in designing treatment, and (3) no single glomerular lesion can reflect the entire renal picture.

The most serious complications of renal biopsies include pericapsular hemorrhage or clot obstruction in those patients with lupus procoagulants.

In those patients with WBC, erythrocyte, hyaline, or granular casts a BUN and creatinine are helpful in order to assess renal function. For most patients, renal function early in the course of the disease is normal despite abnormal urine sediment. If the activity of the disease progresses unchecked, these parameters can change rapidly. When proteinuria is found qualitatively, it is helpful to obtain a 24-h urine protein and a creatinine clearance to quantify the amounts. Urine protein is a useful measure of renal lupus activity. An incremental change of 500 mg of protein excretion is significant to renal pathology just as a decrement can herald clinical improvement.

There can be many other reasons for decreased renal function in SLE. These include concomitant infections, use of aspirin, NSAIDs, or ACE inhibitors, all of which might induce a decrease of renal circulation, as well as obstruction, or thrombosis of the renal vein. Sonograms, contrast studies, or renal scans can be helpful in the evaluation of renal function when causes other than lupus nephropathy are suspected. Platt *et al.* [45] suggest that ultrasound might be of utility in the prediction of worsened renal disease.

Less common forms of renal involvement in SLE include interstitial nephritis, which is thought to be mediated by immune complexes [46]. This form of renal disease may not present as renal failure but rather as a disorder of acidification and potassium transport or regulation [47]. Studies of renal pathology indicate that thrombosis of the glomerulus [48] and intraglomerular thrombi [49] are alternate causes of proteinuria and renal failure in SLE.

Renal transplantation in lupus is as successful as that in the non-SLE population. However, renal disease can occur again in the transplanted kidneys [50]. For unknown reasons the alleviation of overall disease activity of lupus erythematosus after dialysis for chronic renal disease has been reported.

CLINICAL INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM

Both cognitive dysfunction and neuropsychiatric lupus are reviewed extensively in Chapters 26, 27, and 28.

Neuropsychiatric manifestations can be found in as high as 66% of patients with SLE [51–54]. The pathophysiology of this clinical manifestation is not widely understood; however, thrombosis and vasculitis are not responsible for the large number of neuropsychiatric manifestations observed. It is likely that another component, nonimmunologic in nature, might be involved [55]. Central nervous system (CNS) manifestations include seizures, psychiatric illness, and disorders of the cranial nerves [56]. The frequency of organic CNS manifestations in SLE has been reported as between 35 and

75% [57]. The peripheral nervous system is involved in as high as 18% of patients [58].

Seizures are found in 15–20% of SLE patients [59]. These can be the result of the disease process, such as lupus vasculitis or acute thrombosis, steroid therapy and its attendant hypertension, or a concomitant metabolic problem such as uremia [56]. Grand-mal tonic–clonic seizures are the most common, although other seizures, such as Jacksonian, psychomotor, and absence attacks, have all been reported [58]. On occasion, patients with SLE can present with status epilepticus. Treatment of seizures in SLE requires anticonvulsants. Corticosteroid therapy itself, particularly pulse therapy, has been associated with the onset of status epilepticus and should be used with caution [60].

Lupus can cause profound psychiatric disturbances in 50–67% of patients [56, 58]. Overt psychosis can occur in 12% of cases as well as a variety of organic brain syndromes [61]. Severe depression is common to lupus patients and is thought to be a disease manifestation rather than reactive depression that results from chronic disease. Sleep disturbances are common in lupus and need not be related to depression. Steroid psychosis is common in lupus patients on high-dose steroids for long periods [62]. Antiribosomal P protein antibodies are positive in over 60% of patients with SLE-related psychosis [63] and can help distinguish these patients from those with steroid psychosis [62].

Ten percent of patients can have cranial nerve abnormalities that can be the presenting symptom in an even smaller number [56]. Although spinal cord involvement in SLE is rare, three types of cord involvement are seen: transverse myelitis [64], demyelination [65], and spinal cord occlusion because of thrombosis [66]. The latter three spinal manifestations are all commonly associated with antiphospholipid antibodies [67].

In the first 5 years of the disease, the incidence of cerebrovascular accident (CVA) is high (6.6% occurring in the first year alone) [68]. Patients with antiphospholipid antibodies have an increased risk of stroke.

Even though movement disorders are not common in lupus, chorea is common in children with SLE and has been described in adults and children with phospholipid antibody [69–71]. It is virtually indistinguishable from Sydenham's chorea. Parkinsonism and cerebellar ataxia are rare [56, 72]. Rarer forms of CNS involvement include pseudo-tumor cerebri, hypothalamic dysfunction (especially due to thalamic infarcts), aseptic meningitis (particularly related to NSAID use), myasthenia, Eaton–Lambert syndrome, and a TTP-like syndrome. The presence of microadenomata in the pituitary gland of some patients with hyperprolactinemia represents a separate condition found in an SLE-like syndrome caused by hyperprolactinemia.

Peripheral nervous system disease is found in 3–18% of patients and is largely a sensory only or combined sensory-motor neuropathy [73]. A Guillain–Barre syndrome, mononeuropathy, or mononeuritis multiplex has also been reported [74].

The laboratory diagnosis of central nervous system disease in SLE is difficult [75]. Spinal fluid pleocytosis and/or high spinal fluid protein levels are the only helpful indicators that CNS disease is present [76]. MRI and position electrom tomography scanning show the most promise for diagnosing disease of the brain. Use of the newer modalities, such as Tc-99-HMAAQ brain SPECT, may have better utility in the diagnosis of CNS SLE [77]. Infarctions and demyelinating lesions of the brain are best found with these modalities [78]. CT scans are good for detecting focal lesions but are often unreliable. Antibodies to ribosomal P protein and antineuronal antibodies, as well as the finding of cytotoxic lymphocytes against myelin in blood, may eventually prove useful, but now are not very specific for cerebritis [63].

CLINICAL PRESENTATION OF CARDIOVASCULAR LUPUS

Cardiac involvement is very common in SLE, as 30–50% of all patients suffer from some form of heart disease [12, 79]. Pericarditis is the most common form of involvement and occurs in some 19–48% [80] of patients. Pleural–pericardial pain can occur at any time. Although pericardial tamponade can be the initial presentation, this is quite rare and most patients present with pain and small effusions. Most pericarditis in SLE can be managed with NSAIDS and/or low doses of corticosteroids. Echocardiography is the best diagnostic test for this manifestation. The differential diagnosis of pericardial effusion in SLE should include tuberculosis or bacterial peritonitis if the patient is immunosuppressed, and in instances when pericardiocentesis is necessary, the appropriate cultures should be sent. Myocarditis is rare in SLE, involving only 5–10% of patients and usually presents with fever, conduction abnormalities, elevated CPK, skeletal myositis, and serositis [81]. In the SLE patient with hypertensive cardiomyopathy, the differential diagnosis can be difficult and may require extensive investigation until a final diagnosis is made. Myocardial infarctions [82] and conduction abnormalities can be secondary to the myocardiopathy. A myocardial biopsy would give a definitive answer, but as with most invasive procedures carries a high morbidity in the SLE patient.

Systolic cardiac murmurs are heard in up to 70% of SLE patients [83]. These may be related to anemia, fever, or hypoxemia and are found with Libman–Sacks endocarditis, a component more frequent with antiphospholipid antibodies [84]. The mitral and aortic valves are involved most commonly [85]. Pulmonary hypertension is quite common in patients with phospholipid antibodies [86], and a pulmonic murmur or a loud second heart sound in the presence of an elevated PTT are clues to this diagnosis, which should be confirmed by echocardiography or catheterization. Interventional pulmonary artery pharmacological dilatation may be required in choosing a proper therapy. Ultimately, those patients who have profound cardiac failure or unrelenting pulmonary hypertension may benefit only from heart and lung transplantation. The most common cardiac murmur in the SLE patient is likely to be the mitral valve prolapse, which may have relationship to antiphospholipid antibodies. Disturbed autonomic function is common in SLE [87] and may be a reason for many of the cardiac symptoms such as tachycardia. Diastolic impairment may also be common in patients that do not have active disease [88].

Vasculitis is common in SLE and may be reflected in the presence of splinter hemorrhages, digital infarcts, or ecthymic skin lesions [89] (Table 7). Involvement of small- and medium-sized arteries may mimic polyarteritis nodosa and produce localized signs. This variant can be life-threatening. Examples include vasculitis of the coronary or mesenteric vessels.

SLE in the human results in a higher incidence of myocardial infarction because of accelerated atherosclerosis, coronary vasculitis, or coronary emboli [90–92].

TABLE 7 Vasculitic Involvement in SLE

Manifestation	Vessels involved and site
Leukocytoclastic angiitis (urticaria, palpable purpura)	Postcapillary venules, upper dermis
Atrophie blanche, livedo vasculitis	Small vessels, middle and lower dermis
Subcutaneous nodules	Medium-sized arteries, panniculus, and lower dermis
Livedo reticularis	Medium-sized arteries, panniculus, and lower dermis
Coronary arteritis	Coronary arteries
Mononeuritis multiplex	Vasa nervorum
Cerebral infarcts	Primarily small vessels
Mesenteric arteritis	Small and medium-sized arteries
Retinopathy	Arterioles, venules

Raynaud's phenomenon in SLE is not as common as in related diseases and is present in 20% of patients [12] and is often associated with pulmonary hypertension. It may itself be a sign of overlap syndrome [93]. Digital vasoconstriction can produce digital ulcers, gangrene, and autoamputation. This phenomenon is often treated with vasodilators, calcium channel blockade, or, in severe cases, laser sympathectomy.

The most common cause of death in SLE is early onset cardiovascular disease. Hyperlipidemia is often implicated as one of the reasons for early ASCVD [94]. Aranow and Ginzler [95] reviewed this topic in detail. Risk factors for accelerated ASCVD are under intense study [96].

PULMONARY DISEASE AND LUPUS

The lungs are commonly affected in lupus patients [97]. Over 50% of SLE patients have some form of pleural disease in their lifetime. Pleural effusions, which are mostly exudative (>3 g protein), are less common than the pain and findings associated with simple pleuritis. The pain of pleuritis can be quite severe and must be distinguished from pulmonary embolus, infectious, or other forms of pneumonia.

While many abnormalities of lung function can exist in SLE, the most common pulmonary abnormality in lupus is reduced carbon monoxide diffusion capacity (DLCO). Evidence of severe restrictive or obstructive disease in SLE is unusual, although this can be the major manifestation in some variant forms of the disease [98].

Less commonly, parenchymal lung involvement occurs suddenly and presents as acute pneumonitis, dyspnea, and pleuritic pain. Subsequent to such a presentation, the patient might continue with extensive pulmonary problems [99]. For example, interstitial pneumonitis and chronic fibrosis can be the result of such a syndrome [100]. Generally, such parenchymal disease can be treated with high-dose corticosteroids, and a good response in pulmonary function is achieved as the desired result. Pulmonary capillaritis is also part of parenchymal disease [101]. Hemoptysis and overt pulmonary hemorrhage are emergencies in SLE patients and can be the result of numerous reversible processes, such as pneumonitis or pulmonary embolus. There is also an association of alveolar hemorrhage with renal microangiopathy [102]. Shrinking lung found on X-rays in some lupus patients is the result of diaphragmatic weakness [103] or paralysis and is thought by many experts to be a sign typical for SLE lung.

Pulmonary hypertension, even in patients with minimal lupus activity, can be severe and progressive [104, 105].

HEMATOLOGY OF SLE

The cellular elements of the blood and the coagulation pathway can be affected in the SLE patient. The latter is largely the result of the antiphospholipid syndrome and is covered elsewhere.

Sixty to 80% of lupus patients have anemia of chronic disease. Other kinds of anemia, such as autoimmune hemolytic anemia, are rare and are found in less than 10% of patients; however, a positive Coombs test can be found in 20–60% of patients [15, 106] and confuse the picture.

Leucopenia can be found in over 50% of patients with SLE and is associated with either granulocytopenia or lymphopenia [107]. Antibodies can be directed to either of these cellular elements at any point in their maturation pathways. When directed against stem cells, they are a cause of aplastic anemia. Most low cell counts in SLE can be reversed with immunosuppressive therapy. Leucopenia, often a good general sign of disease exacerbation, can also occur in response to cytotoxic lupus therapy and must be differentiated from that effect.

Thrombocytopenia, found in 30–50% of SLE patients [106], is related either to antiplatelet antibodies [107] or to phospholipid antibodies. Either can cause profound thrombocytopenia (<50,000). The latter usually responds to corticosteroid, immunosuppressive therapy, or intravenous γ-globulin. Platelet transfusions are contraindicated in most SLE patients except on rare occasion because of the possibility that the patients will be exposed to new antigens. Anticlotting factor antibodies have been found in SLE and are often associated with bleeding. Antibodies are directed most commonly to factors II, VIII, IX, XI, or XII. Acquired von Willebrand syndrome is also seen. Lupus anticoagulants are found commonly in patients with SLE and are associated with mild to profoundly elevated partial thromboplastin times. This abnormality is usually associated with coagulation and not with bleeding [108]. Associations have been observed, and the triad of the lupus anticoagulant, recurrent abortions, and the presence of false-positive tests for syphilis is often found in patients. Patients with lupus can be hypercoagulable for a variety of reasons other than procoagulant antibodies and these include hereditary deficiencies of factors C and S or antithrombin III. One acquired reason is the loss of antithrombin III in the urine of patients with nephrotic syndrome.

Laparoscopy is the best way to remove the spleen i n most cases of refractive thrombocytopenia. Open laparotomy is not advisable in any except the most complicated cases.

CLINICAL PRESENTATION OF LUPUS OF THE SKIN

Ninety percent of lupus patients have some involvement of the skin. Only 40% of patients experience sensitivity to ultraviolet (UV) light and these are mostly Caucasians [15, 41]. Black patients are less sensitive to UV light [109]. The actual percentage prevalence is 57% Caucasian vs 11% Black. The lupus band test, considered by many to be the definitive test for lupus, measures immunoglobulin and complement deposition at the dermal epidermal junction in nonlesional skin of greater than 60% of patients [110]. However, false positives are encountered in rosacea, rheumatoid arthritis, MCTD, renal diseases, and many other disorders. Its true value is probably the differentiation of discoid lupus from SLE. In the former disease, only lesional skin stains positive, whereas in SLE, both lesional and nonlesional skin stain with immunoglobulin at the dermal–epidermal junction.

Acute cutaneous lupus (30–50%) and subacute cutaneous lupus (10–15%) comprise the vast majority of patients with dermal disease. The butterfly malar rash found in 40% of patients is part of acute cutaneous lupus. This rash is acute in onset and usually heals without scarring. Widespread morbilliform eruptions or bullous lesions can be confused with drug eruptions or erythema multiforme. The bullous lesions usually respond to Dapsone therapy.

Subacute cutaneous lupus (SCLE) is an annular, widespread, nonscarring or papulosquamous/psoriasiform lesion that is worsened by sun exposure. This form of lupus is associated with HLA-DR3, anti-Ro antibody, and high titers of ANA. SCLE has also been associated with complement component deficiencies of C2 [111], C1q, and C1s.

Chronic forms of lupus skin disease include several forms of discoid lupus and *lupus profundus*. These discoid lesions are usually localized to the head, scalp, and external ear, but involvement that is more widespread is possible. Unlike those of subacute cutaneous lupus, these lesions can occur in nonsun-exposed areas. Discoid lesions can be chronic and not associated with the other manifestations of SLE. Patients with isolated discoid lupus have a 2–10% chance of developing systemic disease, whereas 10–20% of SLE patients have discoid lesions. Discoid LE is more common in African-Americans.

Lupus panniculitis or *lupus profundus* is an unusual form of chronic cutaneous lupus that is manifested by indurated subcutaneous nodules on the extremities. It is rare, occurs in 2% of patients, and responds to Dapsone therapy.

Alopecia occurs in all patients with SLE at some time in their disease presentation and can be the result of therapy. It is usually reversible alopecia. Livido reticularis, another skin lesion, is not restricted to lupus but also to the antiphospholipid syndrome.

GASTROINTESTINAL TRACT AND LIVER DISEASE IN SLE

The most common manifestation of gastrointestinal lupus is the occurrence of painless ulcers in the nose and mouth of patients. Almost 100% of patients develop these ulcers at some time during the course of their disease. These occurrences often indicate a flare of disease in known patients [112].

Esophageal ulcerations and dysphagia are rarely found [113]. Thirty percent of patients [114] have nausea and vomiting.

Abdominal pain in SLE can result from a variety of causes [115, 116]. These include pancreatitis [114], ischemic bowel, perforation, or mesenteric vasculitis [117, 118]. Each of these can suggest a surgical abdomen and mandate a laparotomy. The tumor marker CA125 can be elevated in cases of SLE-induced gastrointestinal ischemia [119]. Serositis and, in some cases, vasculitis may simulate a surgical abdomen, but these may not require exploratory surgery. A prudent trial of steroids before laparotomy may obviate invasive abdominal surgery [120, 121]. Abdominal surgery is associated with an extremely high mortality rate in the active SLE patient.

Lupus peritonitis is the result of small vessel involvement in the bowel serosa or retro peritoneum or the result of actual perforation of the bowel. Rebound tenderness, fever, nausea, vomiting, and diarrhea can be found [122]. Bacterial peritonitis is quite common in patients with nephrotic syndrome [123]. Paracentesis is of diagnostic help in such instances.

Parenchymal liver disease as a result of lupus is uncommon and more likely represents chronic active hepatitis or cirrhosis [63]. Lupoid hepatitis is a separate entity and is not part of SLE [124]. Liver function studies may be very abnormal in patients with lupus. These abnormalities are usually secondary to drug therapy [63], aspirin ingestion [125], and rarely with thrombosis secondary to phospholipid syndrome.

THE EYE AND SLE

The eye is not commonly involved in SLE [126]. Only 10% or less of patients have episcleritis or conjunctivitis. In a prospective study, retinopathy was detected in 7% of SLE patients [127]. This retinopathy consists of microangiopathic lesions with cotton wool spots and hemorrhages that can be a significant problem in someone with a bleeding diathesis or one who is anticoagulated. Optic neuritis, papilledema, and retinal vein occlusion are also major problems [128]. Lupus retinopathy is common in patients with active SLE (88%) and in those with lupus cerebritis (73%). Patients can also have uveitis, cytoid bodies, and angle-closure glaucoma.

MISCELLANEOUS ORGAN INVOLVEMENT

Less common in the patient with SLE is sicca syndrome. Patients who are older are particularly affected. Patients with lupus can have asymptomatic parotid gland enlargement with abnormal labial biopsies that suggest Sjogren syndrome [129, 130]. Many of these patients have a positive anti-Ro antibody and a Sjogren syndrome overlap. Patients with lupus may have vocal cord paralysis or present with hoarseness because of vasculitis of the recurrent laryngeal nerve.

Lupus may be a cause of sensori neural learning loss. The mechanism of ear damage is not known [131, 132].

References

1. Stahl, J. I., Klippel, J. H., and Decker, J. L. (1979). Fever in systemic lupus erythematosus. *Am. J. Med.* **67**, 933.
2. Robb-Nicholson, L. C., Liang, M. H., Daltroy, L., Eaton, H., Gall, V., Schwartz, J., *et al.* (1989). Effects of aerobic conditioning in lupus fatigue: A pilot study. *J. Rheum.* **28**, 500.
3. Dobkin, P. L., Da Costa, D., Fortin, P. R., Edworthy, S., Barr, S., Esdaile, J. M., *et al.* (2001). Living with lupus: A prospective pan-Canadian study. *J. Rheumatol.* **28**(11), 2442–2448.
4. Godaert, G. L., Hartkamp, A., Geenen, R., Garssen, A., Kruize, A. A., Bijlsma, J. W., *et al.* (2002). Fatigue in daily life in patients with primary Sjogren's syndrome and systemic lupus erythematosus. *Ann. N. Y. Acad. Sci.* **966**, 320–326.
5. Piura, B., Tauber, E., Dror, Y., Sarov, B., Buskila, D., Slor, H., *et al.* (1991). Antinuclear autoantibodies in healthy nonpregnant and pregnant women and their offspring. *Am. J. Reprod. Immunol.* **26**, 28–31.
6. Hochberg, M. C. (1997). Updating the American College of Rheumatology revised criteria for the classification of

systemic lupus erythematosus. *Arthritis Rheum.* **40**(9), 1725.
7. Parodi, A., and Rebora, A. (1997). ARA and EADV criteria for classification of systemic lupus erythematosus in patients with cutaneous lupus erythematosus. *Dermatology* **194**(3), 217–220.
8. Asherson, R. A., Cervera, R., and Lahita, R. G. (1991). Latent, incomplete or Lupus at all? *J. Rheum.* **18**(12), 1783–1786.
9. Gilboe, I. M., and Husby, G. (1999). Application of the 1982 revised criteria for the classification of systemic lupus erythematosus on a cohort of 346 Norwegian patients with connective tissue disease. *Scand. J. Rheumatol.* **28**(2), 81–87.
10. Smith, E. L., and Shmerling, R. H. (1999). The American College of Rheumatology criteria for the classification of systemic lupus erythematosus: Strengths, weaknesses, and opportunities for improvement. *Lupus* **8**(8), 586–595.
11. Wilson, W. A., Gharavi, A., Koike, T., Lockshin, M. C., Branch, D. W., Piette, J. C., *et al.* (2000). International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: Report of an international workshop. *Arthritis Rheum.* **42**(7), 1309–1311.
12. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85.
13. Dubois, E. L., and Tuiffanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus: Computer analysis of 500 cases. *JAMA* **190**, 104.
14. Hoffman, G. S. (1978). Polyarthritis: The differential diagnosis of rheumatoid arthritis. *Semin. Arthritis Rheum.* **8**, 115.
15. Harvey, A. M., Shulman, L. E., and Tumulty, P. A. (1954). Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine* **33**, 291.
16. Sakane, T. (1992). Physiopathology of systemic lupus erythematosus. *Nippon Naika Gakkai Zasshi* **81**, 1509–1513.
17. Perry, S., and Miller, F. (1992). The psychiatric aspects of systemic lupus erythematosus. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), p. 845. Churchill Livingstone, New York.
18. Keisler, L. W., Kier, A. B., and Walker, S. E. (1991). Effects of prolonged administration of the 19-nor-testosterone derivatives norethindrone and norgestrel to female NZB/W mice: Comparison with medroxyprogesterone and ethinyl estradiol. *Autoimmunity* **9**, 21–32.
19. Arnett, F. C., and Shulman, L. E. (1976). Studies in familial systemic lupus erythematosus. *Medicine* **55**, 313.
20. Blumenthal, D. E. (2002). Tired, aching, ANA-positive: Does your patient have lupus or fibromyalgia? *Cleve. Clin. J. Med.* **69**(2), 143.
21. Alarcon, G. S. (1997). Arthralgias, myalgias, facial erythema, and a positive ANA: Not necessarily SLE. *Cleve. Clin. J. Med.* **64**(7), 361–364.
22. Honey, M. (1956). Systemic lupus erythematosus presenting with sulfonamide hypersensitivity. *Br. Med. J.* **1**, 1272.
23. Hochberg, M. C., Boyd, R. E., and Ahearn, J. M. (1985). Systemic lupus erythematosus: A review of clinical laboratory features and genetic markers in 150 patients with emphasis on demographic subsets. *Medicine* **64**, 285.
24. Dunselman, G. A., Willebrand, D., and Evers, J. L. (1992). Immunohistochemical analysis of oestrogen and progesterone receptors of eutopic and ectopic endometrium in the rabbit model of endometriosis: The effect of pregnancy. *Hum. Reprod.* **7**, 73–75.
25. Urowitz, M. B., and Gladman, D. D. (1992). Antinuclear antibody negative lupus. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), p. 561. Churchill Livingstone, New York.
26. Sharpe, K. L., Zimmer, R. L., Khan, R. S., and Penney, L. L. (1992). Proliferative and morphogenic changes induced by the coculture of rat uterine and peritoneal cells: A cell culture model for endometriosis. *Fertil. Steril.* **58**, 1220–1229.
27. Stollar, B. D. (1981). Anti DNA antibodies. *Clin. Immunol. Allergy* **1**, 243.
28. Hofig, A., Simmen, F. A., Bazer, F. W., and Simmen, R. C. (1991). Effects of insulin-like growth factor-I on aromatase cytochrome P450 activity and oestradiol biosynthesis in preimplantation porcine conceptuses *in vitro*. *J. Endocrinol.* **130**, 245–250.
29. van Vugt, R. M., Derksen, R. H., Kater, L., and Bijlsma, J. W. (1998). Deforming arthropathy or lupus and rhupus hands in systemic lupus erythematosus. *Ann. Rheum. Dis.* **57**(9), 540–544.
30. McMurray, R., Keisler, D., Kanuckel, K., Izui, S., and Walker, S. E. (1991). Prolactin influences autoimmune disease activity in the female B/W mouse. *J. Immunol.* **147**, 3780–3787.
31. Egol, K. A., Jazrawi, L. M., De Wal, H., Su, E., Leslie, M. P., and Di Cesare, P. E. (2001). Orthopaedic manifestations of systemic lupus erythematosus. *Bull. Hosp. Jt. Dis.* **60**(1), 29–34.
32. Gladman, D. D., Urowitz, M. B., Chaudhry-Ahluwalia, V., Hallet, D. C., and Cook, R. J. (2001). Predictive factors for symptomatic osteonecrosis in patients with systemic lupus erythematosus. *J. Rheumatol.* **28**(4), 761–765.
33. Vogelweid, C. M., Johnson, G. C., Besch-Williford, C. L., Basler, J., and Walker, S. E. (1991). Inflammatory central nervous system disease in lupus-prone MRL/lpr mice: Comparative histologic and immunohistochemical findings. *J. Neuroimmunol.* **35**, 89–99.
34. Hahn, D. W., Carraher, R. P., Foldesy, R. G., and McGuire, J. L. (1986). Experimental evidence for failure to implant as a mechanism of infertility associated with endometriosis. *Am. J. Obstet. Gynecol.* **155**, 1109–1113.
35. Isenberg, D. (1984). Myositis in other connective tissue diseases. *Clin. Rheum. Dis.* **10**, 151.
36. Yood, R. A., and Smith, T. W. (1985). Iclusion body myositis and systemic lupus erythematosus. *J. Rheum.* **12**, 568.
37. Askari, A., Vignos, P. J., and Moskowitz, R. W. (1976). Steroid myopathy in connective tissue disease. *Am. J. Med.* **61**, 485.

38. Appel, G. B., Silva, F. G., and Pirani, C. L. (1978). Renal involvement in systemic lupus erythematosus (SLE): A study of 56 patients emphasizing histologic classification. *Medicine* **57**, 371.
39. Koffler, D., Agnello, V., and Carr, R. I. (1969). Variable patterns of immunoglobulin and complement deposition in the kidneys of patients with systemic lupus erythematosus. *Am. J. Pathol.* **56**, 305.
40. Mahajan, S. K., Ordonez, N. G., and Feitelson, P. J. (1977). Lupus nephropathy with clinical renal involvement. *Medicine* **56**, 493.
41. Baldwin, D. S., Lowenstein, J., and Rothfield, N. F. (1970). The clinical course of the proliferative and membranous forms of lupus. *Ann. Intern. Med.* **73**, 929.
42. Gladman, D. D., Urowitz, D. D., and Cole, E. (1989). Kidney biopsy in SLE. I. A clinical–morphologic evaluation. *Quart. J. Med.* **73**, 1125.
43. Bajaj, S., Albert, L., Gladman, D. D., Urowitz, M. B., Hallett, D. C., and Ritchie, S. (2000). Serial renal biopsy in systemic lupus erythematosus. *J. Rheumatol.* **27**(12), 2822–2826.
44. Yoo, C. W., Kim, M. K., and Lee, H. S. (2000). Predictors of renal outcome in diffuse proliferative lupus nephropathy: Data from repeat renal biopsy. *Nephrol. Dial. Transplant.* **15**(10), 1604–1608.
45. Platt, J. F., Rubin, J. M., and Ellis, J. H. (1997). Lupus nephritis: Predictive value of conventional and Doppler US and comparison with serologic and biopsy parameters. *Radiology* **203**(1), 82–86.
46. Suzuki, T., Suzuki, N., Engleman, E. G., Mizushima, Y., and Sakane, T. (1995). Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes in patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **99**, 251–255.
47. DeFronzo, R. A., Cooke, C. R., and Goldberg, M. (1977). Impaired renal tubular potassium secretion in systemic lupus erythematosus. *Ann. Intern. Med.* **86**, 268.
48. Uwonkunda, M. R., Cosyns, J. P., Devogelaer, J. P., and Houssiau, F. A. (1998). Glomerular thrombosis: An unusual cause of renal failure in systemic lupus erythematosus. *Acta Clin. Belg.* **53**(6), 371–373.
49. Bhandari, S., Harnden, P., Brownjohn, A. M., and Turney, J. H. (1998). Association of anticardiolipin antibodies with intraglomerular thrombi and renal dysfunction in lupus nephritis. *Quart. J. Med.* **91**(6), 401–409.
50. Amend, W. J. C., Vincenti, F., and Feduska, N. J. (1981). Recurrent systemic lupus erythematosus involving renal allografts. *Ann. Intern. Med.* **94**, 444.
51. McCune, W. J., and Golbus, J. (1988). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am.* April, 149.
52. Sibbitt, W. L., Jung, R. E., and Brooks, W. M. (1999). Neuropsychiatric systemic lupus erythematosus. *Compr. Ther.* **25**(4), 198–208.
53. Jennekens, F. G., and Kater, L. (2002). The central nervous system in systemic lupus erythematosus. 2. Pathogenetic mechanisms of clinical syndromes: A literature investigation. *Rheumatology (Oxford)* **41**(6), 619–630.
54. Jennekens, F. G., and Kater, L. (2002). The central nervous system in systemic lupus erythematosus. 1. Clinical syndromes: A literature investigation. *Rheumatology (Oxford)* **41**(6), 605–618.
55. Harrison, M. J., and Ravdin, L. D. (2002). Cognitive dysfunction in neuropsychiatric systemic lupus erythematosus. *Curr. Opin. Rheumatol.* **14**(5), 510–514.
56. Feinglass, E. J., Arnett, F. C., and Dorsch, C. A. (1976). Neuropsychiatric manifestations of systemic lupus erythematosus: Diagnosis, clinical spectrum, and relationship to other features of the disease. *Medicine* **55**, 323.
57. Keisler, L. W., and Walker, S. E. (1987). Suppression of reproductive function in autoimmune NZB/W mice: Effective doses of four contraceptive steroids. *Am. J. Reprod. Immunol. Microbiol.* **14**, 115–121.
58. Johnson, R. T., and Richardson, D. P. (1968). The neurological manifestations of systemic lupus erythematosus. *Medicine* **47**, 337.
59. Sergent, J. S., Lockshin, M. D., Klempner, M. S., and Lipsky, B. A. (1975). Central nervous system disease in systemic lupus erythematosus. Therapy and Prognosis. *Am. J. Med.* **58**, 644.
60. Saso, L., Silvestrini, B., Lahita, R., and Cheng, C. Y. (1993). Changes of immunoreactivity in alpha 1-antitrypsin in patients with autoimmune diseases. *Inflammation* **17**, 383–400.
61. Klippel, J. H., and Zwaifler, N. J. (1975). Neuropsychiatric abnormalities in systemic lupus erythematosus. *Clin. Rheum. Dis.* **1**, 621.
62. D'Hooghe, T. M., Bambra, C. S., Cornillie, F. J., Isahakia, M., and Koninckx, P. R. (1991). Prevalence and laparoscopic appearance of spontaneous endometriosis in the baboon (*Papio anubis*, *Papio cynocephalus*). *Biol. Reprod.* **45**, 411–416.
63. Gutierrez, M. A., Molina, J. F., Jara, L. J., Garcia, C., Gutierrez-Urena, S., Cuellar, M. L., *et al.* (1996). Prolactin-induced immunoglobulin and autoantibody production by peripheral blood mononuclear cells from systemic lupus erythematosus and normal individuals. *Int. Arch. Allergy Immunol.* **109**(3), 229–235.
64. Penn, A. S., and Rowan, A. J. (1968). Myelopathy in systemic lupus erythematosus. *Arch. Neurol.* **18**, 337.
65. Fulford, K. W. M., Catterall, R. D., and Delhanty, J. J. (1972). A collagen disorder of the nervous system presenting as multiple sclerosis. *Brain* **95**, 373.
66. Levine, S. R., and Welch, K. M. A. (1987). The spectrum of neurologic disease associated with anti-phospholipid antibdoies. *Arch. Neurol.* **44**, 876.
67. Lavalle, C., Pizarro, S., and Drenkard, C. (1990). Transverse myelitis: A manifestation of systemic lupus erythematosus strongly associated with antiphospholipid antibodies. *J. Rheum.* **17**, 34.
68. Simmen, F. A., Simmen, R. C., Geisert, R. D., Martinat-Botte, F., Bazer, F. W., and Terqui, M. (1992). Differential expression, during the estrous cycle and pre- and post-implantation conceptus development, of messenger ribonucleic acids encoding components of the pig uterine insulin-like growth factor system. *Endo*. **130**, 1547–1556.

69. Borenstein, D. G., Fye, W. B., and Arnett, F. C. (1978). Myocarditis of systemic lupus erythematosus: Association with myositis. *Ann. Intern. Med.* **89**, 619.
70. Groothius, J. R., Groothius, D. R., and Mukhopadhyay, D. (1977). Lupus associated chorea in childhood. *Am. J. Dis. Child.* **131**, 1134.
71. Lahat, E., Eshel, G., and Azizi, E. (1989). Chorea associated with systemic lupus erythematosus in children: A case report. *Israel J. Med. Sci.* **25**, 568.
72. Brechnian, B. (1982). CNS lupus. *Clin. Rheum. Dis.* **8**, 183.
73. Russell, A. R., Bain, M. D., and Periera, R. S. (1992). Simultaneous occurrence of mucopolysaccharide type II disease (Hunter's syndrome) and systemic lupus erythematosus in a paediatric patient. *J. R. Soc. Med.* **85**(2), 109–110.
74. Hellmann, D. B., Laing, T. J., and Petri, M. (1988). Mononeuritis multiplex: The yield of evaluations for occult rheumatic diseases. *Medicine* **67**, 145.
75. Omdal, R. (2002). Some controversies of neuropsychiatric systemic lupus erythematosus. *Scand. J. Rheumatol.* **31**(4), 192–197.
76. Small, P., Mass, M. F., and Kohler, P. T. (1977). Central nervous system involvement in systemic lupus erythematosus, diagnostic profile and clinical features. *Arthritis Rheum.* **20**, 869.
77. Lass, P., Koseda, M., and Lyczak, P. (1998). Technetium-99m-HMPAO brain SPECT in systemic lupus erythematosus with central nervous system involvement. *J. Nucl. Med.* **39**(5), 930–931.
78. Sibbet, W. L., Jr., Sibbet, R. R., and Griffey, R. H. (1989). Magnetic resonance and computed tomographic imaging in the evaluation of acute neuropsychiatric disease in SLE. *Ann. Rheum. Dis.* **48**, 1014.
79. Dubois, E. L. (ed.) (1974). The clinical picture of systemic lupus erythematosus. *In* "Lupus Erythematosus," p. 232. University of California Press, Los Angeles.
80. Bygren, P., Rasmussen, N., Isaksson, B., and Wieslander, J. (1992). Anti-neutrophil cytoplasm antibodies, anti-GBM antibodies and anti-dsDNA antibodies in glomerulonephritis. *Eur. J. Clin. Invest.* **22**(12), 783–792.
81. Ito, M., Kugiyama, Y., and Omura, I. (1979). Cardiovascular manifestations in SLE. *Jap. Circ. J.* **43**, 985–994.
82. Berthet, J., Pasquier, D., and Racinet, C. (1992). An original model of experimental endometriosis in the rabbit. *J. Gynecol. Obstet. Biol. Reprod. (Paris)* **21**, 625–628.
83. Laroche, C. M., and Green, M. (1990). Diaphragmatic paresis: Pathophysiology, clinical features, and investigation. *Thorax* **45**, 302–303.
84. Pauzner, R., Urowitz, M. B., Gladman, D. D., and Gough, J. M. (1994). Prolactin in systemic lupus erythematosus. *J. Rheumatol.* **21**(11), 2064–2067.
85. Ginzler, E. M. (1991). Clinical manifestations of disease activity, its measurement, and associated morbidity in systemic lupus erythematosus. *Curr. Opin. Rheumatol.* **3**, 780–788.
86. Marchesoni, A., Messina, K., and Carrieri, P. (1983). Pulmonary hypertension and systemic lupus erythematosus. *Clin. Exp. Rheum*. **1**, 247.
87. Hogarth, M. B., Judd, L., Mathias, C. J., Ritchie, J., Stephens, D., and Rees, R. G. (2002). Cardiovascular autonomic function in systemic lupus erythematosus. *Lupus* **11**(5), 308–312.
88. Astorri, E., Fiorina, P., Contini, G. A., Albertini, D., Ridolo, E., and Dall'Aglio, P. (1997). Diastolic impairment in asymptomatic systemic lupus erythematosus patients. *Clin. Rheumatol.* **16**(3), 320–321.
89. Perez-Vazquez, M. E., Cabiedes, J., Cabral, A. R., and Alarcon-Segovia, D. (1992). Decrease in serum antiphospholipid antibody levels upon development of nephrotic syndrome in patients with systemic lupus erythematosus: Relationship to urinary loss of IgG and other factors. *Am. J. Med.* **92**(4), 357–362.
90. Hosenpud, J. D., Monatanaro, A., and Hart, M. V. (1984). Myocardial perfusion abnormalities in asymptomatic patients with systemic lupus erythematosus. *Am. J. Med.* **77**, 286–292.
91. Manzi, S. (2000). Systemic lupus erythematosus: A model for atherogenesis? *Rheumatology (Oxford)* **39**(4), 353–359.
92. Salmon, J. E., and Roman, M. J. (2001). Accelerated atherosclerosis in systemic lupus erythematosus: Implications for patient management. *Curr. Opin. Rheumatol.* **13**(5), 341–344.
93. Weissman, B. N., Rappaport, A. S., and Sosman, J. L. (1978). Radiographic findings in the hands in patients with SLE. *Radiology* **126**, 313.
94. Wierzbicki, A. S. (2000). Lipids, cardiovascular disease and atherosclerosis in systemic lupus erythematosus. *Lupus* **9**(3), 194–201.
95. Aranow, C., and Ginzler, E. M. (2000). Epidemiology of cardiovascular disease in systemic lupus erythematosus. *Lupus* **9**(3), 166–169.
96. Svenungsson, E., Jensen-Urstad, K., Heimburger, M., Silveira, A., Hamsten, A., de Faire, U., *et al.* (2001). Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation* **104**(16), 1887–1893.
97. Mochizuki, T., Aotsuka, S., and Satoh, T. (1999). Clinical and laboratory features of lupus patients with complicating pulmonary disease. *Respir. Med.* **93**(2), 95–101.
98. Saso, L., Silvestrini, B., Guglielmotti, A., Lahita, R., and Cheng, C. Y. (1993). Abnormal glycosylation of alpha 2-macroglobulin, a non-acute- phase protein in patients with autoimmune diseases. *Inflammation* **17**, 465–479.
99. Chopra, R., Radotra, B. D., Varma, S., Gupta, K. L., and Joshi, K. (1999). Acute pneumonitis with pulmonary hemorrhage an uncommon and potentially fatal complication of systemic lupus erythematosus: A case report. *Indian J. Pathol. Microbiol.* **42**(3), 375–378.
100. Shapeero, L. G., and Norman, A. (1991). Imaging of SLE. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), p. 447. Churchill Livingstone, New York.
101. Franks, T. J., and Koss, M. N. (2000). Pulmonary capillaritis. *Curr. Opin. Pulm. Med.* **6**(5), 430–435.
102. Hughson, M. D., He, Z., Henegar, J., and McMurray, R. (2001). Alveolar hemorrhage and renal microangiopathy in systemic lupus erythematosus. *Arch. Pathol. Lab. Med.* **125**(4), 475–483.

103. Wilcox, P. G., Stein, H. B., and Clarke, S. D. (1988). Phrenic nerve function in patients with diaphragmatic weakness and systemic lupus erythematosus. *Chest* **93**, 352.
104. Kawamura, N., Tsutsui, H., Fukuyama, K., Hayashidani, S., Koike, G., Egashira, K., *et al.* (2002). Severe pulmonary hypertension in a patient with systemic lupus erythematosus and minimal lupus activity. *Intern. Med.* **41**(2), 109–112.
105. Cheng, T. O. (1999). Pulmonary hypertension in systemic lupus erythematosus. *Mayo Clin. Proc.* **74**(8), 845.
106. Budman, D. R., and Steinberg, A. D. (1977). Hematologic aspects of systemic lupus erythematosus: Current concepts. *Ann. Intern. Med.* **86**, 220.
107. Palatynski, A. (1986). Dynamics of morphologic changes in the ovaries of guinea pigs modified by implanted endometrium. *Zentralbl. Gynakol.* **108**, 560–569.
108. Stephenson, D. C., Hansen, P. J., Newton, G. R., Bazer, F. W., and Low, B. G. (1989). Inhibition of lymphocyte proliferation by uterine fluid from the pregnant ewe. *Biol. Reprod.* **41**, 1063–1075.
109. Watson, R. (1989). Cutaneous lesions in systemic lupus erythematosus. *Med. Clin. North Am.* **73**, 1091.
110. Gilliam, J. (1987). Systemic lupus erythematosus and the skin. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), p. 616. Wiley, New York.
111. Provost, T. T., Arnett, F., and Reichlin, M. (1983). C2 deficiency, lupus erythematosus and anticytoplasmic Ro (SSA antibodies). *Arthritis Rheum.* **26**, 1279–1282.
112. Sultan, S. M., Ioannou, Y., and Isenberg, D. A. (1999). A review of gastrointestinal manifestations of systemic lupus erythematosus. *Rheumatology (Oxford)* **38**(10), 917–932.
113. Tatelman, M., and Keech, M. K. (1966). Esophageal motililty in systemic lupus erythematosus, rheumatoid arthritis and scleroderma. *Radiology* **86**, 1041.
114. Wohl, M. J. (1976). Case records of the Massachusetts General Hospital, Case 47–1976. *N. Engl. J. Med.* **195**, 1187.
115. Luman, W., Chua, K. B., Cheong, W. K., and Ng, H. S. (2001). gastrointestinal manifestations of systemic lupus erythematosus. *Singapore Med. J.* **42**(8), 380–384.
116. Lee, C. K., Ahn, M. S., Lee, E. Y., Shin, J. H., Cho, Y. S., Ha, H. K., *et al.* (2002). Acute abdominal pain in systemic lupus erythematosus: Focus on lupus enteritis (gastrointestinal vasculitis). *Ann. Rheum. Dis.* **61**(6), 547–550.
117. Khamashta, M. A., Ruiz-Irastorza, G., and Hughes, G. R. (1997). Systemic lupus erythematosus flares during pregnancy. *Rheum. Dis. Clin. North Am.* **23**(1), 15–30.
118. Rodriguez, L. R., Carpo, G., and Gertner, M. (2001). Acute gastrointestinal vasculitis as the initial presentation of SLE. *Am. J. Emerg. Med.* **19**(1), 90–91.
119. Koh, M. S., Sunil, K., and Howe, H. S. (2002). Late onset systemic lupus erythematosus with elevated CA125 and gastrointestinal ischaemia. *Intern. Med. J.* **32**(3), 117–118.
120. Hallegua, D. S., and Wallace, D. J. (2000). Gastrointestinal manifestations of systemic lupus erythematosus. *Curr. Opin. Rheumatol.* **12**(5), 379–385.
121. Chng, H. H. (2001). Lupus the great mimic: Gastrointestinal manifestations. *Singapore Med. J.* **42**(8), 342–345.
122. Salomon, P., and Mayer, L. (1992). Non-hepatic gastrointestinal manifestations of systemic lupus erythematosus. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), pp. 747–760. Churchill Livingstone, New York.
123. Pollack, V. E., Grove, W. J., and Karkin, R. M. (1958). Systemic lupus erythematosus simulatingf acute surgical condition of the abdomen. *N. Engl. J. Med.* **259**, 258.
124. Bearn, A. G., Kunkel, H. G., and Slater, R. J. (1956). The problem of chronic liver disease in young women. *Am. J. Med.* **21**, 3.
125. Seaman, W. E., and Plotz, P. H. (1976). Effecty of aspirin on liver tests in patients with RA or SLE and in normal volunteers. *Arthritis Rheum.* **19**, 155.
126. Gold, D. H., Morris, D. A., and Henkind, P. (1972). Ocular findings in systemic lupus erythematosus. *Br. J. Opthalmol.* **56**, 800.
127. Stafford-Brady, F., Urowitz, M. B., and Gladman, D. D. (1988). Lupus retinopathy. *Arthritis Rheum.* **31**, 1105.
128. Wong, K., Everett, A., and Verier-Jones, J. (1981). Visual loss as the initial symptom of systemic lupus erythematosus. *Am. J. Opthalmol.* **92**, 238.
129. Kassan, S. S., and Gardy, M. (1978). Sjogren syndrome: An update and overview. *Am. J. Med.* **64**, 1037.
130. Rosenbaum, J. T., and Wernick, R. (1990). The utility of routine screening of patients with uveitis for systemic lupus erythematosus or tuberculosis: A Bayesian analysis. *Arch. Opthalmol.* **108**, 1291.
131. Harari, S., Paciocco, G., and Aramu, S. (2000). Ear and nose involvement in systemic diseases. *Monaldi Arch. Chest Dis.* **55**(6), 466–470.
132. Kastanioudakis, I., Ziavra, N., Voulgari, P. V., Exarchakos, G., Skevas, A., and Drosos, A. A. (2002). Ear involvement in systemic lupus erythematosus patients: A comparative study. *J. Laryngol. Otol.* **116**(2), 103–107.

17

NEONATAL LUPUS SYNDROMES

Jill P. Buyon

INTRODUCTION

In 1901, congenital heart block (CHB) was first reported by Morquio [1] and in 1945 was diagnosed antepartum by Plant and Steven [2]. CHB can occur in association with structural heart disease, such as atrioventricular septal defects, left atrial isomerism, and abnormalities of the great arteries [3], with tumors, such as mesotheliomas [4], or as an isolated defect. In 1928 Aylward [5] reported the occurrence of CHB in two children whose mother "suffered from Mikulicz's disease." This curious clinical observation was further solidified by the 1970s with reports of CHB, absent major structural abnormalities, in children whose mothers had autoimmune diseases [6–8] and by the finding that the maternal sera contained antibodies to SSA/Ro ribonucleoproteins [9–12]. It was subsequently noted that many mothers also had antibodies to SSB/La [13–17] (Table 1). Other abnormalities affecting the skin, liver, and blood elements were reported to be associated with anti-SSA/Ro-SSB/La antibodies in the maternal and fetal circulation and are now grouped under the heading of neonatal lupus syndromes (NLS) [18–21]. Neonatal lupus was so termed because the cutaneous lesions of the neonate resembled those seen in system lupus erythematosus (SLE) [20, 21].

NLS, albeit encountered infrequently by those caring for pregnant patients with SLE, presents a unique clinical challenge to the team of rheumatologist, perinatologist, neonatologist, pediatric cardiologist, and dermatologist. For the immunologist, the study of these syndromes may yield important insights into the pathogenesis of autoimmune-mediated tissue damage, whereas the embryologist may uncover relevant milestones in fetal antigen expression. The biology of human viviparity involves a wide variety of fetal–maternal relationships, several of which may facilitate the occurrence of passively acquired autoimmunity [22]. Tissue injury in the fetus is presumed to be dependent on the transplacental passage of maternal IgG autoantibodies from the mother who has an autoimmune process but who may be clinically asymptomatic (Fig. 1). Disease in the offspring parallels the presence of maternal antibodies in the fetal and neonatal circulation and disappears, except for CHB, with the clearance of the maternal antibodies by the sixth to eighth month of postnatal life. The transient hematologic abnormalities and skin disease of the neonate reflect the effect of passively acquired autoantibodies on those organ systems that have the capacity of continual regeneration. In contrast, these regenerative processes apparently do not occur in cardiac tissue; complete block is irreversible to date. Curiously, the transient manifestations of this passively acquired autoimmune syndrome closely mimic the disease manifestations observed in adolescents or adults with systemic lupus erythematosus, whereas while heart block is rarely, if ever, present in these same patients.

The study of NLS exemplifies not only translational research, which inherently draws upon clinical observations and explores them in the laboratory, but "integrational" research, which attempts to fit critical clinical and basic observations together, even those seemingly at odds. When this chapter was initially written in 1991 and further updated in 1998, many questions based on important clinical observations were raised and are

TABLE 1 Studies Demonstrating the Frequencies of Antibodies to SSA/Ro[a] and SSB/La[a] in Women Having Offspring with and without CHB

	CHB present		CHB absent[b]	
Study	**SSA/Ro**	**SSB/La**	**SSA/Ro**	**SSB/La**
Taylor *et al*. [15]	34/35	29/35	8/29	8/29
Buyon *et al*. [16, 17]	34/34	28/34	53/145	19/145
Silverman *et al*. [14]	17/17	17/17	5/14	0/14
Ramsey-Goldman *et al*. [12]	6/7	NS	41/148	NS
Totals[c]	91/93 (98%)	74/86 (89%)	107/336 (32%)	27/188 (14%)

[a] Antibodies were evaluated by double immunodiffusion, counterimmunoelectrophoresis, ELISA, or immunoblot.
[b] In each study the control group consisted of mothers who were diagnosed as having SLE, SS, or an undifferentiated connective tissue disease with a positive ANA.
[c] Presence of anti-SSA/Ro in CHB versus non-CHB: odds ratio = 78, $P < 0.001$, sensitivity = 0.98, specificity = 0.68, predictive value = 0.46. Presence of anti-SSB/La in CHB versus non-CHB: odds ratio = 35, $P < 0.001$, sensitivity = 0.86, specificity = 0.86, predictive value = 0.73.

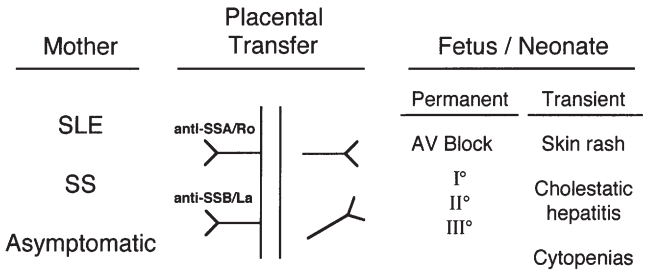


FIGURE 1 Placental transfer of maternal autoantibodies into the fetal circulation may mediate the development of NLS. Hypothetical model.

again reiterated as follows: What accounts for the spectrum of neonatal disease, i.e., why do some offspring remain totally healthy despite circulating maternal autoantibodies whereas others have transient rashes and still others die *in utero* from acute myocarditis? Why is complete heart block irreversible and why is there specificity for the conducting system? Is this an acquired disease that occurs in a previously healthy fetus? Why is the maternal heart unaffected? What is the mechanism of tissue injury? What is the autoantigen–antibody system and how does it relate to the SSA/Ro-SSB/La polypeptides, which are intracytoplasmic cellular components unlikely to be present on the cell surface? Is there a genetic basis for these syndromes? What is the fine specificity of the maternal autoantibody response that is most diagnostically predictive and how can this knowledge guide the approach to therapy? Finally, can we safely tell patients "No antibodies to SSA/Ro or SSB/La, no affected offspring?" Happily, NLS continues to draw tremendous interest and the author is again challenged to address and update these questions by reviewing the results of exciting research recently generated both from the bedside and from the bench.

PASSIVELY ACQUIRED HEART BLOCK: AN IRREVERSIBLE MANIFESTATION OF NEONATAL LUPUS SYNDROMES

Cardiac Histopathology

Histopathologic studies constitute a major basis for formulating hypotheses regarding the pathogenesis of CHB. It appears logical to assume that the time of death relative to initial immune attack may influence the pathologic findings. Evidence of a cellular infiltrate might be present if death occurs close to the time a bradyarrhythmia is first detected, but calcifications may be the sole pathologic finding if death has occurred months later. An inflammatory component is supported by the finding of a mononuclear cell infiltration in the myocardium of a fetus dying *in utero* at 18 weeks of gestation [23] and the demonstration of patchy lymphoid aggregates throughout the myocardium of an infant delivered at 30 weeks and dying in the immediate postnatal period [24]. Moreover, immunofluorescent studies have shown deposition of IgG, complement (including $C1_q$, C4, C3d, C6, and C9), and fibrin [24, 25]. The first cardiac lesion may be a global pancarditis with inflammation of the pericardium, myocardium, and endocardium resulting in subsequent fibrosis of the conducting system clinically manifest as permanent heart block. Litsey and colleagues [25] identified IgG deposits in the epicardial, myocardial, and endocardial tissue of the right atrium on postmortem analysis of a neonate with CHB. Although published literature on serial

echocardiograms in mothers at high risk of a pregnancy complicated by CHB is limited to nonexistent, it has been the general experience that the first clinically apparent abnormality in cardiac function is not myocarditis (i.e., effusions, ventricular dysfunction) but bradycardia. This implies that early inflammation is not clinically detectable and/or that atrioventricular (AV) nodal injury occurs independent of an inflammatory pancarditis.

Specific vulnerability of the conducting system is unexplained [26]. Ho *et al*. [26] described the histopathology of seven hearts with CHB and associated maternal antibodies to the SSA/Ro polypeptide. In all of these hearts there was atrial-axis discontinuity, the AV node was replaced by varying degrees of fibrosis or fatty tissue [26]. The distribution of the distal conducting system was normal.

In five cases of CHB compiled by Carter *et al*. [4] there was disruption of the AV conduction system by a process of uncertain cause. In all five instances the presence of microscopic crystalline structures was associated with the conduction system and with fibrous structures of the heart. These deposits have been designated as products of connective tissue degeneration resulting from an intrauterine inflammatory process. Hogg [27] has reported hematoxylin bodies in the AV node. Further support for an inflammatory process is demonstrated by the findings of calcification along ventricular portions of the conducting system and area of the sinoatrial (SA) node [25, 28]. The origin of diffuse fibroelastosis, which has also been reported in some of these affected babies, is considered to result from dilatation of the cardiac chambers secondary to the compensatory increased stroke volume present in CHB [4]. However, Nield and colleagues [29] have reported 13 CHB patients with endocardial fibroelastosis (EFE), 6 diagnosed *in utero* and 7 in the postnatal period, despite ventricular pacing of all but one infant. EFE is associated with significant mortality and morbidity: 9 (70%) of these 13 patients died and 2 (15%) required heart transplants.

Given the importance of histologic data to infer pathogenic mechanisms, medical records of all families enrolled in the National Research Registry for Neonatal Lupus (established in September 1994) were reviewed to determine the incidence and timing of death, with emphasis on the pathologic findings in the affected fetal hearts [30]. Complete autopsy reports were available in 11 cases. The mean time from detection of CHB to autopsy was 11 weeks. Although in 3 cases there were various lesions of the tricuspid valve, the pathologic descriptions were more suggestive of an imposed injury than a true developmental defect. These included nodularity, dysplasia, hypoplasia and fusion of valve leaflets, and fibrosis. The pulmonary valve was abnormal in 2, 1 was described as stenotic dysplastic, and the other nodular and dysplastic. Aortic valve insufficiency and stenosis and hypoplasia of the mitral valve leaflet were observed in 1. Endocardial fibroelastosis of the right and left ventricles (RV, LV), with or without calcification, was present in 7. Chronic changes in the myocardium were documented in 10 and included biventricular hypertrophy and increased RV and LV walls, thickened but hypoplastic RV, and hyperchromatic nuclei of the myocytes. Abnormalities of the AV node or vicinity were noted in 8 with involution, fibrosis, fatty infiltration, or calcification. However, in 2 the AV node *per se* appeared normal: in one there was calcification in adjacent tissue and in another there was an atrophic His bundle with replacement by dense focally calcified fibrous tissue and scarring of the left and right bundle branches. Although previously unappreciated, autopsies obtained from the research registry revealed a high incidence of valvular abnormalities. While there were sufficient changes in the AV node to account for CHB in most cases, clinical conduction abnormalities may have been secondary to a functional exit block in a normal-appearing node. SA nodal disease expands the spectrum of conduction dysfunction (a fascinating point further addressed later).

These studies leave little doubt that the signature lesion of autoantibody-associated CHB is fibrosis, which can clearly extend beyond the conduction system. As discussed in the update of pathogenesis, the cascade leading to fibrosis is a major focus of investigation. Of interest, slides have been obtained from the heart of an infant diagnosed with CHB *in utero* and dying shortly after birth [31]. The ventricular tissue contained numerous areas of fibrosis and microcalcification in which a predominant infiltrate of transdifferentiated fibroblasts, myofibroblasts, could be readily observed. Macrophages could also be appreciated in areas of scar tissue. In contrast, there were no myofibroblasts or macrophages in the ventricular tissue from an otherwise healthy 24-week abortus. It is especially noteworthy that the fibrosis was not bland but involved an infiltrate of activated myofibroblasts even out to 3 months after the initial insult.

Congenital Heart Block Occurs in an Anatomically Developed Heart and Coincides with Placental Transport

The presence of maternal immunoglobulins in the fetal circulation is directly related to the normal physiology of antibody traffic across the placenta [32]. Maternal antibodies interact with Fc receptors on the trophoblastic cell surface in a specific transport process.

Each Fc receptor has a different ligand specificity and affinity for the IgG subclasses but all receptors bind IgG1 and IgG3 with greater affinity than IgG2 or IgG4 [33]. IgG1, IgG2, and IgG3 are transported relatively early with detectable levels noted at 6–11 weeks of gestation. In contrast, IgG4 is present in the fetal circulation after 19 weeks [34]. The resultant fetal concentrations of total IgG are marginally detectable in the first trimester (<100 mg/dl) and remain low until after 17 weeks, at which time they increase steadily, reaching 400 mg/dl by 24 weeks and 800 mg/dl by 32 weeks as placental transfer becomes more efficient [35]. IgM and IgA antibodies do not cross the placenta.

Perhaps CHB occurs in offspring of mothers whose antibodies are of IgG1 and IgG3 subclasses, while those whose antibodies are predominantly IgG2 and IgG4 are protected. However, no significant differences in subclass distribution have been observed between mothers with anti-SSA/Ro and/or -SSB/La antibodies who had pregnancies complicated by CHB or those who had normal pregnancies [36]. For both groups of mothers, IgG1 antibodies were increased significantly over the other three subclasses in the anti-52-kDa and 60-kDa SSA/Ro responses. IgG1 and IgG3 were the major subclasses represented in the 48-kDa SSB/La responses. All subclasses, including IgG2 and IgG4, were observed in a third to half of the maternal serum with anti-52-kDa and 48-kDa responses. In contrast, anti-60-kDa antibodies were, with rare exception, confined to IgG1. Accordingly, the IgG subclasses of anti-48-kDa SSB/La and 52-kDa or 60-kDa SSA/Ro antibodies do not account for the susceptibility of one fetus versus another for the development of CHB.

The stage of cardiac ontogeny that coincides with the initiation of transplacental passage of maternal autoantibodies into the fetal circulation may influence the extent of tissue injury and permanent dysfunction. The human heart attains most of its adult characteristics by the sixth to eighth week of gestation [37]. Parasympathetic innervation of the heart occurs very early in fetal development, whereas sympathetic innervation develops much later and is completed some months after birth. The SA node can be recognized in the first trimester and by 10 weeks of fetal age attains its own artery. Landmarks of the three internodal pathways from SA to AV nodes appear in the second month of gestation, although the septal course of these pathways does not become fully developed until the closing of the foramen ovale cordis, which occurs shortly after birth. The AV node arises separately from the bundle of His and is joined to it at 8 weeks. The human His bundle undergoes extensive postnatal remodeling to achieve its adult form. Clearly the fetal conduction system has reached functional maturity before maternal antibodies gain access to the fetal circulation.

To support a pathogenic role of maternal autoantibodies in the development of CHB, the onset of bradycardia would be predicted to coincide with heightened placental transport and occur in a previously normal heart. As expected if the hypothesis is correct, Deng *et al*. [38] reported the absence of immunoglobulin deposition in cardiac tissue from 10-week fetuses of two mothers with anti-SSA/Ro antibodies. There is likely to be a "window of vulnerability" when maternal antibodies gain access to the fetal circulation and recognize an antigen, which may be unique to the developing heart that is absent or otherwise inaccessible in the maternal heart.

As seen in Fig. 2, a review of data obtained from the research registry revealed that in 71 (82%) of 87 fetuses, bradycardia was identified before 30 weeks of pregnancy [39]. Detection was clustered most frequently between 20 and 24 weeks. Fourteen (16%) cases were first identified in the third trimester, 5 of which were noted at the time of delivery. The median time of *in utero* detection was 23 weeks. Of the 85 fetuses diagnosed with CHB during pregnancy, 15 were born between 1970 and 1987, 8 of whom were diagnosed before 30 weeks of gestation. Seventy pregnancies occurred between 1988 and 1997, in which 63 were diagnosed with CHB prior to 30 weeks ($P < 0.003$, earlier detection of CHB in pregnancies after 1988 compared to pregnancies before 1988).

Classification of Heart Block

Some confusion still exists regarding the assignment of "congenital" heart block. Webster defines congenital as that "existing at or dating from birth." Data from Hübscher *et al*. [40] strongly suggest that postnatally "acquired" heart block (even without structural abnormalities) is not associated with maternal antibodies to SSA/Ro or SSB/La and is probably best categorized as a separate entity.

Again, the issue of "complete" versus "incomplete" heart block warrants clarification. At the time of writing the initial chapter on NLS, heart block was defined operationally as third degree or congenital *complete* heart block (CCHB), although it was hypothesized that heart block might progress through various stages. Of 187 children in the research registry with CHB associated with anti-Ro/La antibodies in the mother, 9 had a prolonged PR interval on an electrocardiogram (EKG) at birth, 4 of whom progressed to more advanced AV block [41]. A child whose younger sibling had third-degree block was diagnosed with first-degree block at age 10 years at the time of surgery for a broken wrist.

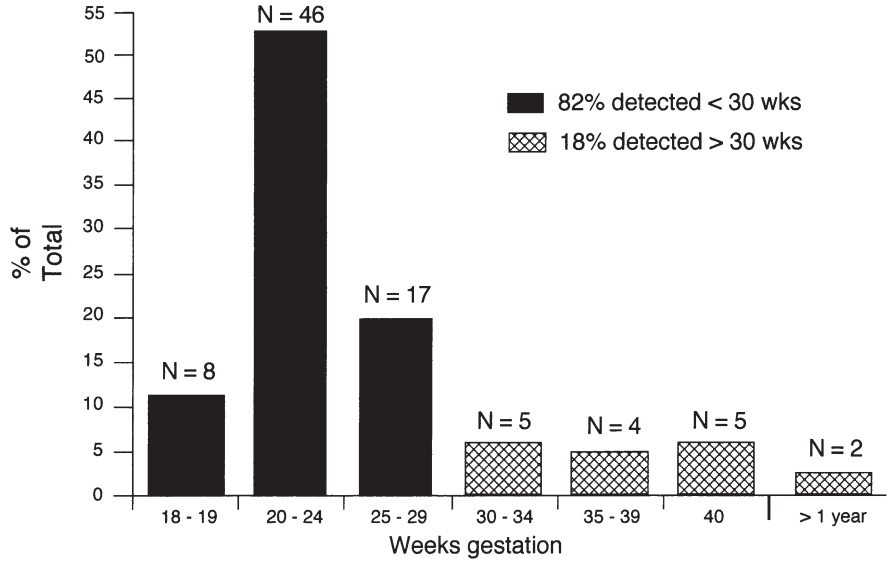


FIGURE 2 Time of detection of CHB. In one child, third-degree block was diagnosed at age 2 years, 7 months; in the other, first-degree block was diagnosed at age 10 years.

Two children diagnosed *in utero* with second-degree block were treated with dexamethasone and reversed to normal sinus rhythm by birth, but ultimately progressed to third-degree block. Four children had second-degree block at birth: of these, two progressed to third-degree block. Accordingly, the general abbreviation "CCHB" is not precise and CHB (*congenital* heart block) has been adopted. The implications of this spectrum of conduction abnormalities from the perspectives of pathobiology and treatment are discussed in subsequent sections.

When CHB is associated with major structural abnormalities, such as transposition of the great vessels, maternal autoantibodies are generally not present. This defect of cardiogenesis occurs prior to the 10th week of gestation and results in the disruption of the development of the AV conducting system. Such cases should be considered as a distinct classification of CHB secondary to abnormal cardiac embryogenesis and not likely the result of passively acquired autoimmunity. Examination of a heart from a child with this type of CHB, whose maternal serum did not contain autoantibodies, revealed nodoventricular discontinuity, with the AV node well formed and normally situated [26]. Similarly, mass lesions developing in the conducting system, such as mesotheliomas of the AV node, would constitute another distinct type of CHB.

THE TRANSIENT SKIN RASH

In striking contrast to the typical late second trimester onset of CHB, skin lesions generally become manifest several weeks into postnatal life. Less commonly the rash is present at birth. Ultraviolet exposure may be an initiating factor and can exacerbate an existing rash [42]. Thornton *et al*. [43] reported that telangiectasia may be a presenting feature and can occur in sun-protected sites independent of "lupus dermatitis." Cutaneous activity, inclusive of erythema and the continued appearance of new lesions, is generally present for several weeks with resolution by 6 to 8 months of age coincident with the clearance of maternal autoantibodies from the baby's circulation. However, hypopigmentation may persist into the second year of life. Lee [42] observed two children with persistent telangiectasias and one child with a small but persistent patch of hyperpigmentation; 11 other children, including several with extensive skin disease, have had complete resolution of the rash.

The rash frequently involves the face and scalp with a characteristic predilection for the upper eyelids (Fig. 3) [44]. In some instances the rash is present in other locations and can cover virtually the entire body. A review of the corporeal distribution of 57 infants (20 males, 37 females) diagnosed with cutaneous NLE (absent heart disease) enrolled in the research registry has been recently reported [45]. All had facial involvement (periorbital region most common) followed by the scalp, trunk, extremities, neck, intertriginous areas, and rarely the palms and soles. In most cases the infant's rash was temporally related to UV exposure; mean age of detection was 6 weeks and duration was 22 weeks. The lesions were described as superficial inflammatory plaques resembling subacute cutaneous lupus erythematosus of the adult [46]. They are typically annular or elliptical with erythema and scaling. Hypopigmentation was frequent and may be a prominent feature. The more characteristic lesions of adult discoid lupus, such as

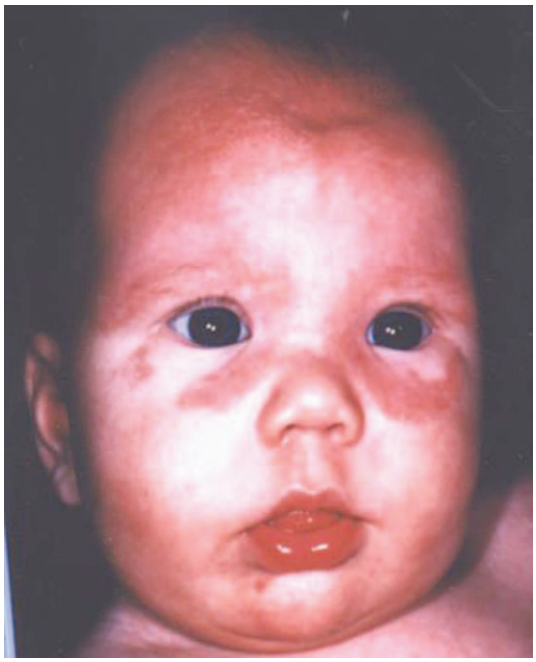


FIGURE 3 Typical skin rash with predilection for the periorbital area. This rash resolved completely without scarring. Photograph courtesy of Dr. Susan Manzi, reproduced from Buyon [44], with permission.

follicular plugging, dermal atrophy, and scarring, were generally not observed in the neonatal skin rash. The lesional histology supports the clinical descriptions of subacute cutaneous lupus with basal cell damage in the epidermis and a superficial mononuclear cell infiltrate in the upper dermis [47, 48]. As observed in subacute cutaneous lupus, immunofluorescence is positive with the finding of a particulate pattern of IgG in the epidermis [42].

LESS COMMONLY ENCOUNTERED MANIFESTATIONS OF NLS

The clinical spectrum of NLS includes hepatic involvement, a manifestation that could well be underestimated as routine neonatal evaluation does not include a liver profile. Permanent sequelae can occur in the liver, but unlike the heart may be clinically insignificant [18, 44–47, 49–52]. Laxer *et al*. [18] have described three living infants and one perinatal death with NLS associated with significant hepatic involvement. The living infants presented with neonatal cholestasis as a major component of their clinical picture. Pathologic changes included giant cell transformation, ductal obstruction, and extramedullary hematopoiesis. The authors speculated that an inflammatory hepatitis proceeding to hepatic fibrosis may ensue, analogous to the mechanism hypothesized to occur in cardiac tissue. Rosh *et al*. [49] reported an infant born with CHB in whom severe neonatal cholestasis developed, requiring surgical exploration to exclude extrahepatic biliary atresia. The clinical picture included an elevation of the serum glutamic-pyruvic transaminase, glutamic oxaloacetic transaminase, alkaline phosphatase, and γ-GTP. A percutaneous liver biopsy revealed mild fibrosis, bile ductular proliferation, and a mixed inflammatory infiltrate in the portal tracts. Lee *et al*. [50] described three infants with hepatic dysfunction; all had laboratory and histologic evidence of significant cholestasis. One occurred in the setting of intractable congestive heart failure. At autopsy immunofluorescence revealed widespread deposits of IgG. A second infant had thrombocytopenia and hepatosplenomegaly at birth followed at 3 weeks of age by a cutaneous eruption characteristic of NLS. Liver biopsy revealed hepatocellular cholestasis, lobular disarray, and mild pseudoacinar formation. A third neonate developed a typical rash at 2 weeks and transaminitis with jaundice by 8 weeks. Liver biopsy revealed canalicular and hepatocellular cholestasis. Lee *et al*. [51] investigated the incidence of hepatobiliary manifestations among 219 NLS patients in the research registry. Nineteen (9%) had probable or possible hepatobiliary disease, appearing as the sole manifestation of NLS in 3 cases and in association with cardiac or cutaneous manifestations in 16 cases. Six (including one reported previously by Schoenlebe *et al*. [52]) of the 19 infants died, either during gestation or within the first few weeks of life. Three clinical variants were observed: (1) severe liver failure present during gestation or in the neonatal period, often with the phenotype of neonatal iron storage disease; (2) conjugated hyperbilirubinemia with mild or no elevations of aminotransferases occurring in the first few weeks of life; and (3) mild elevations of aminotransferases occurring at approximately 2 to 3 months of life. The prognosis for the children in the last two categories was excellent.

These reports and others [53] suggest that the diagnosis of NLS-related liver disease should be considered in situations in which liver enzymes and bilirubin levels are most consistent with cholestasis in the absence of a major structural abnormality of the biliary tree. Reassuringly, in babies that survive cardiac manifestations, the general observation is that hepatic disease resolves.

While the nervous system has not been regarded as an organ characteristically affected in NLS, clinical detection may be a limiting factor. There have been several reports of neurologic sequelae in NLS. Specifically, aseptic meningitis occurred in an infant with CHB

and circulating maternal anti-SSA/Ro and anti-SSB/La antibodies [54]. There has been one case report of NLS and transient hypocalcemia with seizures [55]. Wong and colleagues [56] observed sonographic evidence of infantile lenticulostriate vasculopathy (LSV) in a case of NLS. The authors suggested that sonographic LSV is a nonspecific marker of a previous insult to the developing brain, the clinical significance of which is uncertain. Bourke and Burns [57] described an infant with thrombocytopenia and a generalized annular rash with scattered telangiectases at birth in the setting of antibodies to SSA/Ro and SSB/La. At the age of 1 year the child was found to have an abnormal gait and examination revealed mild spastic diplegia of the lower limbs. The authors appropriately pointed out that the central nervous system abnormalities might have been due to an intracerebral hemorrhage in the neonatal period. This curious observation of late-onset lower limb spasticity has been reported in one other infant with antibodies to SSA/Ro and butterfly rash at birth [58]. In sum, the mechanism of these neurologic sequelae is entirely elusive and awaits further observation in other neonates born to mothers with anti-SSA/Ro-SSB/La antibodies.

Hematologic abnormalities have been described as a manifestation of NLS. Thrombocytopenia has been observed together with other manifestations of NLS [19]. Some of these infants have a petechial or purpuric eruption as the initial feature. Thrombocytopenia was present in 10% of the neonates referred to Lee [42]. Gastrointestinal bleeding occurred in one of these infants. In contrast to the cardiac manifestations, which do not parallel disease in the mother, and the cutaneous manifestations, which occasionally occur in the mother but often not in synchrony with her affected offspring, the hematologic manifestations may more closely parallel maternal disease. However, Watson *et al*. [19] described thrombocytopenia in offspring of anti-SSA/Ro-positive mothers with no apparent history of thrombocytopenia. Despite this, the presence of thrombocytopenia raises some questions as to whether antiplatelet antibodies rather than antibodies to SSA/Ro-SSB/La are targeting the surface of fetal cells. These manifestations, while secondary to passively acquired autoimmunity (and therefore part of NLS), may be more akin to the neonatal thrombocytopenia of idiopathic thrombocytopenic purpura (ITP). The disparate fetal and adult vulnerability appears more pronounced in NLS than in ITP.

Kanagasegar *et al*. [59] reported an infant with neutropenia and mildly abnormal liver functions, but no cardiac or cutaneous manifestations of NLS, born to a mother with anti-SSA/Ro-SSB/La antibodies. The child's neutropenia improved as maternal antibody was metabolized. Sera from this child and mother, as well as sera from two registry mothers who had given birth to infants with CHB and neutropenia, were shown to bind the cell surface of intact neutrophils [59]. Binding to neutrophils was then inhibited (>80%) by incubating the sera with the 60-kDa Ro antigen, suggesting that anti-60-kDa SSA/Ro is directly involved in the pathogenesis of neutropenia.

Wolach *et al*. [60] extended the hematologic spectrum of NLS. They describe a 5-month-old infant with anti-SSA/Ro antibodies and typical cutaneous involvement in the setting of complete marrow aplasia, which recovered at 8 months with the disappearance of anti-SSA/Ro antibodies. However, before this complication is added to the "official list" of manifestations, it is curious that the mother herself was said to have "tested negative" for anti-SSA/Ro antibodies. The child died at 16 months from gram-negative sepsis.

CANDIDATE ANTIGEN–ANTIBODY SYSTEMS IN NLS

Target Autoantigens of the SSA/Ro-SSB/La System

Antibodies to SSA/Ro ribonucleoproteins in the maternal sera, often in association with SSB/La, have been almost universally demonstrated when isolated CHB develops in an offspring [9–11]. Anti-SSA/Ro antibodies are characteristically found in the majority of patients with SS [61] and close to half of those with SLE [62] but can also be detected in asymptomatic individuals [63, 64]. Fritzler *et al*. [63] detected a 0.5% frequency of anti-SSA/Ro positivity after testing several thousand healthy female blood donors of childbearing age. A similar frequency was detected by Harmon *et al*. [64] in a study of 800 asymptomatic pregnant women. Reactivity against SSB/La is also strongly associated with the development of CHB and is frequently detected in patients with SS [65, 66]. Its prevalence in SLE is only about 15% [66].

The candidate autoantigens are ubiquitous and present in all cells. The major antigenic component of SSA/Ro was initially described as a polypeptide of 60 kDa. The gene has been cloned in several laboratories with accumulating evidence that there are isoforms in T-cell lines with at least two slightly different copies in the haploid genome [67–69]. The 60-kDa SSA/Ro contains an RNA-binding protein consensus motif [67, 68], which could account for its direct interaction with small cytoplasmic hY-RNAs [70]. More recent studies demonstrate that the "zinc finger" in human 60-kDa SSA/Ro is not conserved across species [71]. It has been

suggested that 60-kDa SSA/Ro may function as part of a novel quality control or discard pathway for 5S rRNA production in *Xenopus* oocytes [72].

Anti-SSB/La antibodies recognize a 48-kDa polypeptide that does not share antigenic determinants with either 52-kDa or 60-kDa SSA/Ro [73]. The SSB/La polypeptide is composed of at least two structural domains on the native protein, each of which contains a distinct antigenic-binding site [74]. SSB/La facilitates maturation of RNA polymerase III transcripts, directly binds a spectrum of RNAs, and associates at least transiently with 60-kDa SSA/Ro [75, 76].

In addition to the well-characterized 60-kDa SSA/Ro and 48-kDa SSB/La antigens, another target of the autoimmune response in mothers whose children have CHB is the 52-kDa SSA/Ro protein [77]. The full-length protein, 52α, has three distinct domains: an N-terminal region rich in cysteine/histidine motifs containing two distinct zinc fingers known as RING finger and B-box; a central region containing two coiled coils with heptad periodicity, one being a leucine zipper with potential for intramolecular dimerization; and a C-terminal "rfp-like" domain [78, 79]. The 52-kDa SSA/Ro protein has a high degree of homology with the ret finger protein, *rfp*, which is part of the transforming gene *ret*; this raises the question of whether the 52-kDa SSA/Ro protein might have similar transforming potential [78]. Moreover, the *ret* protein has the structure of a cell surface receptor.

An alternatively spliced transcript of full-length 52-kDa SSA/Ro has been identified, 52β [80], in which exon 4 encoding amino acids 168–245, inclusive of the leucine zipper and an immunodominant epitope [81, 82], is deleted. Although there is no evidence that the newly described 52β directly contributes to the pathogenesis of CHB, two requisite conditions for its potential involvement have been satisfied. First, 52β is recognized by autoantibodies from the sera of mothers whose children have CHB [80], consistent with reports of an additional N-terminal epitope on 52α, which is present in 52β [81, 82]. Second, 52β is present in cardiac tissue [80]. In contrast to 52α, 52β mRNA is preferentially expressed between 14 and 16 weeks of gestation, which precedes the clinical detection of CHB by several weeks [83]. Curiously, the increased expression of 52β is not observed in all 14- to 16-week hearts. This could simply reflect inaccuracies in dating the gestational age by sonogram. Alternatively, some fetal hearts may express more 52β at the same gestational age than others, which contributes, in part, to the low percentage (1–2%) of affected fetuses born to mothers with candidate antibodies [12, 84–86].

Cloning and sequencing of a novel autoantigen identified by sera containing predominant reactivity with a 60-kDa SSA/Ro RNA binding protein were found to be >96% identical to rabbit calreticulin [87, 88]. Preliminary work demonstrated that many sera from mothers of children with CHB react with human calreticulin by ELISA [89]. This finding may be of potential relevance, as calreticulin is an acidic high-affinity calcium-binding protein of M_r 46,567 common to both sarcoplasmic reticulum membranes in muscle and endoplasmic membranes in nonmuscle tissues. The carboxyl-terminal end of calreticulin contains the tetrapeptide Lys-Asp-Glu-Leu (KDEL) salvage or endoplasmic reticulum retention signal [88]. Future work on the association of CHB and antibodies to proteins of the endoplasmic reticulum should be of considerable interest.

Eftekhari *et al*. [90] reported that antibodies reactive with the serotoninergic 5-hydroxytryptamine $(5\text{-HT})_{4A}$ receptor, cloned from human adult atrium, also bind 52-kDa SSA/Ro. Moreover, affinity-purified 5-HT_4 antibodies antagonized the serotonin-induced L-type Ca channel activation in human atrial cells. Two peptides in the C terminus of 52-kDa SSA/Ro, amino acids 365–382 and 380–396, were identified that shared some similarity with the 5-HT_4 receptor. The former was recognized by sera from mothers of children with neonatal lupus, and it was this peptide that was reported to be cross-reactive with peptide amino acids 165–185, derived from the second extracellular loop of the 5-HT_4 receptor. These findings are of particular importance, as over 75% of serum from mothers whose children have CHB contain antibodies to 52-kDa SSA/Ro as detected by ELISA, immunoblot, and immunoprecipitation [91, 92].

Given the intriguing possibility that antibodies to the 5-HT_4 receptor might represent the hitherto elusive reactivity, which could contribute directly to atrioventricular block, the 5-HT_4 receptor was examined as a target of the immune response in these mothers. Initial experiments demonstrated mRNA expression of the 5-HT_4 receptor in the human fetal atrium. Electrophysiologic studies established that human fetal atrial cells express functional 5-HT_4 receptors. Sera from 116 mothers enrolled in the research registry, whose children have CHB, were evaluated. Ninety-nine (85%) of these maternal sera contained antibodies to SSA/Ro, 84% of which were reactive with the 52-kDa SSA/Ro component by immunoblot. In sum, none of the 116 sera were reactive with the peptide spanning amino acids 165–185 of the serotoninergic receptor. Rabbit antisera, which recognized this peptide, did not react with 52-kDa SSA/Ro. Accordingly, although 5-HT_4 receptors are present and functional in the human fetal heart, maternal antibodies to the 5-HT_4 receptor are not associated with the development of CHB. These results are in agreement with the findings of Cavill *et al*. [93].

Novel Antigen/Antibody Systems

Maddison and colleagues [94] described a novel antigen of 57 kDa recognized by 8 (38%) of 21 mothers whose children have NLS. Partial sequencing of p57 DNA demonstrated that this antigen is distinct from SSA/Ro and SSB/La. Supporting a potential pathogenic role of this antibody was its absence in the cord blood of an infant with CHB despite readily detectable circulating maternal levels. The authors proposed that this finding could be explained by selective cardiac depletion due to deposition on fetal cardiocytes. Alternatively, the antibody may not have been transported effectively across the placenta.

Although it is not known how maternal antibodies influence the development of cardiac versus cutaneous manifestations of NLS, antibodies to U1RNP in the absence of reactivity to anti-SSA/Ro and/or SSB/La have never been reported in children with CHB. Sheth and colleagues [95] have added two cases to eight reported previously in which anti-U1RNP antibodies but not anti-SSA/Ro-SSB/La antibodies were present in infants with cutaneous disease alone. Furthermore, Solomon *et al.* [96] described anti-U1RNP-positive, anti-SSA/Ro-SSB/La-negative dizygotic twins discordant for cutaneous manifestations of NLS (neither twin had cardiac disease). The segregation of anti-U1RNP antibodies with cutaneous disease may be a useful maternal marker and should guide research efforts in sorting out cardiac versus cutaneous susceptibility to antibody-mediated injury.

Evaluation of the Fine Specificities of the Maternal SSA/Ro-SSB/La Autoantibody Response

Important from the clinician's perspective are issues regarding the management of a pregnancy and the outcome of both the mother and her child. Can our rapidly expanding knowledge of SSA/Ro and SSB/La autoantibodies and their cognate antigens help define the mother at highest risk for having a pregnancy complicated by NLS? While seemingly straightforward, the task is a challenging one. Several laboratories from the United States and abroad have tackled this problem using various techniques, including immunodiffusion, immunoblot of various tissues and cell lysates, ELISA employing recombinant and purified proteins, and immunoprecipitation of radiolabeled *in vitro* translation products and cell lysates.

Our laboratory has used gel separation methods, which vary the quantity of acrylamide to bis-acrylamide to obtain a more precise molecular characterization of the relevant antigenic structures identified by the immune response in women whose children have NLS [17, 91, 97]. Specifically, increasing the ratio of monomer to cross-linker from 37.5 (used in a standard Laemmli buffer system [98]) to 172.4 in a 15% acrylamide solution readily separates the 48-kDa SSB/La polypeptide from the 52-kDa SSA/Ro component. Figure 4 demonstrates representative reactivities (48-kDa SSB/La, 52-kDa SSA/Ro, 60-kDa SSA/Ro) in an immunoblot (using a high ratio gel) of a human fetal heart lysate

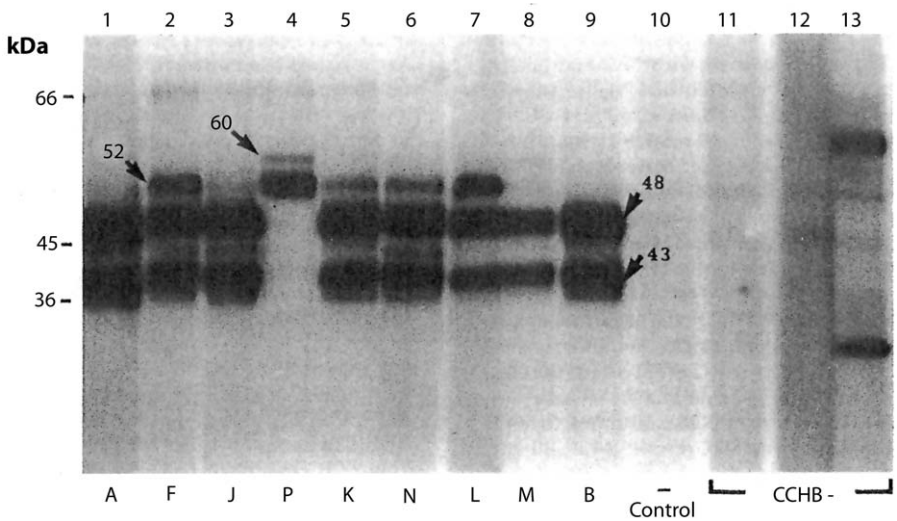


FIGURE 4 Representative immunoblot using fetal cardiac tissue aged 18 weeks and probed with sera from mothers of affected and unaffected offspring. Letters under lanes 1–9 correspond to sera of mothers of children with CHB. Lane 10 was probed with serum from a normal donor. Lanes 11–13 were probed with sera from three pregnant lupus patients carrying healthy fetuses. From Buyon *et al.* [17], with permission.

probed with antisera from mothers of children with CHB.

We have employed this method of gel separation for immunoblot, as well as ELISA, to evaluate antibody frequency, titer, and fine specificity in the sera from four groups of mothers segregated according to the status of the child: 57 whose children had CHB, 12 whose children had transient dermatologic or hepatic manifestations of NLS but no detectable cardiac involvement, 152 with SLE and related autoimmune diseases who gave birth to healthy children, and 30 with autoimmune diseases whose pregnancy resulted in miscarriage, fetal demise, or early postpartum death unrelated to NLS [91]. Anti-SSA/Ro antibodies were identified by ELISA in 100, 91, 47, and 43% of mothers of infants with CHB, transient NLS, healthy children, and fetal demise, respectively. High titers of anti-SSA/Ro antibodies were present more often in mothers of children with cardiac or cutaneous manifestations of NLS than in either of the other two groups. Maternal antibodies to SSB/La were detected by ELISA in 76% of the CHB group, 73% of the cutaneous/hepatic group, 15% with healthy children, and 7% with fetal demise. On immunoblot, 91% of the heart block group who had antibodies to SSA/Ro but not to SSB/La recognized at least one SSA/Ro antigen, with significantly greater reactivity against the 52-kDa component. In contrast, 62% of the anti-SSA/Ro-positive–SSB/La-negative responders in the healthy group recognized either the 52-kDa and/or 60-kDa components. Although there was no profile of anti-SSA/Ro response unique to the mothers of children with heart block or cutaneous manifestations of NLS, only 1% of normal infants were born of mothers with antibodies directed to both the 52-kDa SSA/Ro and 48-kDa SSA/La antigens and not to the 60-kDa SSA/Ro antigen, compared to 21% with cardiac and 25% with cutaneous manifestations of NLS.

Dörner *et al*. [99] investigated quantitative and qualitative differences of anti-SSA/Ro-SSB/La antibodies by ELISA in sera from 16 infants with CHB and their mothers compared to 8 healthy anti-SSA/Ro-positive infants born to SLE mothers. No serum sample contained IgM autoantibodies. All 16 (100%) CHB infants had anti-52-kDa SSA/Ro antibodies, 14 (88%) had anti-SSB/La, and 9 (56%) had anti-60-kDa SSA/Ro compared to 6 (75%) anti-52, 3 (38%) anti-48, and 2 (25%) anti-60 in the control infants. The anti-52-kDa SSA/Ro and anti-SSB/La antibody levels were significantly higher in CHB infants than in the controls. The anti-60-kDa SSA/Ro IgG levels of sera from infants and mothers from the CHB and control groups were similar.

Based on analysis by immunoblot and ELISA of sera from 14 mothers of children with CHB and 12 lupus patients with healthy offspring, Meilof *et al*. [100] concluded that the fine specificity of the autoantibody response to SSA/Ro and SSB/La proteins does not predict the occurrence of CHB. These results are not surprising, as it can be predicted from the disparity of disease expression in twins and the low recurrence rate in subsequent pregnancies (see later) that another factor (probably fetal) is operative and that the discovery of a unique risk profile is unlikely.

In a study of sera from 31 mothers of children with CHB, Julkunen *et al*. [92] demonstrated 97% reactivity with 52-kDa SSA/Ro by ELISA, 77% with 60-kDa SSA/Ro, and 39% with SSB/La. Compared to 45 mothers with SLE and healthy children, mothers of CHB children had higher titers of antibodies to recombinant 52- and 60-kDa SSA/Ro proteins. However, compared to 19 mothers with primary SS and healthy offspring, the autoantibody responses were similar. No differences in the titer of anti-SSB/La antibodies were found between mothers within either of these three groups. In agreement with previous data, these investigators did not find a specific antibody profile unique to CHB.

Silverman and colleagues [101] evaluated the maternal antibody profile in two groups of sera: 41 obtained from mothers whose children had manifestations of NLS (21 CHB, 20 cutaneous) and 19 from lupus patients known to have anti-SSA/Ro and/or anti-SSB/La antibodies and healthy children. Significantly higher levels of anti-SSB/La and anti-52-kDa SSA/Ro antibodies were demonstrated in the mothers of affected children. Mothers whose children had cutaneous manifestations had higher titers of anti-SSB/La antibodies than mothers whose children had CHB. Fine delineation of the anti-SSB/La responses revealed that a small carboxyl terminus polypeptide of recombinant SSB/La, DD, was recognized by 30% of mothers whose children had NLS but none who had healthy children.

Defining the risk of CHB based on a particular antibody profile depends in part on the control group chosen. For example, if the control group is mothers with SLE, as in our study done in 1993 [91] and that of Dörner *et al*. [99], it would not be surprising to find a greater frequency of anti-48-kDa SSB/La and 52-kDa SSA/Ro in CHB mothers. It has been reported previously that anti-60-kDa responses predominate over anti-52-kDa responses in SLE [102]. If the control mothers have SS, the antibody profiles of the CHB mothers and those with healthy offspring might be equivalent as supported by the studies of Julkunen *et al*. [92, 103]. In effect, these observations reinforce the findings of most studies that the serologic profile of mothers whose children have CHB closely resembles that of SS. One approach to defining risk would be to subset the mothers of children with CHB and compare "SS-CHB" to "SS-healthy" and "SLE-CHB" to "SLE-healthy."

However, because many mothers are asymptomatic and only identified by the birth of their affected child, an appropriate control group is difficult to assemble.

The fact that SDS immunoblot favors the recognition of denatured epitopes complicates the interpretation of antibody reactivity. It is likely that all anti-SSA/Ro responses would include reactivity with the 60-kDa SSA/Ro component if immunoprecipitation of the native protein was the assay employed [104]. Perhaps instead of defining a high-risk profile we should define a low-risk profile. In the author's experience, mothers who do not have anti-SSB/La antibodies and have anti-SSA/Ro antibodies of low titer that do not recognize either the 60-kDa or the 52-kDa component on SDS immunoblot appear to be at lower risk [105]. As of this writing, sera have been evaluated from 150 mothers whose children have CHB. All had antibodies to SSA/Ro or SSB/La by ELISA. An isolated response to the denatured 60-kDa component on immunoblot was observed in one (<1%) serum and only two sera (<2%) did not recognize any component of SSA/Ro or SSB/La on immunoblot (unpublished observation). However, this response represents only a small fraction of the anti-SSA/Ro responders in general.

A note of caution: reporting of antibody profiles is highly dependent on the assays employed. For example, Neidenbach and Sahn [106] reported an infant with cutaneous manifestations of NLS associated with maternal antibodies to SSB/La in the absence of associated antibodies to SSA/Ro. Testing was done by ELISA and immunoblot. In the author's experience and that of others, immunoprecipitation of the native 60-kDa SSA/Ro antigen has always been positive when anti-SSB/La antibodies are present [105], and such may well be the case in this "unique" infant.

Isacovics and Silverman [107] have utilized limiting dilution experiments of Epstein–Barr virus (EBV)-infected peripheral blood mononuclear cells to examine the frequency of anti-SSA/Ro and anti-SSB/La antibody secreting cells in mothers whose children have NLS [107]. These authors reported that a low anti-SSA/Ro or anti-SSB/La antibody B-cell precursor frequency tends to be associated with the birth of a child with NLS. Additionally, mothers of children with NLS who later developed SLE have higher anti-SSA/Ro and anti-SSB/La antibody-committed B cells than mothers who remained asymptomatic.

PROPOSED MECHANISMS OF IMMUNE INJURY

This model of passively acquired autoimmunity offers an exceptional opportunity to examine the effector arm of immunity and to define the pathogenicity of an autoantibody in mediating tissue injury. A molecular scenario in which maternal anti-SSA/Ro-SSB/La antibodies convincingly contribute to the pathogenesis of cardiac scarring has yet to be formulated. Two points, one clinical and the other cellular, are particularly difficult to reconcile. Only 2% of neonates born to mothers with the candidate antibodies have CHB [86], yet these antibodies are present in over 85% of mothers whose fetuses are identified with conduction abnormalities in a structurally normal heart [105]. Accordingly, the antibodies are necessary but insufficient to cause CHB. The final pathway leading to fibrosis may be variable, kept totally in check in most fetuses (normal sinus rhythm), subclinical in others (first degree block), and fully executed in very few (advanced block). The intracellular location of the target antigens raises questions regarding accessibility to maternal antibodies.

An overview of a proposed cascade to cardiac injury initiated by maternal anti-SSA/Ro-SSB/La antibodies, novel autoantibodies unique to the conduction system, or cross-reactive antibodies is shown in Fig. 5.

Accessibility of Fetal Antigen to Maternal Antibody

A mechanism whereby antibodies might interrupt critical intracellular events in fetal cardiomyocytes or specialized Purkinje cells is largely unknown. As stated previously by Tan [108], "the question is whether autoantibody reacts with intrinsic antigens to perturb the biologic function of normal cells." For anti-SSA/Ro and -SSB/La antibodies to be causal in the development of NLS, three basic requirements should be satisfied: (1) the candidate antigens must be present in the target fetal tissues; (2) the cognate maternal autoantibodies must be present in the fetal circulation; and (3) these antigens must be accessible to the maternal antibodies.

Earlier studies have firmly demonstrated reactivity of anti-SSA/Ro antibodies with fetal cardiac tissues, including the conduction system [17, 38, 109] (see Fig. 4). The preferential vulnerability of the fetal heart versus the adult heart is addressed by a study demonstrating that a 23-week fetal heart contained a greater quantity of SSA/Ro per milligram protein than 18- to 22-week hearts or an adult heart [110]. The presence of SSA/Ro and SSB/La antigens in the fetal heart is well established and therefore satisfies the first requirement. Similarly the second requirement has been fulfilled by studies demonstrating anti-SSA/Ro-SSB/La antibodies in the fetal circulation as assessed by measurements in cord blood [36, 111].

The third requirement, accessibility, has been more difficult to establish. It has been suggested that autoantibodies can penetrate living cells, subsequently alter

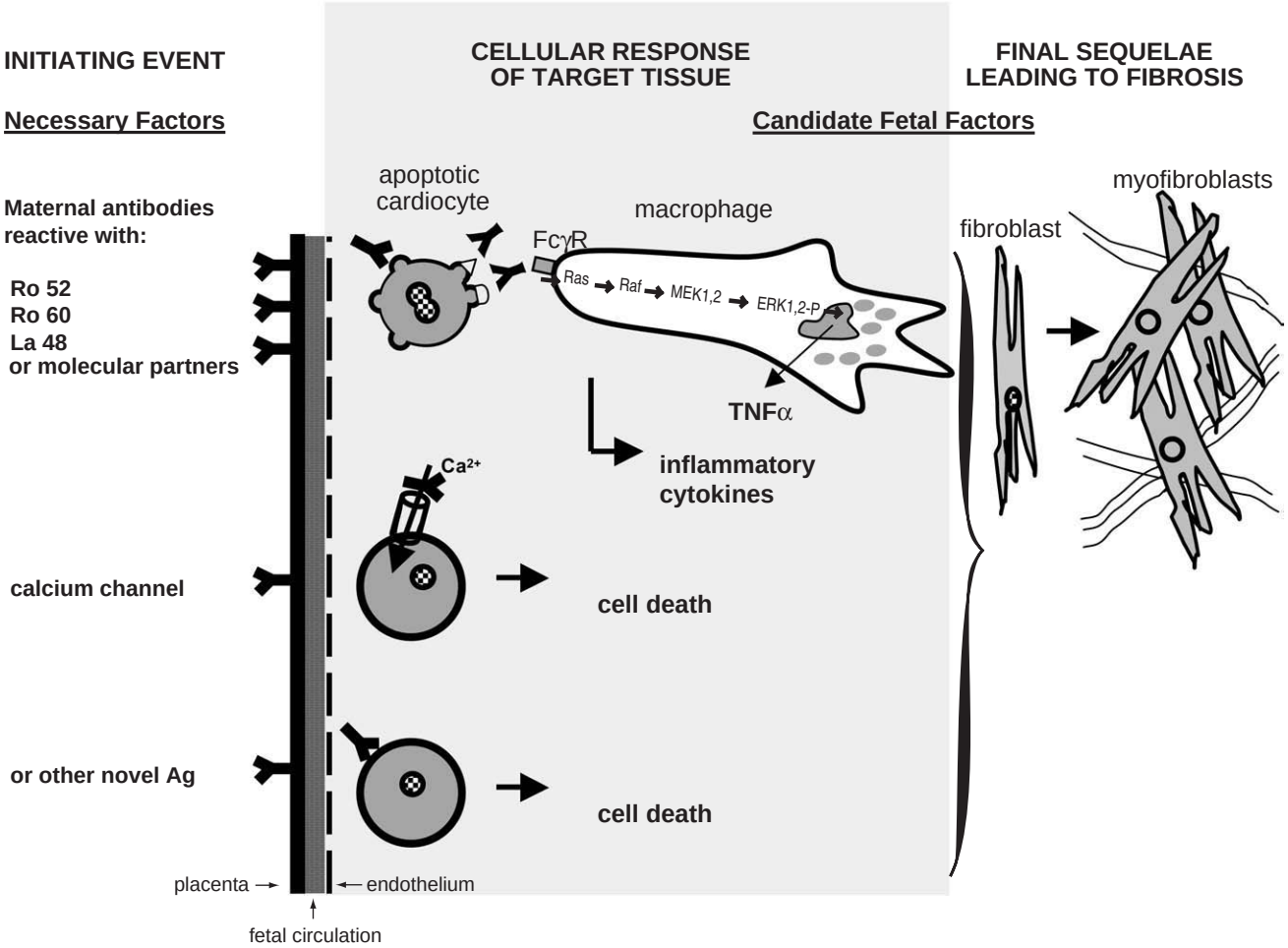


FIGURE 5 Putative mechanisms of autoantibody-induced injury leading to CHB. An overview of a proposed cascade to cardiac injury initiated by maternal anti-SSA/Ro-SSB/La antibodies, novel autoantibodies unique to the conduction system, or cross-reactive antibodies.

function, and cause cell death [112], but this notion still remains controversial. Alternatively, if the antibody cannot cross the cell membrane, then is the antigen trafficked to the cell surface? Finally, anti-SSA/Ro-SSB/La antibodies could cross-react with other surface cardiac antigens.

Several lines of evidence have been advanced to support the possibility that otherwise sequestered intracellular autoantigens can be expressed on the cell surface. Baboonian *et al*. [113] have demonstrated the sequential expression of the SSB/La antigen from the nucleus through the cytoplasm and ultimately on the cell surface of Hep 2 cells infected with adenovirus. Accumulating data support the surface expression of SSA/Ro in keratinocytes, after exposure to ultraviolet light [114–116] or following incubation with TNF-α [117]. Perhaps most relevant to NLS, 17β-estradiol at 10^{-7} M, a concentration reached during the third trimester of pregnancy [118], enhances binding of anti-SSA/Ro antibodies to keratinocytes [119] and induces up to five-fold increases in the expression of 52α and 60Ro mRNA [120]. Biologic significance is strengthened by the reports of estrogen receptors in cardiac tissue [121, 122]. Furthermore, one mismatch consensus estrogen response element was identified upstream of the transcriptional start site in the gene encoding 52-kDa SSA/Ro, and an estrogen response element similar to that of c-*myc* was detected in the human gene encoding 60-kDa SSA/Ro [120].

Reichlin *et al*. [123] have bolstered support for the accessibility of the candidate antigens to the respective maternal antibodies by the finding of antibodies to native 60-kDa SSA/Ro and denatured 52-kDa SSA/Ro in acid eluates of a heart from a fetus with CHB who died at 34 weeks gestation. The enrichment was apparently selective, as these antibodies were not detected in

eluates from the brain, kidney, or skin. Furthermore, Horsfall *et al*. [124] have demonstrated maternal IgG-bearing anti-SSB/La idiotypes on the surface of fetal myocardial fibers on autopsy of a neonate with CHB.

Apoptosis has been traditionally conceptualized from an immunologic point of view as either a means of maintaining B- and T-cell tolerance [125, 126] or as a mechanism for providing accessibility of intracellular antigens to induce an immune response [127]. Casciola-Rosen *et al*. [127] have demonstrated that autoantigens are clustered in two distinct populations of surface blebs on keratinocytes. The larger blebs, so-called apoptotic bodies derived from the apoptotic nucleus, contain both SSA/Ro and SSB/La proteins with SSB/La detected at the cell surface surrounding large blebs in the later stages of apoptosis. The 52-kDa protein was not specifically identified but rather deduced, as evaluation was done with a patient serum considered "monospecific" for 52-kDa SSA/Ro antibodies. The smaller blebs, arising from fragmented rough endoplasmic reticulum and ribosomes, contain SSA/Ro presumably of cytoplasmic origin. SSB/La was not contained in these blebs.

Apoptosis may be relevant in the pathogenesis of NLS. It is a selective process of physiological cell deletion in embryogenesis and normal tissue turnover and plays an important role in shaping morphological and functional maturity [128]. Apoptosis is a process that affects scattered single cells rather than tracts of contiguous cells. In the normal adult myocardium, apoptosis has been observed only rarely [129, 130]. In contrast, apoptosis does occur during the development of the heart. In the 1970s, Pexeider [131] extensively characterized the temporal and spatial distribution of cell death in the hearts of chicken, rat, and human embryos. Major foci included the AV cushions and their zones of fusion, the bulbar cushions and their zones of fusion, and the aortic and pulmonary valves. Albeit much of the cell death was noted in nonmyocytes, a focus of myocyte death was apparent in the muscular interventricular septum as it grew toward the AV cushions in midgestation. Takeda and colleagues [132] demonstrated apoptosis in midgestational rat hearts using terminal deoxynucleotidyl transferase dUTP nick end labeling, an *in situ* technique that detects DNA strand breaks in tissue sections. Although not coincident with the precise timing of CHB, it has also been suggested that apoptosis contributes to the postnatal morphogenesis of the SA node, AV node, and His bundle [133]. Perhaps a novel view of apoptosis is that it facilitates the placing of cardiac target autoantigens in a location accessible to previously generated maternal autoantibodies. Tissue damage might be a consequence of being in the right place at the wrong time.

To investigate the hypothesis that apoptosis indeed facilitates the accessibility of SSA/Ro and SSB/La in the heart, cultured human fetal cardiac myocytes were incubated with staurosporine or 2,3-dimethoxy-1,4-naphthoquinone (DMNQ) [134]. Using phase-contrast microscopy, morphologic signs of early apoptosis were observed in 40% of the cardiocytes after approximately 4 h and increased to 95% after 7 h. The cellular topology of SSA/Ro and SSB/La was evaluated with confocal microscopy and was determined in nonapoptotic and apoptotic cardiocytes by indirect immunofluorescence using two previously characterized antisera: one "monospecific" anti-SSB/La and the other recognizing both 52- and 60-kDa SSA/Ro with goat antihuman IgG-FITC as the secondary antibody (Fig. 6). In nonapoptotic cardiocytes, SSA/Ro was predominantly nuclear with minor cytoplasmic staining and SSB/La was confined to the nucleus. In early apoptotic cardiocytes, condensation of the SSA/Ro- or SSB/La-stained nucleus was observed accompanied in some cells by a "ring" of bright green fluorescence around the periphery. In the later stages of apoptosis, nuclear SSA/Ro and SSB/La staining became weaker. Blebs could now be seen emerging from the cell surface, stained with both SSA/Ro or SSB/La. Scanning electron microscopy unambiguously confirmed the surface expression of SSA/Ro and SSB/La on cultured human fetal apoptotic cardiocytes (Fig. 7).

In vivo studies have confirmed the observations made *in vitro*. Tran and colleagues [135] have demonstrated the translocation of SSB/La in apoptotic cardiocytes in the conduction system of the unmanipulated mouse fetal heart. Clustering of SSB/La near the surface of apoptotic bodies occurs *in vivo* under physiological conditions. To access proof of concept and examine whether SSB/La and/or SSA/Ro epitopes on apoptotic cells are accessible for binding by Abs *in vivo*, these same investigators have exploited a murine passive transfer model in which the fate of human autoantibodies actively transported across the placenta could be traced in fetal tissues known to have high rates of apoptosis [136]. Specifically, BALB/c pregnant mice were injected with human anti-SSA/Ro-SSB/La serum, monospecific anti-Ro60 serum, affinity-purified anti-SSB/La, anti-dsDNA, or normal human serum. Apoptotic cells identified in the fetal conducting tissue (present under normal physiologic conditions of remodelling) showed redistribution of SSB/La from the nucleus to the surface of apoptotic bodies. Fetuses from anti-SSA/Ro-SSB/La Ab-injected mothers showed a striking colocalization of human IgG with apoptotic cells in the atrium, AV node, liver, skin (with particulate epidermal deposition), and newly forming bone. The IgG–apoptotic cell complexes were organ specific and

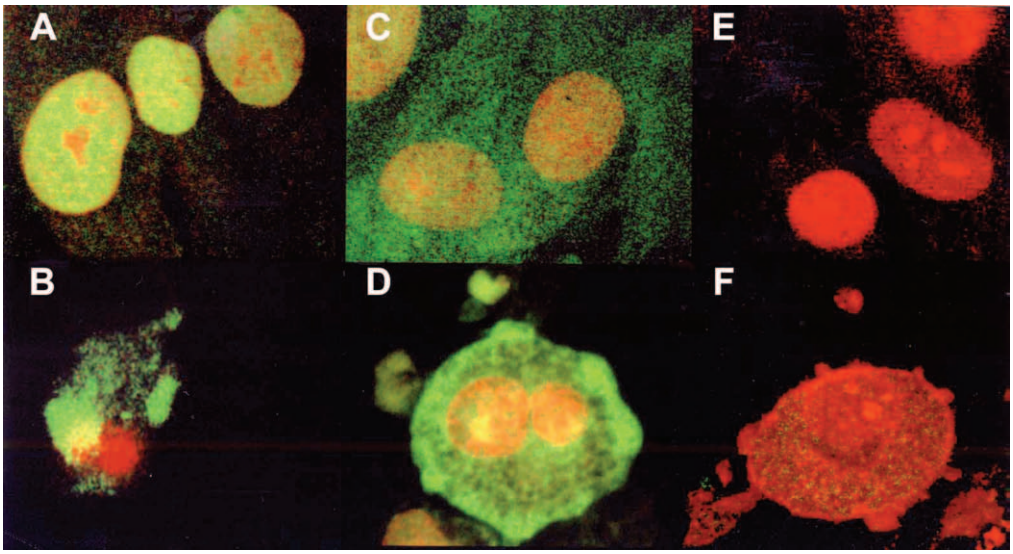


FIGURE 6 Cellular topology of SSA/Ro and SSB/La in nonapoptotic and apoptotic cultured human fetal cardiocytes. Cells were doublelabeled with PI (red) and antisera (FITC). (**A**) SSB/La stains the large nuclei in nonapoptotic cells. (**B**) It can be appreciated that SSB/La is increased in the cytoplasm and also concentrates in blebs. (**C**) SSA/Ro stains the nuclei and cytoplasm of nonapoptotic cells, whereas in apoptotic cells (**D**) there is translocation of SSA/Ro to the cell periphery and strong staining of blebs. (**E** and **F**) Normal human serum did not stain control or apoptotic cells as evidenced by the absence of green fluorescence.

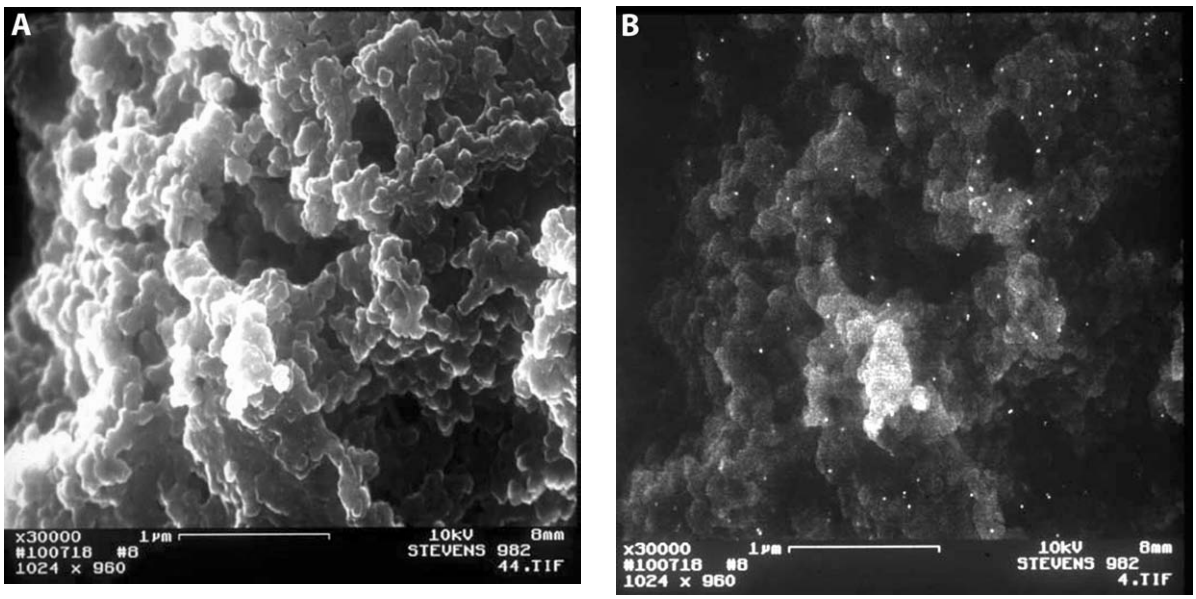


FIGURE 7 Scanning electron micrographs of cultured human fetal cardiocytes labeled with immunogold attached to a maternal serum containing anti-52-kDa and -60-kDa SSA/Ro. (**A**) High-power magnification showing the topography of an apoptotic human fetal cardiocyte. (**B**) Backscatter image demonstrating that the apoptotic surface is bound by sera containing anti-48-kDa SSB/La.

were not detected in thymus, lung, or gut. No IgG deposits were identified in fetuses from mothers injected with anti-dsDNA, anti-Ro60, or normal sera. Experiments with affinity-purified anti-SSB/La and anti-SSA/Ro-SSB/La Abs absorbed with SSB/La confirmed the specificity of deposited IgG as anti-SSB/La.

The demonstration (*in vitro* and *in vivo*) of antigen–antibody binding at the cell surface [135–137] supports the hypothesis that maternal antibodies to components of the SSA/Ro-SSB/La complex are not simply markers of disease but play an active part in the pathogenesis of CHB. A molecular explanation for subsequent damage to the specialized cells of the conduction system and working myocardium remains speculative. In this context, a mechanism is envisioned whereby unexpected circumstances convert the physiologic process of apoptosis into one in which an inflammatory component is evoked. Perhaps the unexpected event is opsonization. These findings suggest that circulating maternal autoantibodies opsonize cells undergoing physiological apoptosis, which then changes otherwise innocent degradation products into proinflammatory stimuli. This cascade could result in damage and ultimately permanent scarring in those tissues with low regenerative capacity.

To address the functional consequences of opsonization of apoptotic cardiocytes, we designed coincubation experiments with human macrophages [138]. The Th1 cytokine, TNF-α, was chosen as a readout of inflammation. Basal production of TNF-α by the macrophages was 9.7 ± 0.9 SEM pg/ml and decreased to 3.3 ± 0.3 SEM pg/ml after coincubation with apoptotic cells, which was not observed in initial experiments using cardiocytes rendered necrotic after hypotonic lysis. Apoptotic cardiocytes preincubated with normal human IgG acted functionally as nontreated apoptotic cells; TNF-α production by the macrophages was 5.7 ± 0.9 SEM pg/ml. In contrast, when macrophages were cocultured with apoptotic cardiocytes incubated with affinity-purified antibodies to each of the components of the Ro/La complex, TNF-α production was increased by 3- to 5-fold over basal levels and 10- to 14-fold over that secreted after culture with apoptotic cells alone. Nonapoptotic cardiocytes incubated with medium alone or serum containing antibodies reactive with 48-kDa SSB/La, 52-kDa SSA/Ro, and 60-kDa Ro did not modify the basal production of TNF-α by the macrophages. Preliminary studies demonstrated that macrophage-derived factors induce phenotypic changes in cardiac fibroblasts supportive of scarring [139].

Li and colleagues [140] reported that affinity-purified anti-SSB/La antibodies but not anti-52-kDa SSA/Ro antibodies recognize laminin, a high molecular weight, noncollagenous structural glycoprotein present on the sarcolemmal membrane of cardiocytes [140]. Several anti-SSB/La antibodies bound to the sarcolemmal membrane of human fetal cardiocytes aged 9–15 weeks of gestation as demonstrated by immunofluorescence on frozen sections. In contrast, binding to adult hearts was not observed. Experimental data suggested that the cros-sreactivity was dependent on the native SSB/La structure, as laminin was not recognized on the SDS immunoblot. These results support the hypothesis that molecular mimicry between laminin and SSB/La may account, in part, for the vulnerability of the fetal and not adult heart.

Complement Regulatory Proteins

With regard to the mechanism of tissue injury, consideration should be given to the possibility that protective molecules may be diminished on the surface of fetal cells. In adulthood the cells of an organism are protected from complement-mediated damage by membrane-bound proteins such as decay accelerating factor (DAF, CD55), protectin (CD59), and membrane cofactor protein (MCP, CD46) [141–143]. DAF regulates C3/C5 convertases, and CD59 regulates assembly of the terminal components of the membrane attack complex. MCP patrols cell membranes, inactivating the C4b and C3b that is bound inadvertently by acting as a cofactor for their factor I-mediated cleavage [144]. MCP is expressed on a wide variety of cells, including those of the epithelial, fibroblast, and endothelial cell lineage [145].

To examine fetal characteristics that might influence autoantibody-mediated diseases acquired *in utero*, such as heart block in NLS, the tissue expression of MCP was studied [146]. Immunoblots of organs from six fetuses (aged 19–24 weeks) probed with rabbit anti-MCP antibodies revealed a band at 60 kDa in addition to the known 65- and 55-kDa isoforms that comprise the codominant allelic system of MCP. Five fetuses expressed the most common MCP polymorphism (predominance of the 65-kDa isoform, upper band α phenotype) in the kidney, spleen, liver, and lung. In contrast, all hearts from these five fetuses demonstrated a different pattern in which there was a marked decrease in the intensity of the 65-kDa band and accentuation of the lower molecular weight bands. In a sixth fetus that expressed the second most common polymorphism (equal expression of the 65- and 55-kDa MCP isoforms, αβ phenotype), the heart was similar to the other tissues. Preferential expression of the MCP β isoform in five of six fetal hearts, irrespective of the phenotype of other organs, suggests tissue-specific RNA splicing or posttranslational modification. The clinical significance of this tissue-specific phenotype is unknown at present

but may provide an important clue to the susceptibility of the fetal heart to antibody-mediated damage.

Arrhythmogenicity of Maternal Autoantibodies and Perturbation of L-Type Calcium Channels

Two earlier publications, both in animal models, indirectly invoked arrhythmogenic effects of anti-SSA/Ro-SSB/La antibodies. Alexander *et al.* [147] reported that superfusion of newborn rabbit ventricular papillary muscles with IgG-enriched fractions from sera containing anti-SSA/Ro-SSB/La antibodies specifically reduced the plateau phase of the action potential consistent with an alteration of calcium influx. Using isolated adult rabbit hearts, Garcia *et al.* [148] showed that IgG fractions from women with anti-SSA/Ro-SSB/La antibodies induced conduction abnormalities and reduced the peak slow inward current (I_{Ca}) in patch-clamp experiments of isolated rabbit ventricular myocytes. Because conduction in the AV node is essentially dependent on calcium electrogenesis, an AV block would be expected to result from treatments leading to reduction of the I_{Ca} in ventricular myocytes. The L-type calcium channel is mainly responsible for I_{Ca} in ventricular myocytes and for the propagation of the action potential in the AV node.

These intriguing publications led to a collaborative effort with Dr. Mohamed Boutjdir (Brooklyn VA Medical Center, Brooklyn, NY) in which attention focused on the human fetal heart [149]. To assess the effect of IgG fractions and affinity-purified antibodies on conduction and heart rate, EKG recordings were obtained from whole human fetal hearts, aged 18–24 weeks (Fig. 8). Baseline EKGs were recorded after a stabilization period of 30–45 min. Perfusion of the heart for 27 min with purified anti-52-kDa SSA/Ro antibodies from three mothers whose children had CHB resulted in bradycardia associated with widening of the QRS complex that could represent a bundle branch block or an intraventricular defect in the conducting system. The average increase in R-R and P-P interval corresponded to 32 and 30%, respectively. At 33 min of perfusion, a complete AV block was diagnosed with the presence of only P waves and missing QRS complexes. Reperfusion of the heart with antibody-free Tyrode's solution for 48 min resulted in partial and slow recovery. In contrast, IgG from four control mothers did not have any measurable effect on AV conduction. These findings were further characterized by studying the effects of IgG fractions and affinity-purified anti-52-kDa SSA/Ro antibodies on whole cell L-type I_{Ca} recorded by the patch-clamp technique [150]. As seen in Fig. 9B, IgG from a mother whose children had CHB,

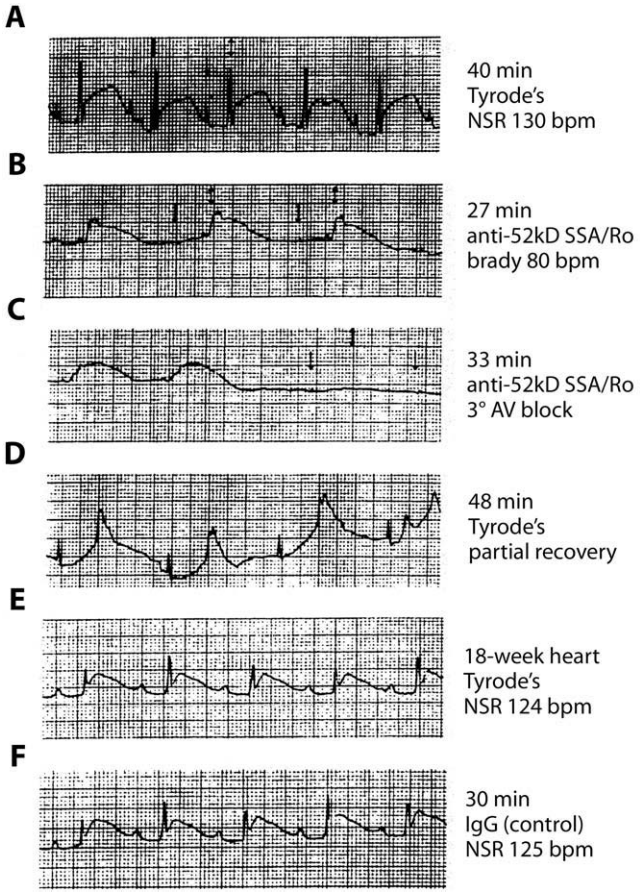


FIGURE 8 Effect of affinity-purified anti-52-kDa SSA/Ro antibody on the EKG recording of a 24-week human fetal heart. After 40 min of stabilization in Tyrode's solution, a regular sinus rhythm at 130 bpm (**A**) was recorded (lead I: horizontal scale, 50 mm/s; vertical scale, 10 mm/mV). Twenty-seven minutes after infusion of anti-52-kDa SSA/Ro antibody (800 µg/ml), there was significant bradycardia (80 bpm) associated with a widening of the QRS complex (**B**) that could represent bundle branch block or an intraventricular defect in the conducting system. (**C**) After 33 min, complete AV block was diagnosed with the presence of only P waves (indicated by single-headed arrows) and missing QRS (indicated by double-headed arrow). Perfusion of the heart with antibody-free Tyrode's solution for 48 min resulted in partial recovery (**D**). (**E** and **F**) EKG recordings from an 18-week human fetal heart during control conditions (**E**) and after 30 min of perfusion with 800 µg/ml of normal IgG (**F**). Normal IgG did not induce any conduction abnormalities or change in heart rate (124 bpm during control vs 125 bpm with normal IgG). From Boutjdir *et al.* [149], with permission.

but not from a control mother, inhibited peak I_{Ca} at all voltages tested. The average inhibition at 0 mV was 59%. Similarly, affinity-purified anti-52-kDa SSA/Ro antibodies from three CHB mothers inhibited peak I_{Ca} by 56% at 0 mV (Fig. 9C). Accordingly, inhibition of I_{Ca} by the autoantibodies in isolated myocytes further

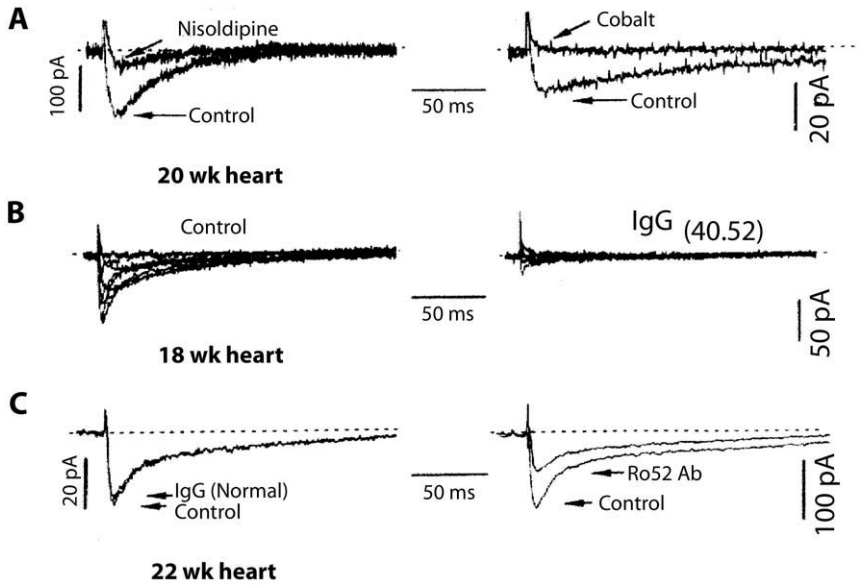


FIGURE 9 Effect of IgG and affinity-purified anti-52-kDa SSA/Ro antibodies on whole cell I_{Ca}. (**A**, left) I_{Ca} tracings elicited by a 200-ms test pulse to 0 mV preceded by a 100-ms prepulse to -40 mV from a holding potential of -80 mV every 10 s. I_{Ca} on the left and right were recorded from a cell of 20- and 18-week hearts, respectively. Application of nisoldipine (2 μmol/liter) or cobalt (5 mmol/liter) inhibited I_{Ca}. (**B**) A series of time- and voltage-dependent I_{Ca} tracings recorded from a cell of an 18-week heart at voltages ranging between –30 and 160 mV with a 10-mV increment during control and during the steady-state effect of 80 μg/ml of IgG from a mother whose child has CHB. (**C**) The lack of IgG effect from a control mother on I_{Ca} in a cell from a 22-week heart. However, the affinity-purified anti-52-kDa SSA/Ro antibody from a second CHB mother inhibited peak I_{Ca} by 46% in another cell from a 22-week heart. From Boutjdir *et al.* [149], with permission.

supports the contribution of Ca^{2+} channels to the conduction abnormalities observed in the whole heart.

The biophysical properties by which the autoantibodies inhibited whole cell I_{Ca} were then investigated at the single channel level using the cell-attached configuration of the patch-clamp method [150]. Barium currents were recorded through Ca^{2+} channels as described [151]. Bath application of affinity-purified anti-52-kDa SSA/Ro antibody from two CHB mothers produced a significant decrease in the Ca^{2+} channel activity and the ensemble average current. The ensemble average currents decreased from 0.23 to 0.13 pA (–43%, $P < 0.02$). Similar inhibition was obtained with IgG from two CHB mothers, but no significant effect was observed with IgG from three control mothers. Analysis of single channel kinetics indicated that this inhibition was the result of shorter open times and longer closed times, which could also explain the basis of the whole cell I_{Ca} inhibition by the autoantibody. The effect of the affinity-purified antibody and IgG from mothers whose children have CHB was less pronounced in the cell-attached than the whole-cell recordings, suggesting involvement of a diffusible cytosolic constituent in mediating the response to autoantibodies. Xiao and colleagues [152] have extended this work and reported a direct interaction of maternal anti-SSA/Ro-SSB/La antibodies with the pore-forming α (1)-subunit of Ca channels using transfected *Xenopus* oocytes, a finding of interest that remains to be confirmed.

Given data in the rabbit and human heart it is tempting to conclude that inhibition of L-type Ca^{2+} channels explains the pathogenicity of anti-SSA/Ro (perhaps anti-SSB/La) antibodies in the development of CHB. Several facts are highly supportive of this conclusion. AV nodal electrogenesis is dependent on L-type Ca^{2+} currents. Ca^{2+} channel density is lower and sarcoplasmic reticulum less abundant in fetal compared to adult cardiac cells, increasing the dependency on transsarcolemmal Ca^{2+} entry [153]. Prolonged exposure of fetal Ca^{2+} channels to maternal anti-SSA/Ro-SSB/La antibodies may lead to internalization and degradation of the channel, cell death, and ultimately fibrosis. Inhibition of ventricular Ca^{2+} channels may result in decreased contraction and congestive failure. Alternatively, antibody binding to the channel may result in opsonization and phagocytosis by macrophages with inflammatory/fibrotic sequelae (similar to the apoptosis theory described earlier).

SA nodal electrogenesis is also dependent on L-type Ca^{2+} currents [154]. Interestingly, detailed evaluation of autopsies done on children in the Research Registry for Neonatal Lupus revealed pathology in some cases at the SA node (as described earlier) [30]. Mazel *et al.* [155] observed sinus bradycardia in a murine model of passive immunity with anti-SSA/Ro antibodies. Highly relevant to these histologic and functional observations is Brucato's identification of sinus bradycardia in 4 of 24 EKGs from otherwise healthy newborns born to the cohort of mothers with SSA/Ro antibodies [86]. Of 187 cases in the research registry [41], atrial rates from postnatal EKGs were available for 40 neonates; the mean rate was 137 bpm ± 20 SD (range 75–200). The one slow rate of 75 bpm was obtained during sleep and increased to 140 bpm when awake. In an additional child, the records stated sinus bradycardia; however, no EKG was available and subsequent records were not sent to the registry. In both the author's review and the study by Brucato the sinus bradycardia was not permanent. Although this suggests that the nature of the initial insult and/or the subsequent reparative processes may be different for the SA and AV nodes, normal atrial rates may reflect the functioning of other pacer foci in the atria.

Murine Model of CHB

While clinical data leave little doubt regarding the association of anti-SSA/Ro and/or SSB/La antibodies with the development of CHB, and experimental data are beginning to suggest pathogenicity, efforts to establish an animal model have been limited. Kalush *et al.* [154] reported that offspring of BALB/c mice immunized with the monoclonal anti-DNA idiotype 16/6 had conduction abnormalities. Of 31 pups born to mothers with experimental SLE, 8 had first-degree heart block, 2 had second-degree heart block, 2 had complete block, 10 had bradycardia, and 8 demonstrated widening of the QRS complex. None of these disorders could be detected in the 20 offspring of healthy control mice. One of the difficulties in interpreting these findings is that the immunized mothers synthesized a variety of autoantibodies, including antibodies reactive with 16/6 Id, ss/dsDNA, Sm, RNP, cardiolipin, SSA/Ro, and SSB/La. Accordingly, it was not possible to segregate out which specific antibody might be responsible for the arrhythmias detected in these pups. The electrocardiographic data are provocative; however, no histologic data were provided to assess the status of the SA or AV node or the presence of myocarditis.

To further establish an antibody-specific murine model to correlate arrhythmogenic effects of maternal autoantibodies with the *in vivo* genesis of CHB, female BALB/C mice were immunized with 100 µg of one of the following 6× His human recombinant proteins purified by Ni^{2+} affinity chromatography: 48-kDa SSB/La, 60-kDa SSA/Ro, 52-kDa SSA/Ro (52α full-length), and 52β [157]. Control animals were given the same injections with a Ni^{2+} affinity-purified polypeptide encoded by pET-28 alone. Following primary immunization in complete Freund's adjuvant and two boosters (50 µg) in incomplete Freund's adjuvant, high-titer immune responses to the respective antigens were established by ELISA and immunoblot of recombinant antigens, and immunoprecipitation of [^{35}S]methionine-labeled *in vitro* translation products. Sera from mice immunized with either 52α or β immunoprecipitated radiolabeled murine 52-kDa SSA/Ro, confirming that these mice were specifically reactive with the murine homologue. Moreover, immunoblot of a newborn murine heart demonstrated the presence of 52-kDa SSA/Ro. Mice were mated and boosters continued every 3 weeks to ensure continued high-titer antibody responses. EKGs were performed on all pups using standard limb leads at birth or within 2 days postpartum. Maternal antibodies to the primary immunogens were detected by ELISA in the pups.

Results are summarized in Table 2. Of 54 pups born to six fertile mice immunized with 60-kDa SSA/Ro, none had CHB; of 27 pups born to three fertile mice immunized with 48-kDa SSB/La, none had CHB. In contrast, of 78 pups born to five fertile mice immunized with 52α and 86 pups born to five fertile mice immunized with 52β, 1 and 5 pups, respectively, had CHB. Accordingly, this antibody-specific animal model provides strong preliminary evidence for a pathogenic role of antibodies reactive with 52-kDa SSA/Ro, particularly the 52β form, in the development of CHB. Moreover, analogous to the frequency of 1–5% given for women with SLE who have anti-SSA/Ro and/or SSB/La antibodies [84–86], this model suggests that additional factors promote disease expression.

Lessons from Monozygotic and Dizygotic Twins

The study of twin gestations further emphasizes the complexity of NLS. These informative *in vivo* experiments of nature provide clues to the true relevance of the maternal antibody and host factors. Discordance of disease expression in monozygotic twins would be particularly intriguing because the placenta is shared and the fetal genetics are identical. Twins born to mothers with anti-SSA/Ro-SSB/La antibodies provide a unique opportunity to gauge the effect of a specific antibody profile on disease phenotype. Regardless of whether twins share a common placenta or not, it is likely that

TABLE 2 Murine Model of CHB

Immunogen	Mothers immunized (*N*)	Mothers fertile (*N*)	Pups (*N*)	AV block		
				I (*N*)	II (*N*)	III (*N*)
48-kDa SSB/La	10	3	27	2	0	0
52α SSA/Ro	8	5	78	3	1	1
52β SSA/Ro	5	5	86	5	0	5[a]
60-kDa SSA/Ro	7	6	54	10	0	0
β-Galactosidase	3	2	11	0	0	0
Vector	4	4	43	0	0	0

[a] One 52β mother had three pups with CHB in the same litter; another mother had one CHB pup.

even if a mother had three types of antibodies (type A causing CHB, type B causing skin rashes, and type C being nonpathogenic) the proportion of these antibodies should be similar in each fetal circulation. Types A and B by definition must be of the correct subclass and isotype (*i.e.,* IgG, subclasses 1–4) to be transported across the trophoblast, whereas type C might not be transported at all. One might envision quantitative differences in each twin, but it is difficult to conceive of a mechanism to explain why one placenta would transport antibodies more selectively than another unless Fc receptors were expressed differentially.

Of 23 twin pairs and one triplet set published [3, 6, 10, 39, 85, 96, 110, 158–169], only 6 are entirely concordant for disease expression (summarized in Table 3). Gawkrodger and Beveridge [158] described disparate disease expression in male twins who were in the same amniotic sac and shared a common placenta. The mother had anti-SSA/Ro and anti-SSB/La antibodies. One infant presented at 8 weeks of age with Stokes Adams attack and second-degree heart block, a facial rash, and evidence of hemolytic anemia, whereas his twin brother had merely a "peeling" facial rash at 15 weeks of age. Cooley *et al.* [159] reported two sets of monozygotic twins discordant for CHB. Watson *et al.* [163] performed HLA studies and C4 phenotypes on dizygotic twins discordant for isolated CHB. Both infants had identical HLA (DR4,DR7) and C4 phenotypes (C4A3.QOB1.1). Serum from the mother and both cord bloods demonstrated anti-SSA/Ro and SSB/La antibodies with no significant difference in titer noted in the cord blood of either twin when compared with maternal titers. Brucato *et al.* [164] reported a second pair of HLA identical twins discordant for CHB, and a third such pair was reported by Kaaja *et al.* [165]. In the research registry there were three sets of twins [39]. Two sets were dizygotic, with only one twin in each set having CHB. Histologic and genetic analysis of the third set revealed a twin placenta (diamniotic, dichorionic) and monozygosity. One twin had CHB and the other was completely healthy.

Certainly these observations support a fetal contribution to the development of neonatal lupus, but perhaps even more intriguing is that in two sets of twins [158, 162] and the set of triplets [169], all children were affected but with discordant manifestations. Accordingly, even if the antibody profile is an important predictor of risk for one manifestation of neonatal lupus versus another, the fetus (perhaps density of calcium channels, impaired healing, exuberant fibrosis) and/or its immediate environment (low oxygen) provides an additional factor(s) required to convert susceptibility to overt disease. The logical inference from these reports is that developmental events *in utero* strongly contribute to the susceptibility of a particular fetus.

IMMUNOGENETICS OF THE MOTHER AND HOST

The majority of studies to date have documented the near universal presence of DR3 alleles in the mothers of affected offspring, frequently associated with the extended haplotype A1,B8 [11, 103, 110, 170–172]. In addition to the reports of others, 10 of 11 affected mothers studied at our institution were HLA DR3. The one exception was DR7. For comparison, of 25 women with SLE or SS who gave birth to healthy offspring, 48% were DR3, (odds ratio = 16, $P < 0.003$). Several investigators have noted an increased frequency of HLA-DR2 in anti-SSA/Ro-positive mothers of normal infants [11, 171]. This is not surprising, as sera from the majority of mothers whose offspring have CHB contain both anti-SSA/Ro and -SSB/La antibodies and not anti-SSA/Ro alone [14, 17, 91]. It is the former subgroup that is more strongly associated with the linked HLA alleles B8, DR3, DRw52, DQw2 and the latter subgroup with DR2, DQw1 [173].

TABLE 3 Neonatal Lupus in Twins/Triplets

Study	Maternal serology	Zygosity	Disease manifestation[a]		
			Sib 1	Sib 2	Sib 3
Gawkrodger and Beveridge [158]	SSA/Ro, SSB/La	Monozygotic	CHB/rash/anemia	Rash	
Cooley *et al*. [159]	SSA/Ro	Monozygotic	CHB	Healthy	
Cooley *et al*. [159]	SSA/Ro	Monozygotic	CHB	Healthy	
Buyon *et al*. [39]	SSA/Ro, SSB/La	Monozygotic, dichorionic	CHB	Healthy	
Siren *et al*. [160]	SSA/Ro, SSB/La	Monozygotic, HLA Cw3	CHB	CHB	
Shimosegawa *et al*. [161]	SSA/Ro, SSB/La	Monozygotic, HLA Cw3	Rash	Rash	
Machado *et al*. [3]	SSA/Ro	Not reported	CHB	CHB	
McCue *et al*. [6][b]	+RF, –ANA	Not reported	CHB-death	CHB	
Siren *et al*. [160]	SSA/Ro, SSB/La	Dizygotic, HLA Cw3	CHB	CHB	
Silverman [162]	SSA/Ro, SSB/La	Dizygotic	CHB	Rash	
Scott *et al*. [10]	SSA/Ro	Dizygotic	CHB	Healthy	
Harley *et al*. [110]	SSA/Ro	Dizygotic	CHB	Healthy	
Watson *et al*. [163]	SSA/Ro, SSB/La	Dizygotic, HLA identical	CHB	Healthy	
Brucato *et al*. [164]	SSA/Ro	Dizygotic, HLA identical	CHB	Healthy	
Kaaja *et al*. [165]	SSA/Ro	Dizygotic, HLA identical	CHB	Healthy	
Buyon *et al*. [39]	SSA/Ro	Dizygotic	CHB	Healthy	
Buyon *et al*. [39]	SSA/Ro	Dizygotic	CHB	Healthy	
Eronen *et al*. [166]	Not reported	Dizygotic	CHB	Healthy	
Lockshin *et al*. [85]	SSA/Ro	Dizygotic	CHB	Healthy	
Lockshin *et al*. [85]	SSA/Ro	Dizygotic	Rash	Healthy	
Solomon *et al*. [96]	U1RNP	Dizygotic	Rash	Healthy	
Callen *et al*. [167]	SSA/Ro	Dizygotic	Rash	Healthy	
Lawrence *et al*. [168]	SSA/Ro, SSB/La	Dizygotic	Rash	Rash	
Yazici *et al*. [169]	SSA/Ro	Trizygotic	Rash	Rash	Rash, thrombocytopenia, liver

[a] One, 2 and 3 arbitrarily refer to the twin or triplet siblings but do not imply sequence of birth.
[b] Both twins had CHB recognized at birth; twin 2 had associated fibroelastosis and was asymptomatic at 1 year of age, twin 1 had L-transposition, ventricular septal defect, coarctation, and hypoplastic right ventricle, and died at 15 days of age.

As has been suggested for other HLA-linked diseases, it is possible that several genes within a particular haplotype may contribute to enhanced susceptibility. Arnaiz-Villena *et al.* [174] found that class III antigens BS and C4QOB1 are increased together with the A1;B8;DR3 haplotype in SSA/Ro-positive mothers whose offspring have CHB. Moreover, these class II genes were not increased significantly in a group of SSA/Ro-positive mothers whose offspring did not have CHB. Of particular interest was the observation that the most common DR3-bearing haplotype found in the Spanish population for adult SLE (A30; B18; DR3; BfF1; C2C,; C4ABQ) was not increased among the mothers of offspring who had CHB. This study did not find an increased frequency of DR2 in the anti-SSA/Ro-positive mothers of healthy offspring, *i.e.,* $DR2^+ = 3/15$ (20%) versus 23% in their normal population.

Brucato *et al.* [172] reviewed the literature and reported that of 28 mothers whose children have CHB, 50% were A1, 62% were B8, and 96% were DR3. In their own cohort of 15 Italian mothers of children with CHB, Brucato and co-workers [172] found a significantly increased prevalence of DR3 and DQ2 and B8/DR3, DR3/DQ2 and A1/Cw7/B8/DR3/DQ2 haplotypes. In a study of 31 Finnish mothers (all anti-SSA/Ro and/or SSB/La positive) of children with CHB, HLA B8 and DR3 were present in 71 and 74% of the affected mothers, respectively [103]. Thus, the genetic background of CHB mothers is similar in Anglo-Saxon populations and populations from southern and northern Europe [103]. The same Finnish study demonstrated that as a group, CHB mothers were genetically related more closely to primary SS than to SLE [103].

At the present time a role for fetal genetic differences in the major histocompatibility complex influence susceptibility is not firmly established. In five earlier reports of affected neonates, only two were DR3 [11, 110, 170, 171]. These data emphasize the passive nature of NLS and the difference between acquiring manifestations of lupus and actually developing the disease. However, continued investigations of larger numbers of affected mothers add complexities to the picture. In the study of Arnaiz-Villena *et al.* [174], four of five offspring with CHB were DR3 positive compared to two of five unaffected siblings [174].

Perhaps fetal HLA, not as an isolated risk factor, but as it relates to maternal HLA, is a fetal factor contributing to injury. A plausible hypothesis might be that tissue damage occurs when the HLA relationship between the fetus and mother is either bidirectionally compatible or unidirectionally compatible from the child's perspective. Relevant to the hypothesis that macrophage phagocytosis of apoptotic cardiocytes opsonized by autoantibodies initiates an inflammatory cascade leading to CHB, HLA class II compatible maternal Th1 cells would be able to provide help to fetal macrophages. In support of this notion, Miyagawa and colleagues [175] reported on a limited study of 13 Japanese families in which children with CHB shared both HLA class II alleles with their mothers significantly more often than children without CHB (4/9 CHB vs 0/12 healthy siblings, $P < 0.02$). Furthermore, Stevens *et al.* [176] demonstrated maternal cells in the AV node, myocardium, and liver of two infants with NLS who died shortly after birth, possibly representing alloreactive hematopoietic cells that trigger inflammation leading to tissue distruction.

MATERNAL DISEASE AT IDENTIFICATION OF NEONATAL LUPUS AND PROGRESSION

In 1987, McCune and colleagues [177] assessed the health status of 21 mothers and their children with NLS. This study suggested that all mothers eventually developed symptoms of a rheumatic disease but larger series have not reached similar conclusions [92, 178–180].

To date, 110 mothers of children with CHB enrolled in the research registry have been evaluated; at the time CHB was identified, 39 mothers were asymptomatic, 17 had an undifferentiated autoimmune syndrome (UAS), 20 had SLE, 24 had SS, 5 had SLE/SS, and for 5 the diagnosis is unknown. At a mean follow-up of 8.4 years, 14 (36%) of the 39 initially asymptomatic mothers had developed symptoms of a rheumatic disease: 1 developed UAS, 5 SLE, 7 SS, and 1 SLE/SS. Twenty-five (64%) remained asymptomatic. Of the 17 mothers initially diagnosed with UAS, 1 developed SLE and 4 SS. Three mothers with SS at the birth of the affected child progressed to SLE/SS at follow-up.

Similar results were reported by Julkunen *et al.* [92]. Fifteen (48%) of 31 mothers whose children had CHB were asymptomatic before the index delivery, 7 of whom remained asymptomatic after a mean follow-up of 8 years. Two mothers had SLE, 1 mother had definite primary SS, 1 had probable SS, 1 had autoimmune hypothyroidism, and 1 had Grave's disease. Six (19%) gave a self-reported diagnosis of a chronic autoimmune disease antedating the index delivery. Of 2 mothers who died, 1 subsequently developed SLE and died of a fatal cardiac arrhythmia 5 years after the birth of the affected child. The other died of alcoholic liver disease and had developed SLE after 11 years. No patient with SLE had nephritis. Importantly, these authors noted that as a group, mothers of CHB children had clinical and immunologic characteristics more closely related to primary SS than SLE.

In a study of 64 CHB mothers from Toronto, 42 (66%) were asymptomatic, 2 (3%) had SLE, 2 (3%) had linear scleroderma, 2 (3%) had RA, 3 (5%) had a history of rheumatic fever, 1 (2%) had SS, and 12 (2%) had UAS [178]. Three of the 12 mothers with UAS progressed to SLE and 2 developed SS. Thirty-six of the 42 initially healthy mothers remained well, 1 developed SLE, 1 hyperthyroidism, 1 ankylosing spondylitis, and 3 UAS. Unlike the other larger studies, not all mothers included in this cohort were documented to have anti-SSA/Ro-SSB/La antibodies.

Taken together, it can be concluded that asymptomatic mothers do not invariably become ill (at least over 8 years or more of follow-up) and if an asymptomatic mother does develop SLE, it is not likely to be life-threatening. Although a more formal epitope analysis of the autoantigens has not been done with regard to clinical outcome, the fine specificity of anti-SSA/Ro-SSB/La antibodies as assessed by immunoblot is highly stable for years, independent of the maternal clinical status [179].

Maternal health status may also be of interest with regard to the discordant clinical manifestations of neonatal lupus. A link between the health status of the mother and child might aid to the researcher in defining risk for a given mother, an enormous benefit to the practitioner faced with family counseling, and in identifying a pathogenetic mechanism of injury. Maternal disease might reflect the fine specificity of an antibody response, which in turn could account for the preferential vulnerability of one particular organ versus another. Lawrence *et al.* [180] compared a cohort of 24 women with anti-SSA/Ro-SSB/La antibodies whose children

have only cutaneous manifestations of neonatal lupus to their previously published cohort of 32 mothers with similar antibodies whose children have only cardiac manifestations [178]. Maternal health status was considered as either symptomatic (inclusive of varied rheumatic diseases such as systemic lupus erythematosus, Sjogren's syndrome, and undifferentiated autoimmune diseases) or completely asymptomatic. A significantly greater number of mothers whose children had CHB were asymptomatic (75%) compared to mothers of children with skin rash alone (42%). At follow-up, mothers of children with CHB were more likely to remain asymptomatic (59%) than were mothers of children with skin rash (25%).

This same question has also been addressed by review of data in the research registry [39]; included were 105 mothers whose children had only CHB and 47 whose children had only a skin rash [181]. All mothers had documented antibodies to SSA/Ro and/or SSB/La. Initially, 37% of the "CHB mothers" and 28% of the "skin mothers" were asymptomatic. After at least 5 years of follow-up, 24% of the 105 "CHB mothers" and 13% of the 47 "skin mothers" remained symptom-free. There was a trend to more symptomatic disease in the mothers whose children had cutaneous manifestations, but the differences between "skin mothers" and "CHB mothers" in the registry did not reach statistical significance. These data are not completely in accord with those of Lawrence *et al*. [180], the difference being in the distribution of maternal disease in the CHB groups. There was a significantly greater proportion of symptomatic "CHB mothers" in the registry than in Toronto. Interestingly, when Fisher's exact test was used to compare the "skin mothers," the proportion of symptomatic individuals did not differ significantly between the Toronto and registry databases. The referral patterns of the two studies could plausibly explain their divergent findings with regard to the health of "CHB mothers." All subjects in the Toronto database are from within a single city catchment area, not from outside sources; the research registry, in contrast, includes the entire United States. While the referral source for the Toronto patients is not included in the report by Lawrence *et al*. [180], 42% of the registry CHB cases and 54% of registry skin cases were referred by rheumatologists (P = NS, CHB vs skin). Data from two separate studies suggest that mothers who have children with cutaneous disease are more likely to be symptomatic than asymptomatic. However, this could be explained by the fact that recognizing a neonatal rash as NLS is more likely in a mother with rheumatic disease. Whether maternal health status (i.e., absence of clinical disease) is a marker for heart disease remains to be established.

IDENTIFICATION AND MANAGEMENT OF THE HIGH-RISK PREGNANCY

An overall schema for management is presented in Fig. 10. These recommendations are considered experimental, based on the author's experience, and have not been tested in a controlled trial.

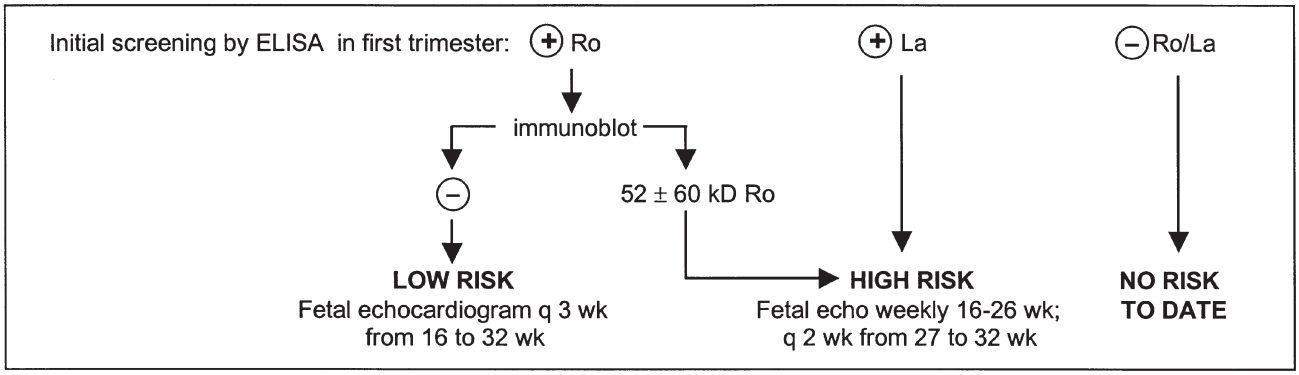
Prenatal Considerations

Ideally, because CHB is identified most often from 18 to 24 weeks of gestation, intrauterine therapy should eventually be possible. The clinical approach to cardiac manifestations of NLS includes obstetric and rheumatologic management of (1) the fetus identified with CHB and (2) the fetus with a normal heart beat but at high risk of developing CHB.

To address the treatment of identified CHB, one needs to know if the presence of bradycardia represents an irreversible fibrotic process and if continued autoimmune tissue injury will cause progressive damage. McCue *et al*. [6] reported a neonate described with first-degree heart block at birth that resolved at 6 months. In contrast, Geggel and colleagues [182] reported an infant born with second-degree heart block that progressed to third-degree block by 9 weeks of age. As discussed in preceding sections, data from the research registry also emphasize that an incomplete AV block is not always fixed and the degree of block is variable [39, 41]. There is a spectrum of conduction abnormalities even at the time of initial detection and *in utero* injury can have continued sequelae in some cases, despite clearance of maternal antibodies from the neonatal circulation.

The rationale for the treatment of identified heart block and the prevention of potential heart block is to diminish a generalized inflammatory insult and reduce or eliminate maternal autoantibodies. Accordingly, several intrauterine therapeutic regimens have been tried, including dexamethasone, which is not metabolized by the placenta and is available to the fetus in an active form, and plasmapheresis. Although there is not yet documentation in the literature regarding the reversal of third-degree heart block (complete fibrosis of the AV node would not be reversible) by maternal treatment with dexamethasone alone, the potential for diminishing an inflammatory fetal response attacking the myocardium is plausible. This would be an effect independent of a decrease in antibody titer. A precedent for this therapeutic rationale is the resolution of associated pleuropericardial effusions and ascites reported in separate investigations [13, 23, 183]. In the largest retrospective study published to date, it was observed that fluorinated glucocorticoids ameliorated

A. Laboratory evaluation of pregnant women with autoimmune disorders, and guidelines for management



B. Therapeutic approach to CHB diagnosed *in utero*

Situation	Treatment
1. Degree of block at presentation	
• 3rd degree (>3 wk from detection)	• Evaluate by weekly echocardiography and obstetrical sonography; no therapy is initiated.
• 3rd degree (<3 wk from detection)	• 4 mg p.o. dexamethasone daily for 6 wk. If no change, taper dose to discontinue. If reversal to 2nd degree or better, continue until delivery, then taper.
• Alternating 2nd/3rd degree	• 4 mg p.o. dex daily for 6 wk. If progression to 3rd degree, taper. If reversal to 2nd degree or better, continue until delivery, taper.
• 2nd degree • Prolonged mechanical PR interval (1st degree)	• 4 mg p.o. dex daily until delivery, then taper. If progression to 3rd degree, taper after 3rd degree persists for 6 wk.
2. Block associated with early signs of myocarditis, congestive heart failure, and/or hydropic changes	• 4 mg p.o. dex daily until improvement, then taper.
3. Severely hydropic fetus	• 4 mg p.o. dex daily, with apheresis as a last resort to rapidly remove maternal antibodies, or consider terbutaline, digoxin, diuretics, direct fetal pacing. **Deliver if lungs are mature.**

FIGURE 10 Current laboratory evaluation and clinical management of neonatal lupus.

incomplete atrioventricular block and hydropic changes in autoimmune-associated congenital heart block but did not reverse established third-degree block [184]. Specifically, data included 47 mothers whose sera contained anti-SSA/Ro-SSB/La antibodies and their 50 children diagnosed with isolated CHB *in utero* and in whom at least four echocardiograms were performed thereafter. In 28 pregnancies, the mothers were treated with 4–9 mg/day dexamethasone for 3–19 weeks or with 12–24 mg/week betamethasone for >6 weeks (group A). In 22 pregnancies, fluorinated steroids were not used (group B). Third-degree block was present in 21 fetuses in group A and in 18 fetuses in group B; none were reversible despite steroids. Three fetuses in group A and two in group B progressed from second-degree block alternating with third-degree block to permanent third degree at birth and postnatally. Notably, of four fetuses in group A with second-degree block at presentation, all reverted to first degree by birth. Of two fetuses in group B with second-degree block at presentation, both progressed to permanent third-degree block postnatally. Initial echocardiographic evaluation revealed pericardial effusions in 13 group A vs 4 group B (P = NS), pleural effusions in 2 group A vs 0 group B (P = NS), ascites in 8 group A vs 0 group B ($P < 0.007$), hydrops in 8 group A vs 0 group B ($P < 0.007$), and intrauterine growth restriction (IUGR) in 1 group A vs 1 group B (P = NS). Pericardial effusions resolved and reappeared

in both groups. Steroid therapy was most effective in the sustained resolution of pleural effusions (2/2), ascites (6/8), and hydrops (5/8). Oligohydramnios ensued in 9 group A and 2 group B (P = NS). Although fetuses in group A had more associated complications at initial presentation than those in group B, there were no significant differences between the groups in the duration of pregnancy (35.7 weeks vs 37.0 weeks), number of deaths (4 vs 1), final degree of block, or requirement for permanent pacing (14 vs 11).

In the author's experience and that of others, apheresis in addition to dexamethasone has not reversed third-degree heart block [13, 23], although titers of maternal anti-SSA/Ro-SSB/La have been decreased profoundly [185]. Maternal risks of dexamethasone are similar to any glucocorticoid and include infection, osteoporosis, osteonecrosis, diabetes, hypertension, and preeclampsia. Fetal risks include oligohydramnios, intrauterine growth retardation, and adrenal suppression. Intervention with glucocorticoids might decrease acute inflammation but not necessarily prevent subsequent fibrosis.

Available data support serial cardiac monitoring of all fetuses with any bradyarrhythmias detected *in utero* and of neonates with incomplete blocks at birth whose mothers are previously known or currently identified to have anti-SSA/Ro-SSB/La antibodies. Fetal echocardiogram is essential to diagnose and follow the course of disease and may suggest the presence of an associated myocarditis by the finding of decreased contractility in addition to the secondary changes associated with myocarditis, such as an increase of cardiac size, pericardial effusions, and tricuspid regurgitation. The obstetric management should be guided by the degree of cardiac failure noted on the ultrasound images. The *in utero* environment is preferred as long as possible because of the low resistance circulatory pathways, thereby affording minimal work to maintain cardiac output.

The initiation of dexamethasone or plasmapheresis as a preventative measure has been considered. With regard to prophylactic therapy of the high-risk mother (documentation of high-titer anti-SSA/Ro and SSB/La antibodies, anti-48-kDa SSB/La and 52-kDa SSA/Ro on immunoblot, and a previous child with NLS), administration of prednisone, dexamethasone, or plasmapheresis is not justified at the present time. Maternal prednisone (at least in low and moderate doses) early in pregnancy does not prevent the development of CHB [186]. This might be anticipated, as prednisone given to the mother is not active in the fetus [187] and levels of anti-SSA/Ro and anti-SSB/La antibodies remain relatively constant during steroid therapy. However, Shinohara *et al.* [188] published uncontrolled data suggesting that prenatal use of maternal glucocorticoids has prophylactic merit. This recommendation was based on the finding that 15 of 61 infants born to 40 mothers with anti-SSA/Ro antibodies who did not receive glucocorticoids had CHB. The unexpectedly high prevalence may be explained by the retrospective nature of the study and potential referral bias. Conversely, CHB did not occur in any of 26 fetuses whose mothers were given steroids prior to the 16th week of gestation. If one accepts the rate of 2%, then at least 40 mothers with anti-SSA/Ro antibodies may need to be followed to find one infant with complete block.

With regard to plasmapheresis as a prophylactic therapy, Barclay and colleagues [189] initiated plasmapheresis during the late second trimester in a woman with anti-SSA/Ro antibodies and a history of four unsuccessful pregnancies, including a 32-week stillbirth with unexplained antenatal bradycardia. The pregnancy resulted in a healthy birth, and the titer of anti-SSA/Ro antibodies was decreased by 75%, although detectable antibodies were present in the cord blood [189]. The author similarly utilized "prophylactic" plasmapheresis in a pregnant woman with SS and a previous child with CHB [185]. In this case the fetus, despite having circulating levels of these antibodies detectable at birth, was exposed to only 10% of the potential maternal antibody load during the second and third trimesters. The child, now 8 years of age, never had any manifestations of NLS. In the absence of controlled studies, which may never be feasible given the rarity of CHB, plasmapheresis should be considered highly experimental and only reserved for those cases where the fetus is in a life-threatening situation with hydrops and deteriorating cardiac function.

The best approach for a mother with a previous offspring with CHB is unknown. It is suggested that all mothers with anti-SSB/La antibodies or antibodies to components of SSA/Ro (particularly the 52-kDa) on immunoblot should have serial fetal echocardiography done by an experienced pediatric cardiologist weekly from 16 to 26 weeks and every other week until about 34 weeks. Thereafter auscultation should be sufficient. Major advances have been made in fetal echocardiography. Until recently, the *in utero* detection of first-degree block was not technically feasible. However, the EKG equivalent of the PR interval can now be measured by echocardiography [190]. A prospective NIH-supported multicenter study, PR Interval and Dexamethasone Evaluation (PRIDE), in CHB is underway to examine the mechanical PR interval weekly in pregnant woman with anti-SSA/Ro and/or anti-SSB/La antibodies. One of the goals of this trial is to identify the prevalence of first-degree block and to determine whether it is a marker for more advanced destruction of the conducting system. Such information will provide the optimal opportunity for reversibility. It

is strongly recommended that all neonates born to mothers with anti-SSA/Ro-SSB/La antibodies have an EKG at birth to detect first-degree block.

Breast Feeding

Mothers often ask about the risks of breast feeding. Askanase and colleagues [191] have addressed whether human breast milk contains antibodies to components of the SSA/Ro-SSB/La complex and, if so, whether breast feeding might be associated with the postnatal manifestations of neonatal lupus. To accomplish these goals, breast milk from nine mothers with serologic evidence of anti-SSA/Ro and/or SSB/La antibodies was examined by ELISA and immunoblot for the presence of these same antibodies. Five of these breast-fed infants were healthy without any manifestations of neonatal lupus, one had an isolated cardiomyopathy and died, two had cutaneous manifestations of neonatal lupus, and one had both CHB and rash. IgA and IgG antibodies to all components of the SSA/Ro-SSB/La complex were present in breast milk. Not unexpectedly, the antibody profiles of the breast milk paralleled those observed in the serum.

Of 237 mothers enrolled in the registry as of September 2000, 129 mothers answered a questionnaire regarding breast feeding of their 266 children. Neonatal lupus was present in 149 of the children (55 with a skin rash alone, 72 with CHB alone, and 22 with both manifestations) and 117 were unaffected. The frequency of breast feeding in the mothers enrolled in the Research Registry for Neonatal Lupus was slightly lower (51%) than the national average (60%) of mothers breast feeding upon leaving the hospital [192]. This might be explained by the fact that mothers with anti-SSA/Ro-SSB/La antibodies are discouraged from breast feeding by their physicians or, in some cases, may be too ill or on other medications. Overall, a total of 136 children (51%) were breast fed. Of 55 children with neonatal skin rash, 33 were breast fed. Of 22 with both rash and CHB, 12 were breast fed. For the unaffected siblings, 60 of 117 were breast fed. Fisher's exact test revealed no significant differences between breast-fed and nonbreast-fed children with isolated skin rash, or those with rash and CHB, compared to unaffected children. Although there was a trend for the children who were breast fed to have cutaneous manifestation of neonatal lupus appear at a later age than those who had rashes and were formula fed, the difference in mean age of presentation of the rash did not reach statistical significance: 9.69 weeks vs 7.55 weeks (P = NS). The duration of the rash was not influenced by breast feeding: 14.7 weeks in the breast-fed group vs 19 weeks in those not breast fed (P = NS). Not unexpectedly, of 72 children with isolated CHB, 31 were breast fed, which did not differ significantly from unaffected children. Of 7 infants in whom cardiomyopathy was detected after birth, 4 were breast fed.

Accordingly, available data do not suggest that breast feeding has pathologic consequences. Specifically, children with skin rashes were not breast fed more frequently than those who remained healthy. Furthermore, prematurity was not a factor contributing to the development of skin rash in breast-fed infants. There was a trend toward later presentation of the rash in the children who were breast fed but there was no increase in duration compared to children who received formula. Because maternal antibodies transferred to the fetus during gestation would still be present for several months postpartum, the additional contribution of antibodies from breast milk may be inconsequential even if intestinal transport is effective. Mothers should be advised that autoantibodies are present in their breast milk but reassured that, at least within the limits of published literature, breast feeding is not associated with neonatal lupus. However, given the potential for intestinal transport of the maternal antibodies in the unusual circumstance of a worsening skin rash or developing cardiomyopathy, consideration should be given to the discontinuation of breast feeding.

Treatment of Cutaneous Manifestations

Infants with CHB should be protected from excessive sun exposure, as they are at risk for developing skin lesions until 8–12 months of age. In the absence of precise information regarding specific pathogenicity of the maternal antibody response, it is reasonable to consider all offspring of mothers with antibodies to components of the SSA/Ro-SSB/La ribonucleoproteins at risk for cutaneous disease in the first few months of postnatal life. Topical steroids, preferably those that are nonfluorinated, have been recommended for babies who develop lesions [42]. High-potency topical steroids to the skin of an infant can result in systemic effects. Because the lesions are transient and generally benign, systemic therapies such as antimalarials, which have a low toxic-to-therapeutic ratio in young children, have not been recommended [42].

Neiman and colleagues [45] reported on therapy and outcome of 57 infants (20 males, 37 females) diagnosed with cutaneous NLE (absent heart disease) between 1981 and 1997. Thirty-four (60%) were treated. Thirty-one were given only low to medium potency topical corticosteroid preparations. Three children were initially given topical antifungal agents and then treated subsequently with topical steroid preparations. None received systemic glucocorticoid therapy. The active

rash resolved in all children regardless of treatment. In 51 children for whom reliable follow-up data were available, 37 rashes completely resolved without sequelae, of which 21 were treated and 16 received no therapy except avoidance of sun exposure. However, in 14 there were residual skin abnormalities: 10 had telangiectasias, 2 had hyperpigmentation of the affected areas, and 10 had what was described as pitting, scarring, or atrophy after at least 2 years of follow-up. Of these 14 children, 10 were treated and 4 were untreated. Although there was no significant difference in outcome between treated and untreated children by Fisher's exact test, firm conclusions are limited by the small number of cases.

Recurrence Rates

In the research registry as of June 2003, 91 mothers have had pregnancies (lasting longer than 6 months) subsequent to the birth of child with CHB (Table 4). In 65 (71%) of these pregnancies there were no AV conduction abnormalities or reported cutaneous manifestations of NLS in the children. Seventeen (19%) next pregnancies resulted in a second child with CHB, 3 in association with a skin rash. It is of note that in one of these families the first child had first-degree block detected at age 10 and the second child had CHB detected *in utero*. In 7 (8%) next pregnancies, the offspring had cutaneous manifestations alone. Thus, the probability of having a second affected child with any manifestation of NLS was 25%. One subsequent pregnancy ended at 30 weeks, but the fetus was not known to have any conduction abnormalities. Another subsequent pregnancy resulted in the birth of a boy who died at age 2 months. While there were no documented conduction abnormalities, he had severe aortic stenosis and cardiomyopathy, which on biopsy was found to be associated with immunoglobulin deposition [30].

TABLE 4 Outcome of 83 Pregnancies Immediately Subsequent to the Birth of a Child with CHB

Outcome	*N*	%
Healthy	65	71
Manifestations of NLS		
CHB only	14	13
CHB and rash	3	4
Rash only	7	8
Fetal demise	1	2
Neonatal death[a]	1	2

[a] Aortic stenosis and severe cardiomyopathy.

It is also important for the clinician to consider the outcomes of pregnancies subsequent to the birth of a child with NLS rash. Among registry mothers, as of June 2003, there have been 33 pregnancies (lasting longer than 6 months) following the birth of a child with NLS rash (Table 5). In 13 (39%) of the offspring, there were no cardiac or cutaneous manifestations of NLS; 12 (36%) had NLS rash alone. It is striking that 8 (24%) next pregnancies resulted in a child with CHB, 5 in association with rash.

FETAL OUTCOME

Data published from the research registry revealed that 22 (19%) of the 113 offspring with CHB whose mothers were documented to have anti-SSA/Ro and/or SSB/La antibodies have died (12 boys, 10 girls), as illustrated in Fig. 11. Six of these deaths occurred *in utero.* Ten neonates died in the first 3 months of life. Six children died between 3 months and 3 years of age. None of the 67 children between 3 and 10 years old remaining in the cohort have died. Twenty-two children are older than 10 years. However, the mortality is reduced markedly in those children born at later gestational ages. Specifically, only 8 (9%) of 86 children born at or after 34 weeks have died [39]. Those infants who survive the neonatal period have an excellent prognosis. The cumulative probability of survival at 3 years is 79%.

With regard to the morbidity of CHB, 67 (63%) of the 107 children born alive have required pacemakers, 35 within the first 9 days of life. Fifteen additional children have been paced in the first year and 17 after 1 year. One infant has had a cardiac transplant at 8 months of age because of intractable cardiomyopathy. Moak *et al.* [193] underscored the need to recognize late-onset cardiomyopathy as a sequela of CHB with their report of 16 cases that developed despite adequate pacing.

In a study of 15 CHB patients followed by Silverman *et al.* [14] there were 3 neonatal deaths, 2 late deaths due

TABLE 5 Outcome of 33 Pregnancies Immediately Subsequent to the Birth of a Child with NLS Rash

Outcome	*N*	%
Healthy	13	39
Manifestations of NLS		
CHB only	3	9
CHB and rash	5	15
Rash only	12	36

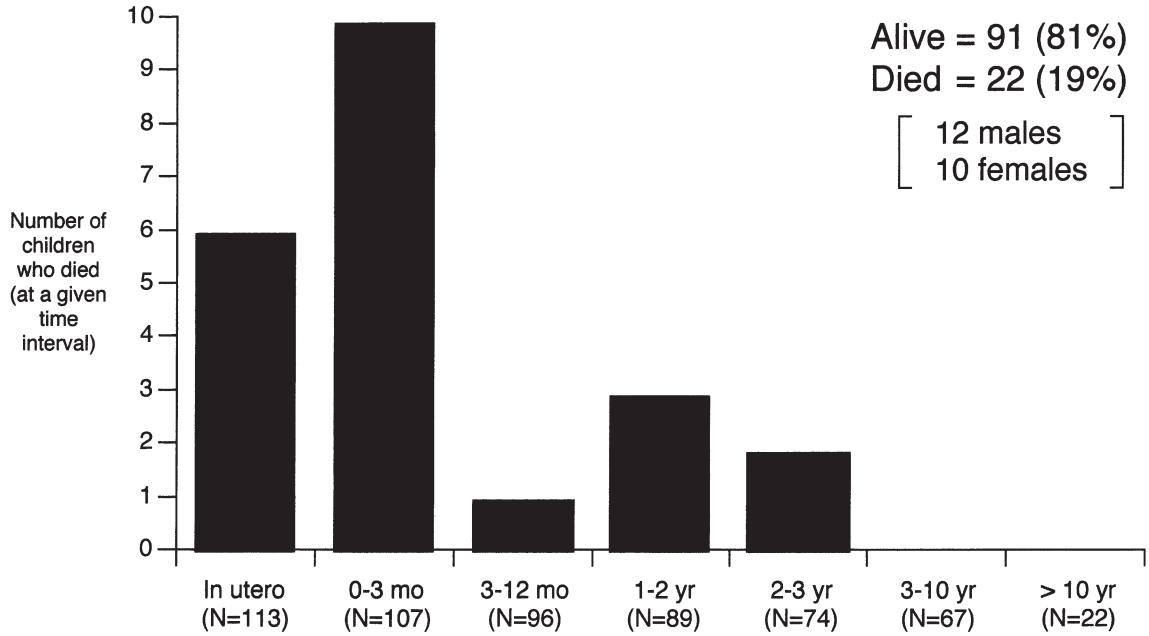


FIGURE 11 Mortality of CHB at different ages. Along the horizontal axis, N is the number of children in this cohort alive at the age specified (0–3 months indicates children up to and including age 3 months, 3–12 months indicates children older than 3 months up to and including age 12 months, etc.)

to pacemaker failure, and 6 who have required pacemaker therapy. Similarly, McCune *et al.* [177] reported a follow-up of 14 neonates with CHB of whom 5 required pacemakers.

Long-Term Follow-up of Children with Varied Manifestations of NLS

Given the rarity of the disease, little information is available on the health outcome of children with neonatal lupus and their unaffected siblings. This is of interest from several perspectives. There is a genetic susceptibility for the development of SLE [194–198], and relatives of SLE patients can have autoantibodies in the absence of clinical disease [199]. Perhaps in addition to autoantibodies, the expression of SLE in the mother increases the risk of autoimmune disease in her offspring independent of whether the child has neonatal lupus or not. This generates the hypothesis that children with neonatal lupus, as well as their unaffected siblings, whose mothers have SLE might be at greater risk for the development of subsequent disease than children whose mothers are asymptomatic. Perhaps vulnerability to neonatal lupus is a marker for susceptibility to, or protection from, the development of actively acquired autoimmunity.

Martin and colleagues [200] reported on the health of children 8 years of age or older who had manifestations of neonatal lupus (affected group) and their unaffected siblings (unaffected group). Questionnaires were sent to mothers (with anti-SSA/Ro-SSB/La antibodies) enrolled in the research registry and to a control group comprising children of healthy mothers referred by the registry enrollees. Responses to the questionnaires were confirmed and expanded by a review of medical records. Fifty-five mothers enrolled in the registry returned questionnaires on 49 children with neonatal lupus and their 45 unaffected siblings. Six children were identified with definite rheumatologic/autoimmune diseases: 2 with juvenile rheumatoid arthritis, 1 with Hashimoto's thyroiditis, 1 with psoriasis and iritis, 1 with diabetes mellitus and psoriasis, and 1 with congenital hypothyroidism and nephrotic syndrome. All had manifestations of neonatal lupus and their mothers have manifestations of autoimmune diseases: 4 SS, 1 SLE/SS, and 1 UAS. In 4 of 55 sera tested, the ANA was positive (2 of 33 affected children and 2 of 22 unaffected children). No serum contained antibodies reactive with SSA/Ro or SSB/La antigens. These data suggest that children with neonatal lupus require continued follow-up, especially prior to adolescence and if the mother herself has an autoimmune disease. While there was no apparent increased risk of SLE, the development of some form of autoimmune disease (systemic or organ specific) in early childhood may be of concern. During adolescence and young adulthood, individuals with

neonatal lupus and their unaffected siblings do not appear to have an increased risk of developing systemic rheumatic diseases.

In one small series, Brucato *et al*. [164] found that at a mean follow-up of 18 years none of 13 children with CHB developed clinical symptoms or serological abnormalities suggesting immune disease.

Despite these encouraging results, there have been seven cases published in which an autoimmune disease developed in a child with manifestations of neonatal lupus. All the children were female and in each instance except one the mother had SLE. Specifically, Esscher and Scott [201] reported a female with CHB who developed SLE at age 15 years. Jackson and Gulliver [202] reported an infant with cutaneous neonatal lupus who developed SLE at age 13, and Fox *et al.* [203] described a similar patient who developed SLE at age 19 years. In a patient described by Waterworth [204], CHB was identified at age 6 years and SLE diagnosed at age 13 years; no information was provided on the mother. Lanham *et al*. [205] reported two children with CHB; one subsequently developed primary SS at age 23 years and the other arthritis, positive ANA, and antibodies to dsDNA at age 19 years. Hübscher *et al.* [206] reported the development of scleroderma of the face, puffy hands, and Raynaud's phenomenon in a 13-year-old girl with CHB. At age 15 she was found to be seropositive for anti-SSA/Ro and U1 RNP. It should be emphasized that maternal anti-SSA/Ro-SSB/La antibodies were not actually documented for any of these mothers.

CONCLUSIONS

The presence of antibodies reactive with several components of the SSA/Ro-SSB/La ribonucleoprotein particle, notably the 48-kDa SSB/La and the 52-kDa SSA/Ro antigens by immunoblot, is to date a near-universal characteristic of all mothers giving birth to offspring with NLS. Although NLS is rare, its discussion is an integral part of all pregnancy counseling of women with SLE, SS, and undifferentiated autoimmune syndromes. Overall, studies suggest that there is an unacceptable morbidity and mortality. A major clue to defining the pathogenesis of antibody-mediated damage is the selective vulnerability of the fetal heart. Vulnerability could relate to a direct or indirect antigen target expressed differentially in the developing human heart. Alternatively, but not mutually exclusively, biologic events operative during fetal life, such as apoptosis, could facilitate accessibility of intracellular antigens to the extracellular environment. Opsonization of apoptotic cardiocytes might alter the normal events of cell removal by macrophages. Inadvertent activation of the macrophage and subsequent dysregulation of the fibroblast may lead to permanent injury. Perturbation of L-type calcium channels, which propagate the action potential in the AV node, provides a clue to a definable pathogenetic effect of anti-SSA/Ro antibodies. How apoptosis and calcium channel dysfunction relate, if at all, is not intuitive; there may be more than one mechanism involved in pathogenesis. A reproducible murine model of CHB would be a critical tool to define antibody pathogenicity at the histologic and molecular levels with subsequent application for testing prophylactic and therapeutic interventions. The availability of a research registry devoted to NLS should provide an invaluable resource for basic and clinical research. The reporting of recurrence rates, mortality and morbidity, and maternal outcomes in a large number of patients has facilitated family counseling greatly. We eagerly await the results of the PRIDE study in the hope that with more detailed echocardiographic data, risk can be better defined and reversibility of early injury a reality.

Acknowledgments

The work reported herein has been supported in part by NIH/NIAMS Contract NO1-AR-4-2220 (Research Registry for Neonatal Lupus), NIH Grant RO1 AR42455, the S.L.E. Foundation, Inc., and the American Heart Association New York chapter. The author thanks Ann Rupel for her continued labor of love in preparation of the manuscript, Peg Katholi for tireless work for the registry, and the Lee and Driscoll families for their generous support.

References

1. Morquio, L. (1901). Sur une maladie infantile et familiale characterisee par des modifications permanentes du pouls, des attaques syncopales et epileptiformes et al mort subite. *Arch. Med. Inf.* **4**, 467–475.
2. Plant, R. K., and Steven, R. A. (1945). Complete A–V block in a fetus. *Am. Heart J.* **30**, 615–618.
3. Machado, M. V. L, Tynan, M. J., Curry, P. V. L., and Allan, L. D. (1988). Fetal complete heart block. *Br. Heart J.* **60**, 512–515.
4. Carter, J. B., Blieden, L. C., and Edwards, J. E. (1974). Congenital heart block: Anatomic correlations and review of the literature. *Arch. Pathol.* **97**, 51–57.
5. Aylward, R. D. (1928). Congenital heart-block. *Br. Med. J.* **1928**, 943.
6. McCue, C. M., Mantakas, M. E., and Tingelstad, J. B. (1977). Congenital heart block in newborns of mothers with connective tissue disease. *Circulation* **56**, 82–90.
7. Chameides, L., Truex, R. C., Vetter, V., Rashkind, W. J., Galioto, F. M., and Noonan, J. A. (1977). Association of maternal systemic lupus erythematosus with congenital complete heart block. *N. Engl. J. Med.* **297**, 1204–1207.

8. Winkler, R. B., Nora, A. H., and Nora, J. J. (1977). Familial congenital complete heart block and maternal systemic lupus erythematosus. *Circulation* **56**, 1103–1107.
9. Lee, L. A., Reed, B. R., and Harmon, C. (1983). Autoantibodies to SS-A/Ro in congenital heart block. *Arthritis Rheum.* **20**, S24.
10. Scott, J. S., Maddison, P. J., Taylor, P. V., Esscher, E., Scott, O., and Skinner, R. P. (1983). Connective-tissue disease, antibodies to ribonucleoprotein, and congenital heart block. *N. Engl. J. Med.* **309**, 209–212.
11. Watson, R. M., Lane, A. T., Barnett, N. K., Bias, W. B., Arnett, F. C., and Provost, T. T. (1984). Neonatal lupus erythematosus: A clinical, serological and immunogenetic study with review of the literature. *Medicine (Baltimore)* **63**, 362–378.
12. Ramsey-Goldman, R., Hom, D., Deng, J.-S., Ziegler, G. C., Kahl, L. E., Steen, V. D., LaPorte, R. E., and Medsger, T. A., Jr. (1986). Anti-SS-A antibodies and fetal outcome in maternal systemic lupus erythematosus. *Arthritis Rheum.* **29**, 1269–1273.
13. Buyon, J. P., Swersky, S., Fox, H., Bierman, F., and Winchester, R. J. (1987). Intrauterine therapy for presumptive fetal myocarditis with acquired heart block due to systemic lupus erythematosus: Experience in a mother with a predominance of SSB/La antibodies. *Arthritis Rheum.* **30**, 44–49.
14. Silverman, E. D., Mamula, M. J., Hardin, J. A., and Laxer, R. M. (1991). The importance of the immune response to the Ro/La particle in the development of complete heart block and neonatal lupus erythematosus. *J. Rheumatol.* **18**, 120–124.
15. Taylor, P. V., Taylor, K. F., Norman, A., Griffiths, S., and Scott, J. S. (1988). Prevalence of maternal Ro(SS-A) and La(SS-B) autoantibodies in relation to congenital heart block. *Br. J. Rheum.* **27**, 128–132.
16. Buyon, J. P., Slade, S. G., Elkon, K., Chan, E. K. L., Winchester, R., and Lockshin, M. (1990). Combined assays for Ro/La identify risk of congenital heart block (CHB). *Arthritis Rheum.* **33**, S28.
17. Buyon, J. P., Ben-Chetrit, E., Karp, S., Roubey, R. A. S., Pompeo, L., Reeves, W. H., Tan, E. M., and Winchester, R. (1989). Acquired congenital heart block: Pattern of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La system in neonatal lupus. *J. Clin. Invest.* **84**, 627–634.
18. Laxer, R. M., Roberts, E. A., Gross, K. R., Britton, J. R., Cutz, E., Dimmick, J., Petty, R. E., and Silverman, E. D. (1990). Liver disease in neonatal lupus erythematosus. *J. Pediatr*. **116**, 238–242.
19. Watson, R., Kang, J. E., May, M., Hudak, M., Kickler, T., and Provost, T. T. (1988). Thrombocytopenia in the neonatal lupus syndrome. *Arch. Dermatol.* **124**, 560–563.
20. McCuistion, C. H., Schoch, E. P. (1954). Possible discoid lupus erythematosus in a newborn infant: Report of a case with subsequent development of acute systemic lupus erythematosus in the mother. *Arch. Dermatol. Syph.* **70**, 782–785.
21. Kephardt, D. C., Hood, A. F., and Provost, T. T. (1987) Neonatal lupus erythematosus: New serologic findings. *J. Invest. Derm.* **77**, 331–333.
22. Buyon, J., and Szer, I. (1986). Passively acquired autoimmunity and the maternal fetal dyad in systemic lupus erythematosus. *Semin. Immunopathol.* **9**, 283–304.
23. Herreman, G., and Galezewski, N. (1985). Maternal connective tissue disease and congenital heart block. *N. Engl. J. Med.* **312**, 1329.
24. Lee, L. A., Coulter, S., Erner, S., and Chu, H. (1987). Cardiac immunoglobulin deposition in congenital heart block associated with maternal anti-Ro antibody. *Am. J. Med.* **83**, 793–796.
25. Litsey, S. E., Noonan, J. A., O'Connor, W. N., Cottrill, C. M., and Mitchell, B. (1985). Maternal connective tissue disease and congenital heart block: Demonstration of immunoglobulin in cardiac tissue. *N. Engl. J. Med.* **312**, 98–100.
26. Ho, Y. S., Esscher, E., Anderson, R. H., and Michaelsson, M. (1986). Anatomy of congenital complete heart block and relation to maternal anti-Ro antibodies. *Am. J. Cardiol.* **58**, 291–294.
27. Hogg, G. R. (1957). Congenital acute lupus erythematosus associated with subendocardial fibroelastosis: Report of a case. *Am. J. Clin. Pathol.* **28**, 648–654.
28. Singsen, B. H., Akhter, J. E., Weinstein, M. M., and Sharp, G. S. (1985). Congenital complete heart block and SSA antibodies: Obstetric implications. *Am. J. Obstet. Gynecol.* **152**, 655–658.
29. Nield, L. E., Silverman, E. D., Taylor, C. P., Smallhorn, J. F., Mullen, J. B., Silverman, N. H., Finley, J. P., Law, Y. M., Human, D. G., Seaward, P. G., Hamilton, R. M., and Hornberger, L. K. (2002). Maternal anti-Ro and anti-La antibody-associated endocardial fibroelastosis. *Circulation* **105**, 843–848.
30. Tseng, C., Friedman, D., and Buyon, J. P. (1997). Spectrum of cardiac histopathology in cases of autoimmune-associated congenital heart block (CHB) obtained from the Research Registry for Neonatal Lupus. *Arthritis Rheum.* **40(Suppl.)**, S333. [Abstract]
31. Clancy, R. M., Askanase, A. D., Kapur, R. P., Chiopelas, E., Azar, N., Miranda-Carus, M. E., and Buyon, J. P. (2002). Transdifferentiation of cardiac fibroblasts a fetal factor in anti-SSA/Ro-SSB/La antibody-mediated congenital heart block. Submitted for publication.
32. Rote, N. S. (1985). Maternal fetal immunology. In "Immunology in Obstetrics and Gynecology" (J. R. Scott and N. S. Rote, eds.), p. 55. Appelton-Crofts, Norwalk, CT.
33. Lin, C. T., Shen, Z., Boros, P., and Unkeless, J. C. (1992). Fc receptor-mediated signal transduction. *J. Clin. Immunol.* **14**, 1–13.
34. Wells, M., and Fox, H. (1992). Immunology and immunopathology of the maternofetal interface. In "Immunological Obstetrics" (C. B. Coulam, W. P. Faulk, and J. A. McIntyre, eds.), pp. 166–176. W.W. Norton & Company, New York.
35. Stiehm, E. R. (1975). Fetal defense mechanisms. *Am. J. Dis. Child* **129**, 438–443.

36. Tseng, C., Caldwell, K., Feit, S., Chan, E. K. L., and Buyon, J. P. (1996). Subclass distribution of maternal and neonatal anti-SSA/Ro and -SSB/La antibodies in congenital heart block. *J. Rheum.* **23**, 925–932.
37. James, T. N. (1970). Cardiac conduction system: Fetal and postnatal development. *Am. J. Cardiol.* **25**, 213–226.
38. Deng, J. S., Bair, L. W., Jr., Shen-Schwartz, S., Ramsey-Goldman, R., and Medsger, T., Jr. (1987). Localization of Ro(SS-A) antigen in the cardiac conduction system. *Arthritis Rheum.* **30**, 1232–1238.
39. Buyon, J. P., Hiebert, R., Copel, J., Craft, J., Friedman, D., Katholi, M., Lee, L., Provost, T., Reichlin, M., Rider, L., Rupel, A., Weston, W., and Skovron, M. L. (1998). Autoimmune-associated congenital heart block: Mortality, morbidity, and recurrence rates obtained from a national neonatal lupus registry. *J. Am. Coll. Cardiology* **31**, 1658–1666.
40. Hübscher, O., Batista, N., Rivero, S., Marletta, C., Arriagada, M., Boire, G., Menard, H. A., and Arana, R. M. (1995). Clinical and serological identification of 2 forms of complete heart block in children. *J. Rheum.* **22**, 1352–1355.
41. Askanase, A. D., Friedman, D. M., Dische, M. R., Dubin, A., Starc, T., Katholi, M. C., and Buyon, J. P. (2002). Spectrum and progression of conduction abnormalities in infants born to mothers with anti-SSA/Ro-SSB/La antibodies. *Lupus* **11**, 145–151.
42. Lee, L. A. (1990). Maternal autoantibodies and pregnancy. II. The neonatal lupus syndrome. In "Bailliere's Clinical Rheumatology, Pregnancy and the Rheumatic Diseases" (A. L. Parke, ed.), pp. 69–84. Bailliere Tindall.
43. Thornton, C. M., Eichenfield, L. F., Shinall, E. A., Siegfried, E., Rabinowitz, L. G., Esterly, N. B., Lucky, A. W., and Friedlander, S. F. (1995). Cutaneous telangiectases in neonatal lupus erythematosus. *J. Am. Acad. Dermatol.* **33**, 19–25.
44. Buyon, J. P. (1998). Neonatal lupus. In "Rheumatology" (J. H. Klippel, and P. A. Dieppe, eds.), 2nd Ed. Mosby-Wolfe, London.
45. Neiman, A. R., Lee, L. A., Weston, W. L., and Buyon, J. P. (2002). Cutaneous manifestations of neonatal lupus without heart block: Characteristics of mothers and children enrolled in a national registry. *J. Pediatr.* **37**, 674–680.
46. Lee, L. A., and Weston, W. L. (1988). Neonatal lupus erythematosus. *Semin. Dermatol.* **7**, 66–72.
47. Bangert, J. L., Freeman, R. G., Sontheimer, R. D., and Gilliam, J. N. (1984). Subacute cutaneous lupus erythematosus and discoid lupus erythematosus: Comparative histopathologic findings. *Arch. Dermatol.* **120**, 332–337.
48. David, K. M., Bennion, S. D., DeSpain, J. D., Golitz, L. E., and Lee, L. A. (1989). Immunoreactants in lesions and uninvolved skin in lupus. *Clin. Res.* **37**, 748A. [Abstract]
49. Rosh, J. R., Silverman, E. D., Groisman, G., Dolgin, S., and LeLeiko, N. S. (1993). Intrahepatic cholestasis in neonatal lupus erythematosus. *J. Pediatr. Gastroenterol. Nutr.* **17**, 310–312.
50. Lee, L. A., Reichlin, M., Ruyle, S. Z., and Weston, W. L. (1993). Neonatal lupus liver disease. *Lupus* **2**, 333–338.
51. Lee, L. A., Sokol, R. J., and Buyon, J. P. (2002). Hepatobiliary disease in neonatal lupus erythematosus: Prevalence and clinical characteristics in cases enrolled in a national registry. *Pediatrics* **109**, e11.
52. Schoenlebe, J., Buyon, J. P., Zitelli, B. J., Friedman, D., Greco, M. A., and Knisely, A. S. (1993). Neonatal hemochromatosis associated with maternal autoantibodies against Ro/SS-A and La/SS-B ribonucleoproteins. *Am. J. Dis. Child.* **147**, 1072–1075.
53. Evans, N., and Gaskin, K. (1993). Liver disease in association with neonatal lupus erythematosus. *J. Paed. Child Health* **29**, 478–480.
54. Wallace, S. A., Aron, A. M., and Taff, I. (1984). Neonatal lupus involving the central nervous system. *Ann. Neurol.* **16**, 399.
55. Moudgil, A., Kishore, K., and Srivastava, R. N. (1987). Neonatal lupus erythematosus, late onset hypocalcaemia, and recurrent seizures. *Arch. Dis. Childhood* **62**, 736–739.
56. Wong, H. S., Kuo, M. F., and Chang, T. C. (1995). Sonographic lenticulostriate vasculopathy in infants: Some associations and a hypothesis. *Am. J. Neuroradiol.* **16**, 97–102.
57. Bourke, J. F., and Burns, D. A. (1993). Neonatal lupus erythematosus with persistent telangiectasia and spastic paraparesis. *Clin. Exp. Dermatol.* **18**, 271–273.
58. Kaye, E. M., Butler, I. J., and Conley, S. (1987). Myelopathy in neonatal and infantile lupus erythematosus. *J. Neurol. Neurosurg. Psych.* **50**, 923–926.
59. Kanagasegar, S., Cimaz, R., Kurien, B. T., Brucato, A., and Scofield, R. H. (2002). Neonatal lupus manifests as isolated neutropenia and mildly abnormal liver functions. *J. Rheumatol.* **29**, 187–191.
60. Wolach, B., Choc, L., Pomeranz, A., Ben Ari, Y., Douer, D., and Metzker, A. (1993). Aplastic anemia in neonatal lupus erythematosus. *Am. J. Dis. Child.* **147**, 941–944.
61. Reichlin, M. (1994). Antibodies to ribonuclear proteins in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **20**, 29–43.
62. Reichlin, M. (1992). Antibodies to cytoplasmic antigens. In "Systemic Lupus Erythematosus" (R. Lahita, ed.), 2nd Ed., pp. 237–246. Churchill Livingstone, London.
63. Fritzler, M. J., Pauls, J. D., Kinsella, T. D., and Bowen, T. J. (1985). Antinuclear, anticytoplasmic, and anti-Sjögren's syndrome antigen A (SS-A/Ro) antibodies in female blood donors. *Clin. Immunol. Immunopathol.* **36**, 120–128.
64. Harmon, C. E., Lee, L. A., Huff, J. C., Norris, D. A., and Weston, W. L. (1984). The frequency of autoantibodies to the SS-A/Ro antigen in pregnancy sera. *Arthritis Rheum.* **27**, S20. [Abstract]
65. Keech, C. L., McCluskey, J., Gordon, T. P. (1996). SS-B(La) autoantibodies. In "Autoantibodies" (J. B. Peter, and Y. Shoenfeld, eds.), pp. 789–797. Elsevier, Amsterdam.
66. Harley, J. B. (1989). Autoantibodies in Sjögren's syndrome. *J. Autoimmun.* **2**, 283–394.

67. Ben-Chetrit, E., Gandy, B. J., Tan, E. M., and Sullivan, K. F. (1989). Isolation and characterization of a cDNA clone encoding the 60-kD component of the human SS-A/Ro ribonucleoprotein autoantigen. *J. Clin. Invest.* **83**, 1284–1292.
68. Deutscher, S. L., Harley, J. B., and Keene, J. D. (1988). Molecular analysis of the 60 kDa human Ro ribonucleoprotein. *Proc. Natl. Acad. Sci. USA* **85**, 9479–9483.
69. Chan, E. K. L., Hamel, J. C., Peebles, C. L., and Tan, E. M. (1990). Molecular heterogeneity in SS-A/Ro autoantigens. *Arthritis Rheum.* **33**, S73.
70. Lerner, M. R., Boyle, J. A., Hardin, J. A., and Steitz, J. A. (1981). Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* **211**, 400–402.
71. Wang, D., Buyon, J. P., and Chan, E. K. L. (1996). Cloning and expression of mouse 60 kDa ribonucleoprotein SS-A/Ro. *Mol. Biol. Rep.* **23**, 205–210.
72. O'Brien, C. A., and Wolin, S. L. (1994). A possible role for the 60-kD Ro autoantigen in a discard pathway for defective 5 s rRNA precursors. *Genes Dev.* **8**, 2891–2903.
73. Chambers, J. C., Kenan, D., Martin, B. J., and Keene, J. D. (1988). Genomic structure and amino acid sequence domains of the human La autoantigen. *J. Biol. Chem.* **263**, 18043–18051.
74. Chan, E. K. L., Francour, A. M., and Tan, E. M. (1986). Epitopes, structural domains and asymmetry of amino acid residues in SS-B/La nuclear protein. *J. Immunol.* **136**, 3744–3749.
75. Gottlieb, E., and Steitz, J. A. (1989). Function of the mammalian La protein: Evidence for its action in transcription termination by RNA polymerase III. *EMBO J.* **8**, 851–861.
76. Boire, G., and Craft, J. (1990). Human Ro ribonucleoprotein particles: Characterization of native structure and stable association with the La polypeptide. *J. Clin. Invest.* **85**, 1182–1190.
77. Ben-Chetrit, E., Chan, E. K. L., Sullivan, K. F., and Tan, E. M. (1988). A 52 kD protein is a novel component of the SS-A/Ro antigenic particle. *J. Exp. Med.* **162**, 1560–1571.
78. Chan, E.K.L., Hamel, J. C., Buyon, J. P., and Tan, E. M. (1991). Molecular definition and sequence motifs of the 52-kD component of human SS-A/Ro autoantigen. *J. Clin. Invest.* **87**, 68–76.
79. Itoh, K., Itoh, Y., and Frank, M. B. (1991). Protein heterogeneity in the human Ro/SSA ribonucleoproteins. *J. Clin. Invest.* **87**, 177–186.
80. Chan, E. K. L., DiDonato, F., Hamel, J. C., Tseng, C. E., and Buyon, J. P. (1995). 52-kD SS-A/Ro: Genomic structure and identification of an alternatively spliced transcript encoding a novel leucine zipper-minus autoantigen expressed in fetal and adult heart. *J. Exp. Med.* **182**, 983–992.
81. Buyon, J. P., Slade, S. G., Reveille, J. D., Hamel, J. C., and Chan, E. K. L. (1994). Autoantibody responses to the "native" 52 kD SS-A/Ro protein in neonatal lupus syndromes, systemic lupus erythematosus and Sjögren's syndrome. *J. Immunol.* **152**, 3675–3684.
82. Blange, I., Ringertz, N. R., and Pettersson, I. (1994). Identification of antigenic regions of the human 52 kD Ro/SS-A protein recognized by patient sera. *J. Autoimmun.* **7**, 263–274.
83. Buyon, J. P., Tseng, C.-E., Di Donato, F., Rashbaum, W., Morris, A., and Chan, E. K. L. (1997). Cardiac expression of 52β, an alternative transcript of the congenital heart block-associated 52-kD SS-A/Ro autoantigen, is maximal during fetal development. *Arthritis Rheum.* **40**, 655–660.
84. Lee, L. A. (1993). Neonatal lupus erythematosus. *J. Invest. Derm.* **100**, 9s–13s.
85. Lockshin, M. D., Bonfa, E., Elkon, K., and Druzin, M. L. (1988). Neonatal risk to newborns of mothers with systemic lupus erythematosus. *Arthritis Rheum.* **31**, 697–701.
86. Brucato, A., Frassi, M., Franceschini, F., Cimaz, R., Faden, D., Pisoni, M. P., Muscara, M., Vignati, G., Stramba-Badiale, M., Catelli, L., Lojacono, A., Cavazzana, I., Ghirardello, A., Vescovi, F., Gambari, P. F., Doria, A., Meroni, P. L., and Tincani, A. (2001). Risk of congenital congenital heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis. *Arthritis Rheum.* **44**, 1832–1835.
87. McCauliffe, D. P., Lux, A. F., Lieu, T., Sanz, I., Hanke, J., Newkirk, M. M., Bachinski, L. L., Itoh, Y., Siciliano, M. J., Reichlin, M., Sontheimer, R. D., and Capra, J. D. (1990). Molecular cloning, expression, and chromosome 19 localization of a human Ro/SS-A autoantigen. *J. Clin. Invest.* **85**, 1379–1391.
88. Fliegel, L., Burns, K., MacLennan, D. H., Reithmeier, R. A. F., and Michalak, M. (1989). Molecular cloning of the high affinity calcium-binding protein (calreticulin) of skeletal muscle sarcoplasmic reticulum. *J. Biol. Chem.* **264**, 21522–21528.
89. Sontheimer, R. D., Nguyen, T. O., Buyon, J. P., Lee, L. A. Hall, R. P., Yang, Y. S., and Capra, J. D. (1996). Clinical correlations of autoantibodies to a recombinant, hYRNA-binding form of human calreticulin. *Arthritis Rheum.* **39(Suppl.)**, S38. [Abstract]
90. Eftekhari, P., Salle, L., Lezoualc'h, F., Mialet, J., Gastineau, M., Briand, J. P., Isenberg, D. A., Fournie, G. J., Argibay, J., Fischmeister, R., Muller, S., and Hoebeke, J. (2000). Anti-SSA/Ro52 autoantibodies blocking the cardiac 5-HT_4 serotoninergic receptor could explain neonatal lupus congenital heart block. *Eur. J. Immunol.* **30**, 2782–2790.
91. Buyon, J. P., Winchester, R. J., Slade, S. G., Arnett, F., Copel, J., Friedman, D., and Lockshin, M. D. (1993). Identification of mothers at risk for congenital heart block and other neonatal lupus syndromes in their children. *Arthritis Rheum.* **36**, 1263–1273.
92. Julkunen, H., Kurki, P., Kaaja, R., Heikkila, R., Ilkka, I., Chan, E. K. L., Wallgren, E., and Friman, C. (1993). Isolated congenital heart block: Long-term outcome of mothers and characterization of the immune response to SS-A/Ro and to SS-B/La. *Arthritis Rheum.* **36**, 1588–1598.

93. Cavill, D., Waterman, S., and Gordon, T. P. (2002). Failure to detect antibodies to the second extracellular loop of the serotonin 5-HT4 receptor in systemic lupus erythematosus and primary Sjögren's syndrome. *Lupus* **11**, 197–198.
94. Maddison, P. J., Lee, L., Reichlin, M., Sinclair, A., Wasson, C., and Schemmer, G. (1995). Anti-p57: A novel association with neonatal lupus. *Clin. Exp. Immunol.* **99**, 42–48.
95. Sheth, A. P., Esterly, N. B., Ratoosh, S. L., Smith, J. P., Hebert, A. A., and Silverman, E. (1995). U_1RNP positive neonatal lupus erythematosus: Association with anti-La antibodies? *Br. J. Dermatol.* **132**, 520–526.
96. Solomon, B. A., Laude, T. A., and Shalita, A. R. (1995). Neonatal lupus erythematosus: Discordant disease expression of U_1RNP-positive antibodies in fraternal twins. *J. Am. Acad. Dermatol.* **32**, 858–862.
97. Buyon, J. P., Slade, S. G., Chan, E. K. L., Tan, E. M., and Winchester, R. J. (1989). Effective separation of the 52 SSA/Ro polypeptide from the 48 kD polypeptide by altering conditions of gel electrophoresis. *J. Immunol. Methods* **129**, 207–210.
98. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
99. Dörner, T., Chaoui, R., Feist, E., Göldner, B., Yamamoto, K., and Hiepe, F. (1995). Significantly increased maternal and fetal IgG autoantibody levels to 52 kD Ro(SS-A) and La(SS-B) in complete congenital heart block. *J. Autoimmun.* **8**, 675–684.
100. Meilof, J. F., Frohn-Mulder, I. M. E., Stewart, P. A., Szatmari, A., Hess, J., Veldhoven, C. H. A., Smeenk, R. J. T., and Swaak, A. J. G. (1993). Maternal autoantibodies and congenital heart block: No evidence for the existence of a unique heart block-associated anti-Ro/SS-A autoantibody profile. *Lupus* **2**, 239–246.
101. Silverman, E. D., Buyon, J., Laxer, R. M., Hamilton, R., Bini, P., Chu, J. L., and Elkon, K. B. (1995). Autoantibody response to the Ro/La particle may predict outcome in neonatal lupus erythematosus. *Clin. Exp. Immunol.* **100**, 499–505.
102. Ben-Chetrit, E., Fox, R. I., and Tan, E. M. (1990). Dissociation of immune responses to the SS-A/Ro 52-kD and 60-kD polypeptides in systemic lupus erythematosus and Sjogrens syndrome. *Arthritis Rheum.* **33**, 349–355.
103. Julkunen, H., Siren, M. K., Kaaja, R., Kurki, P., Friman, C., and Koskimies, S. (1995). Maternal HLA antigens and antibodies to SS-A/Ro and SS-B/La: Comparison with systemic lupus erythematosus and primary Sjögren's syndrome. *Br. J. Rheum.* **34**, 901–907.
104. Itoh, Y., and Reichlin, M. (1992). Autoantibodies to the Ro/SSA antigen are conformation dependent. I. Anti-60 kD antibodies are mainly directed to the native protein; anti-52 kD antibodies are mainly directed to the denatured protein. *Autoimmunity* **14**, 57–65.
105. Buyon, J. P. (1993). Congenital complete heart block. *Lupus* **2**, 291–295.
106. Neidenbach, P. J., and Sahn, E. E. (1993). La (SS-B)-positive neonatal lupus erythematosus: Report of a case with unusual features. *J. Am. Acad. Dermatol.* **29**, 848–852.
107. Isacovics, B., and Silverman, E. D. (1993). Limiting dilution analysis of Epstein–Barr virus infectable B cells secreting anti-Ro/SSA and anti-La/SSB antibodies in neonatal lupus erythematosus and systemic lupus erythematosus. *J. Autoimmun.* **6**, 481–494.
108. Tan, E. M. (1989). Do autoantibodies inhibit function of the cognate antigens in vivo? *Arthritis Rheum.* **32**, 924–925.
109. Taylor, P. V., Scott, J. S., Gerlis, L. M., Path, F. R. C., Esscher, E., and Scott, O. (1986). Maternal antibodies against fetal cardiac antigens in congenital complete heart block. *N. Engl. J. Med.* **315**, 667–672.
110. Harley, J. B., Kaine, J. L., Fox, O. F., Reichlin, M., and Gruber, B. (1985). Ro(SS-A) antibody and antigen in a patient with congenital complete heart block. *Arthritis Rheum.* **28**, 1321–1325.
111. Buyon, J. P., Waltuck, J., Crawford, B., Slade, S., Copel, J., and Chan, E. K. L. (1994). Relationship between maternal and neonatal levels of antibodies to 48 kD SSB/La, 52 kD SSA/Ro and 60 kD, SSA/Ro in pregnancies complicated by congenital heart block. *J. Rheumatol.* **21**, 1943–1950.
112. Alarcon-Segovia, D., Ruiz-Arguelles, A., and Liorente, L. (1996). Broken dogma: Penetration of autoantibodies into living cells. *Immunol. Today* **17**, 163–164.
113. Baboonian, C., Venables, P. J. W., Booth, J., Williams, D. G., Roffe, L. M., and Maini, R. N. (1989). Virus infection induces redistribution and membrane localization of the nuclear antigen La (SS-B): A possible mechanism for autoimmunity. *Clin. Exp. Immunol.* **78**, 454–459.
114. LeFeber, W. P., Norris, D. A., Ryan, S. B., Huff, J. C., Lee, L. A., Kubo, M., Boyce, S. T., Kotzin, B. L., and Weston, W. L. (1984). Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultured human keratinocytes. *J. Clin. Invest.* **74**, 1545–1551.
115. Furukawa, F., Kashihara-Sawami, M., Lyons, M. B., and Norris, D. A. (1990). Binding of antibodies to the extractable nuclear antigens of SS-A/Ro and SS-B/La is induced on the surface of human keratinocytes by ultraviolet light (UVL): Implications for the pathogenesis of photosensitive cutaneous lupus. *J. Invest. Dermatol.* **94**, 77–85.
116. Zhu, J. (1996). Ultraviolet B irradiation and cytomegalovirus infection synergize to induce the cell surface expression of 52-kD Ro antigen. *Clin. Exp. Immunol.* **103**, 47–53.
117. Dörner, T., Hucko, M., Mayet, W. J., Trefzer, U., Burmester, G., and Hiepe, F. (1996). Enhanced membrane expression of the 52 kD Ro (SSA) and La (SSB) antigens by human keratinocytes induced by TNF alpha. *Ann. Rheum. Dis.* **54**, 904–909.
118. Levitz, M., and Young, B. K. (1977). Estrogens in pregnancy. *Vit. Horm.* **35**, 109–147.
119. Furukawa, F., Lyons, M. B., Lee, L. A., Coulter, S. N., and Norris, D. A. (1988). Estradiol enhances binding to

cultured human keratinocytes of antibodies specific for SS-A/Ro and SS-B/La. *J. Immunol.* **141**, 1480–1488.
120. Wang, D., and Chan, E. K. L. (1996). 17-β-estradiol increases expression of 52-kDa and 60-kDa SS-A/Ro autoantigens in human keratinocytes and breast cancer cell line MCF-7. *J. Invest. Dermatol.* **107**, 610–614.
121. Ciocca, D. R., and Vargas Roig, L. M. (1995). Estrogen receptors in human nontarget tissues: biological and clinical implications. *Endocr. Rev.* **16**, 35–62.
122. Lin, A. L., and Shain, S. A. (1985). Estrogen-mediated cytoplasmic and nuclear distribution of rat cardiovascular estrogen receptors. *Arteriosclerosis* **5**, 668–677.
123. Reichlin, M., Brucato, A., Frank, M. B., Maddison, P. J., McCubbin, V. R., Wolfson-Reichlin, M., and Lee L. (1994). Concentration of autoantibodies to native 60-kd Ro/SS-A and denatured 52-kd Ro/SS-A in eluates from the heart of a child who died with congenital complete heart block. *Arthritis Rheum.* **37**, 1698–1703.
124. Horsfall, A. C., Venables, P. J. W., Taylor, P. V., and Maini, R. N. (1991). Ro and La antigens and maternal autoantibody idiotype on the surface of myocardial fibres in congenital heart block. *J. Autoimmu.* **4**, 165–176.
125. Watanabe-Fukunaga, R., Brannan, C. L., Copeland, N. G., Jenkins, N. A., and Nagata, S. (1993). Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* **356**, 314–317.
126. Bretscher, P. (1992). The two-signal model of lymphocyte activation twenty-one years later. *Immunol. Today* **13**, 74–76.
127. Casciola-Rosen, L. A., Anhalt, G., and Rosen, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317–1330.
128. Ucker, D. S. (1991). Death by suicide: One way to go in mammalian cellular development? *New Biol.* **3**, 103–109.
129. Kajstura, J., Cheng, W., Reiss, K., Clark, W. A., Sonneblick, E. H., Krajewski, S., Reed, J. C., Olivetti, G., and Anversa, P. (1996). Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. *Lab. Invest.* **74**, 86–107.
130. Cheng, W., Li, B., Kajstura, J., Li, P., Wolin, M. S., Sonneblick, E. H., Hintze, T. H., Olivetti, G., and Anversa, P. (1995). Stretch-induced programmed myocyte cell death. *J. Clin. Invest.* **96**, 2247–2259.
131. Pexeider, T. (1975). Cell death in the morphogenesis and teratogenesis of the heart. *Adv. Anat. Embryo. Cell. Bio.* **51**, 1–100.
132. Takeda, K., Yu, Z. X., Nishikawa, T., Tanaka, M., Hosoda, S., Ferrans, V. J., and Kasajima, T. (1996). Apoptosis and DNA fragmentation in the bulbus cordis of the developing rat heart. *J. Mol. Cell. Cardiol.* **28**, 209–215.
133. James, T. N. (1994). Normal and abnormal consequences of apoptosis in the human heart: From postnatal morphogenesis to paroxysmal arrhythmias. *Circulation* **90**, 556–573.
134. Miranda-Carus, M. E., Tseng, C. E., Rashbaum, W., Ochs, R. L., Casiano, C. A., DiDonato, F., Chan, E. K. L., and Buyon, J. P. (1998). Accessibility of SSA/Ro and SSB/La antigens to maternal autoantibodies in apoptotic human fetal cardiac myocytes. *J. Immunol.* **161**, 5061–6059.
135. Tran, H. B., Ohlsson, M., Beroukas, D., Hiscock, J., Bradley, J., Buyon, J. P., and Gordon, T. P. (2002). Subcellular redistribution of La(SS-B) autoantigen during physiologic apoptosis in the fetal mouse heart and conduction system: A clue to the pathogenesis of congenital heart block. *Arthritis Rheum.* **46**, 202–208.
136. Tran, H. B., Macardle, P. J., Hiscock, J., Cavill, D., Bradley, J., Buyon, J. P., and Gordon, T. P. (2002). Anti-La (SS-B) antibodies transported across the placenta bind apoptotic cells in fetal organs targeted in neonatal lupus. *Arthritis Rheum.* **42**, 1572–1579.
137. Miranda-Carús, M. E., Dinu Askanase, A., Clancy, R. M., Di Donato, F., Chou, T. M., Libera, M. R., Chan, E. K. L., and Buyon, J. P. (2000). Anti-SSA/Ro and anti-SSB/La autoantibodies bind the surface of apoptotic fetal cardiocytes and promote secretion of tumor necrosis factor α by macrophages. *J. Immunol.* **165**, 5345–5351.
138. Miranda-Carus, M. E., Azar, N., Chandrashekhar, S., Clancy, R. M., Askanase, A. D., Chan, E. K. L., and Buyon, J. P. (2002). A role for extracellular-regulated kinase (ERK) activation by opsonized apoptotic cardiocytes in the pathogenesis of congenital heart block. Submitted for publication.
139. Clancy, R., Askanase, A. D., Chiopelas, E., Azar, N., Miranda, M. E., and Buyon, J. P. (2001). Pivotal role of human fetal cardiac fibroblasts in the pathogenesis of autoantibody-associated congenital heart block. *Arthritis Rheum.* **44(Suppl.)**, S160. [Abstract]
140. Li, J. M., Horsfall, A. C., and Maini, R. N. (1995). Anti-La (SS-B) but not anti-Ro_{52} (SS-A) antibodies cross-react with laminin: A role in the pathogenesis of congenital heart block? *Clin. Exp. Immunol.* **99**, 316–324.
141. Nicholson-Weller, A., Burge, J., Fearon, D. T., Weller, P. F., and Austen, K. F. (1982). Isolation of a human erythrocyte membrane glycoprotein with decay accelerating activity for C3 convertases of the complement system. *J. Immunol.* **129**, 184–189.
142. Ballard L. L., Bora, N. S., Yu, G. H., and Atkinson, J. P. (1988). Biochemical characterization of membrane cofactor protein of the complement system. *J. Immunol.* **141**, 3923–3929.
143. Ojicius, D. M., Jiang, S., and Young, J. D. E. (1990). Restriction factors of homologous complement: A new candidate? *Immunol. Today* **11**, 47–49.
144. Seya, T., Turner, J. R., and Atkinson, J. P. (1986). Purification and characterization of a membrane protein (gp 45–70) that is a cofactor for cleavage of C3b and C4b. *J. Exp. Med.* **163**, 837–855.
145. Seya, T., Ballard, L., Bora, N., McNearney, T., and Atkinson, J. P. (1987). Membrane cofactor protein (MCP or gp 45–70): A distinct complement regulatory protein with a wide tissue distribution. *Complement* **4**, 225.
146. Gorelick, A., Oglesby, T., Rashbaum, W., Atkinson, J., and Buyon, J. P. (1995). Ontogeny of membrane cofactor

protein: Phenotypic divergence in the fetal heart. *Lupus* **4**, 293–296.
147. Alexander, E., Buyon, J. P., Provost, T. T., and Guarnieri, T. (1992). Anti-Ro/SS-A antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model: *In vitro* electrophysiologic and immunocytochemical studies. *Arthritis Rheum.* **35**, 176–189.
148. Garcia, S., Nascimento, J. H. M., Bonfa, E., Levy, R., Oliveira, S. F., Tavares, A. V., and Campos deCarvalho, A. C. (1994). Cellular mechanism of the conduction abnormalities induced by serum from anti-Ro/SSA-positive patients in rabbit hearts. *J. Clin. Invest.* **93**, 718–724.
149. Boutjdir, M., Chen, L., Zhang, Z., Tseng, C., DiDonato, F., Rashbaum, W., Morris, A., El-Sherif, N., and Buyon, J. P. (1997). Arrhythmogenicity of IgG and anti-52 kD SSA/Ro affinity purified antibodies from mothers of children with congenital heart block. *Circ. Res.* **80**, 354–362.
150. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. (1981). Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Eur. J. Physiol.* **391**, 85–100.
151. Chen, L., El-Sherif, N., and Boutjdir, M. (1996). α_1-adrenergic activation inhibits β-adrenergic-stimulated unitary Ca^{2+} currents in cardiac ventricular myocytes. *Circ. Res.* **79**, 184–193.
152. Xiao, G. Q., Hu, K., and Boutjdir, M. (2001). Direct inhibition of expressed cardiac L- and T-type calcium channels by IgG from mothers whose children have congenital heart block. *Circulation* **103**, 1599–1604.
153. Mahony, L. (1995). Development of myocardial structure and function. In "Moss and Adams Heart Disease in Infants, Children, and Adolescents, Including the Fetus and Young Adults" (G. C. Emmanouilides, T. A. Riemenschneider, H. D. Allen, and H. P. Gutgesell, eds.), 5th Ed., Vol. I, pp. 17–28. Williams and Wilkins, Baltimore.
154. Josephson, I. R., and Sperelakis, N. (1993). Initiation and propagation of the cardiac action potential. In "Physiology" (N. Sperelakis, and R. O. Banks, eds.), pp. 251–269. Little, Brown and Co., Boston.
155. Mazel, J. A., El-Sherif, N., Buyon, J., and Boutjdir, M. (1999). Electrocardiographic abnormalities in a murine model injected with IgG from mothers of children with congenital heart block. *Circulation* **99**, 1914–1918.
156. Kalush, F., Rimon, E., Keller, A., and Mozes, E. (1994). Neonatal lupus erythematosus with cardiac involvement in offspring of mothers with experimental systemic lupus erythematosus. *J. Clin. Immunol.* **14**, 314–322.
157. Miranda-Carús, M. E., Boutjdir, M., Tseng, C.E., DiDonato, F., Chan, E. K. L., and Buyon, J. P. (1998). Induction of antibodies reactive with SSA/Ro-SSB/La and development of congenital heart block in a murine model. *J. Immunol.* **161**, 5886–5892.
158. Gawkrodger, D. J., and Beveridge, G. W. (1984). Neonatal lupus erythematosus in four successive siblings born to a mother with discoid lupus erythematosus. *Br. J. Dermatol.* **111**, 683–687.
159. Cooley, H. M., Keech, C. L., Melny, B. J., Menahem, S., Morahan, G., and Kay, T. W. (1997). Monozygotic twins discordant for congenital complete heart block. *Arthritis Rheum.* **40**, 381–384.
160. Siren, M. K., Julkunen, H., Kaaja, R., Ekblad, H., and Koskimies, S. (1999). Role of HLA in congenital heart block: Susceptibility alleles in children. *Lupus* **8**, 60–67.
161. Shimosegawa, M., Akasaka, T., and Matsuta, M. (1997). Neonatal lupus erythematosus occuring in identical twins. *J. Dermatol.* **24**, 578–82.
162. Silverman, Earl D., M.D., Hospital for Sick Children, Toronto, Ontario; personal communication.
163. Watson, R. M., Scheel, J. N., Petri, M., Kan, J. S., Provost, T. T., Ratrie, H., III, and Callan, N. A. (1994). Neonatal lupus erythematosus: Report of serological and immunogenetic studies in twins discordant for congenital heart block. *Br. J. Dermatol.* **130**, 342–348.
164. Brucato, A., Gasparini, M., Vignati, G., Riccobono, S., De Juli, E., Quinzanini, M., Bortolon, C., Coluccio, E., and Massari, D. (1995). Isolated congenital heart block: Long-term outcome of children and immunogenetic study. *J. Rheum.* **22**, 541–543.
165. Kaaja, R., Julkunen, H., Ammala, P., Kurki, P., and Koskimies, S. (1993). Congenital heart block in one of two HLA identical twins. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **51**, 78–80.
166. Eronen, M., Siren, M. K., Ekblad, H., Tikanoja, T., Julkunen, H., and Paavilainen, T. (2000). Short- and long-term outcome of children with congenital complete heart block diagnosed in utero or as a newborn. *Pediatrics* **106**, 86–91.
167. Callen, J. P., Fowler, J. F., and Kulick, K. B. (1985). Neonatal lupus erythematosus occurring in one fraternal twin. *Arthritis Rheum.* **28**, 271–275.
168. Lawrence, N., Bligard, C. A., Storer, J., and Courrege, M. L. (1989). Neonatal lupus in twins. *S. Med. J.* **82**, 657–660.
169. Yazici, Y., Onel, K., and Sammaritano, L. (2000). Neonatal lupus erythematosus in triplets. *J. Rheumatol.* **27**, 807–809.
170. Lockshin, M. D., Gibofsky, A., Peebles, C. L., Gigli, I., Fotino, M., and Hurwitz, S. (1983). Neonatal lupus erythematosus with heart block: family study of a patient with anti-SS-A and SS-B antibodies. *Arthritis Rheum.* **26**, 210–213.
171. Lee, L. A., Bias, W. B., Arnett, F. C., Huff, C., Norris, D. A., Harmon, C., Provost, T. T., and Weston, W. L. (1983). Immunogenetics of the neonatal lupus syndrome. *Ann. Intern. Med.* **99**, 592–596.
172. Brucato, A., Franceschini, F., Gasparini, M., De Juli, E., Ferraro, G., Quinzanini, M., Vignati, G., Bortolon, C., Ghessi, A., and Possoli, R. (1995). Isolated congenital complete heart block: Long-term outcome of mothers, maternal antibody specificity and immunogenetic background. *J. Rheum.* **22**, 533–540.
173. Hamilton, R. G., Harley, J., Bias, W., Roebber, M., Reichlin, M., Hochberg, M., and Arnett, F. (1988).

TwoRo(SS-A) autoantibody responses in SLE: Correlation of HLA-DR/DQ specificities with quantitative expression of Ro(SSA) autoantibody. *Arthritis Rheum.* **31**, 496–505.
174. Arnaiz-Villena, A., Vazquez-Rodriguez, J. J., Vicario, J. L., Lavilla, P., Pascual, D., Moreno, F., and Martinez-Laso, J. (1989). Congenital heart block immnogenetics: Evidence of an additional role of HLA class III antigens and independence of Ro autoantibodies. *Arthritis Rheum.* **32**, 1421–1426.
175. Miyagawa, S., Fukumoto, T., Hashimoto, K., Yoshioka, A., Shirai, T., Shinohara, K., Kidoguchi, K. I., and Fujita, T. (1997). Neonatal lupus erythematosus: Haplotypic analysis of HLA class II alleles in child/mother pairs. *Arthritis Rheum.* **40**, 982–983.
176. Stevens, A. M., Hermes, H., and Nelson, J. L. Maternal michrochimerism in neonatal lupus erythematosus. *Arthritis Rheum.* **44(Suppl.)**, S161. [Abstract]
177. McCune, A. B., Weston, W. L., and Lee, L. A. (1987). Maternal and fetal outcome in neonatal lupus erythematosus. *Ann. Intern. Med.* **106**, 518–523.
178. Press, J., Uziel, Y., Laxer, R. M., Luy, L., Hamilton, R. M., and Silverman, E. D. (1996). Long-term outcome of mothers of children with complete congenital heart block. *Am. J. Med.* **100**, 328–332.
179. Tseng, C., Di Donato, F., and Buyon, J. P. (1996). Stability of immunoblot profile of anti-SSA/Ro-SSB/La antibodies over time in mothers whose children have neonatal lupus. *Lupus* **5**, 212–215.
180. Lawrence, S., Luy, L., Laxer, R., Krafchik, B., and Silverman, E. (2000). The health of mothers with cutaneous neonatal lupus erythematosus differs from that of mothers with congenital heart block. *Am. J. Med.*
181. Dinu Askanase, A., Neiman, A., Lee, L. A., and Buyon, J. P. (1999). Clinical parameters of mothers whose children have permanent and transient manifestations of neonatal lupus, and risk of crossover in siblings. *Arthritis Rheum.* **42(Suppl.)**, S225. [Abstract]
182. Geggel, R. L., Tucker, L., and Szer, I. (1988). Postnatal progression from second- to third-degree heart block in neonatal lupus syndrome. *J. Pediatr.* **113**, 1049–1052.
183. Bierman, F. Z., Baxi, L., Jaffe, I., and Driscoll, J. (1988). Fetal hydrops and congenital complete heart block: Response to maternal steroid. *J. Pediatr.* **112**, 646–648.
184. Saleeb, S., Copel, J., Friedman, D., and Buyon, J. P. (1999). Comparison of treatment with fluorinated glucocorticoids to natural history of autoantibody-associated congenital heart block: Retrospective review of the Research Registry for Neonatal Lupus. *Arthritis Rheum.* **42**, 2335–2345.
185. Buyon, J., Roubey, R., Swersky, S., Pompeo, L., Parke, A., Baxi, L., and Winchester, R. (1988). Complete congenital heart block: Risk of occurrence and therapeutic approach to prevention. *J. Rheum.* **15**, 1104–1108.
186. Waltuck, J., and Buyon, J. P. (1994). Autoantibody-associated congenital heart block: Outcome in mothers and children. *Ann. Intern. Med.* **120**, 544–551.
187. Blanford, A. T., and Pearson Murphy, B. E. (1977). In vitro metabolism of prednisolone, dexamethasone, betamethasone, and cortisol by the human placenta. *Am. J. Obstet. Gynecol.* **127**, 264–267.
188. Shinohara, K., Miyagawa, S., Fujita, T., Aono, T., and Kidoguchi, K. (1999). Neonatal lupus erythematosus: Results of maternal corticosteroid therapy. *Obstet. Gynecol.* **93**, 952–957.
189. Barclay, C. S., French, M. A. H., Ross, L. D., and Sokol, R. J. (1987). Successful pregnancy following steroid therapy and plasma exchange in a woman with anti-Ro (SS-A) antibodies: Case report. *Br. J. Obstet. Gynecol.* **94**, 369–371.
190. Glickstein, J. S., Buyon, J. P., and Friedman, D. (2000). The fetal PR interval: Pulsed Doppler echocardiographic assessment. *Am. J. Cardiol.* **86**, 236–239.
191. Askanase, A. D., Miranda-Carus, M. E., Tang, X., Katholi, M., and Buyon, J. P. (2002). The presence of IgG antibodies reactive with components of the SSA/Ro-SSB/La complex in human breast milk: Implications in neonatal lupus. *Arthritis Rheum.* **46**, 269–271.
192. Ryan, A. S. (1997). The resurgence of breastfeeding in the United States. *Pediatrics* **99**, E12.
193. Moak, J. P., Barron, K. S., Hougen, T. J., Cohen, M. H., Nordenberg, A., Van Hare, G. F., Perez, M., Cecchin, F., Schneider, D. S., Nehgme, R. A., and Buyon, J. P. (2001). Congenital heart block: Development of late-onset cardiomyopathy, a previously underappreciated sequela. *J. Am. Coll. Cardiol.* **37**, 238–242.
194. Hochberg, M., Florsheim, P., Scott, J., and Arnett, F. (1985). Familial aggregation of systemic lupus erythematosus. *Am. J. Epidemiol.* **122**, 526–527.
195. Lawrence, J. S., Martins, L., and Drake, G. (1987). A family survey of lupus erythematosus. 1. Heritability. *J. Rheumatol.* **14**, 913–921.
196. Block, S. R., Winfield, J. B., Lockshin, M. D., D'Angelo, W. A., and Christian, C. L. (1975). Studies of twins with systemic lupus erythematosus: A review of the literature and presentation of 12 additional sets. *Am. J. Med.* **59**, 533–552.
197. Arnett, F. C., and Shulman, L. E. (1976). Studies in familial systemic lupus erythematosus. *Medicine* **55**, 313–322.
198. Deapen, D., Escalante, A., Weinrib, L., Horwitz, D., Bachman, B., Roy-Burman, P., Walker, A., and Mack, T. M. (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 311–318.
199. Shoenfeld, Y., Slor, H., Shafrir, S., Krause, I., Granados, J., Villarreal, G. M., and Alarcon-Segovia, D. (1992). Diversity and pattern of inheritance of autoantibodies in families with multiple cases of systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 611–618.
200. Martin, V., Lee, L. A., Katholi, M., and Buyon, J. P. (2002). Long term follow-up of children with neonatal lupus and their unaffected siblings. *Arthritis Rheum.* **42**, 2377–2383.
201. Esscher, E., and Scott, J. S. (1979). Congenital heart block and maternal systemic lupus erythematosus. *Br. Med. J.* **1**, 1235–1238.

202. Jackson, R., and Gulliver, M. (1979). Neonatal lupus erythematosus progressing into systemic lupus erythematosus: A 15 year follow-up. *Br. J. Dermatol.* **101**, 81–86.
203. Fox, R. J., McCuistion, C. H., and Schoch, E. P., Jr. (1979). Systemic lupus erythematosus: Association with previous neonatal lupus erythematosus. *Arch. Dermatol.* **115**, 340. [Abstract]
204. Waterworth, R. F. (1980). Systemic lupus erythematosus occurring with congenital complete heart block. *N. Z. Med. J.* **92**, 311–312.
205. Lanham, J. G., Walport, M. J., and Hughes, G. R. (1983). Congenital heart block and familial connective disease. *J. Rheumatol.* **10**, 823–825.
206. Hübscher, O., Carrillo, D., and Reichlin, M. (1997). Congenital heart block and subsequent connective tissue disorder in adolescence. *Lupus* **6**, 283–284.

18

SYSTEMIC LUPUS ERYTHEMATOSUS IN CHILDHOOD

Paul J. DeMarco
Ilona S. Szer

INCIDENCE

Approximately 25% of all cases of systemic lupus erythematosus (SLE) begin in the first two decades of life [1] but the disease is extraordinarily rare in children under the age of 5. Of 108 patients who developed the disease before the age of 18, 6 became ill before their 5th birthday [2] and of 23 children who had onset prior to their 10th birthday, only 3 were less than 5 years old [3]. After the age of 5, the incidence of the disease increases progressively through adolescence. The average annual incidence rate of SLE in individuals under the age of 24 is 5 in 100,000 compared to the annual rate of 0.53 in children under the age of 14 [4–6]. In the first decade of life, the ratio of girls to boys with SLE is 3:1, increasing to 6:1 in adolescence.

Family studies reveal that relatives of children with SLE exhibit the disease more frequently than family members of adults with SLE. Seventeen percent of children have an affected first-degree relative and 27% of children have an affected first- or second-degree relative [2]. Moreover, 33% of the parents and 39% of the sisters of pediatric patients have positive antinuclear antibodies, specifically antihistone antibodies [7]. Those sisters are also found to have activation of the complement system and may be the relatives at highest risk for the later development of SLE [8–10]. In addition, familial occurrence of increased peripheral blood B cells actively secreting immunoglobulins has been observed [11]. These data suggest that the genetic determinants involved in influencing susceptibility to SLE are more pronounced in childhood-onset disease.

A concordance of 57–69% is reported for identical twins [12–15]. The second twin generally develops the disease within a mean of 3.6 years after the first [15]. Similarly, siblings with SLE are more likely to develop their disease within a few calendar years of each other than they are to develop their disease at approximately the same chronological age [16]. The mean interval in years between the onset of disease in siblings is about the same as that interval in identical twins.

Observations for an underlying etiology for juvenile SLE continue to suggest a complex interaction of a genetically predisposed individual under specific circumstances. Some infectious vectors described in children include Epstein–Barr virus [17] and parvovirus [18]. Children with SLE have a higher seroconversion rate for Epstein–Barr virus than age-matched controls; these same autoantibodies demonstrated cross-reactivity with elements of the viral particle. Reports of parvovirus infection in children reveal induction of antinuclear antibodies (ANA) as well as induction of other autoantibodies, including Sm, RNP, Ro, and La. This report suggests a prolonged lupus-like illness without persistence of symptoms of SLE.

MANIFESTATIONS OF CHILDHOOD SLE

The classification criteria established for SLE have been applied successfully to children. The overall clinical manifestations of SLE occurring in children resemble those in adults (Table 1), although involvement of the reticuloendothelial system has appeared

TABLE 1 Presenting Clinical and Laboratory Features of SLE in Childhood[a]

	Incidence (%)					
	Jacobs [29] (*n* = 35)	**Meislin and Roshfield *et al.* [19] (*n* = 42)**	**King *et al.* [2] (*n* = 108)**	**Platt *et al.* [204] (*n* = 70)**	**Gidden *et al.* [203] (*n* = 55)**	**Total (*n* = 310)**
Clinical manifestation						
Fever	100	90	71	63	55	76
Weight loss[b]	80	88	79	61	51	72
Lymphadenopathy	50	69	39	30	16	41
Hepatosplenomegaly	100	69	28	31	16	49
Renal involvement	70	69	61	71	25	59
Hematologic abnormalities	65	52	46	70	57	58
Pulmonary involvement	60	26	19	Not reported (NR)	18	31
GI symptoms	NR	21	30	NR	NR	25
CNS involvement	35	29	13	14	10	20
Cardiac involvement	25	38	17	NR	15	24
Laboratory finding						
Thrombocytopenia[c]	10	31	NR	21	12[d]	18
Anemia[e]	50	69	NR	62	46	57
Leukopenia[f]	65	53	NR	33	57	52
Proteinuria[g]	70	69	60	65	78	68
VDRL[h]	50	28	NR	NR	9	29
Rheumatoid factor	25	29	NR	NR	12	22
Direct Coombs'	20	44	NR	62	73	49
LE prep or ANA	100	100	100	100	100	100
dsDNA	NR	NR	NR	NR	78	78
Hypocomplementemia	NR	NR	NR	70	NR	70

[a] Age <18 years.
[b] 10% of total body mass.
[c] Platelet count <125,000/mm^3.
[d] Platelet count <100,00/mm^3.
[e] Hb <10 g/100 ml.
[f] WBC <5000/mm^3.
[g] Twenty-four-hour urine protein >200 mg.
[h] Venereal Disease Research Laboratory.

more prominent in children [2, 19–34]. The most recent reevaluations of the differences between adults and children have varying information regarding which clinical manifestations are more likely to be seen in children; however, these recent reports seem to support a much earlier notion that juvenile SLE is more severe than its adult counterpart. Although a study by Manthorpe *et al.* [35] demonstrated a high prevalence of lupus nephritis of 82% in children <11 years versus 53% in adults, two earlier studies reported a similar prevalence in adults and children [19, 36]. Tucker *et al.* [30] reported a statistically significant incidence of hematologic manifestations and double-stranded (ds)DNA as well as a greater prednisone requirement. The greater number of renal manifestations, 27% children versus 17% adults, was not statistically significant. Font *et al.* [31] reported nephropathy to be statistically more common, 20% versus 9% in adults, as well as fever and lymphadenopathy. Rood *et al.* [32] reported arthritis, anemia, and seizures to be the most frequent manifestations, which the author accounted for the 2.6–3.9 times higher chance of more severe disease. Double-stranded DNA was also more common in children than adults in this population, 93% in children versus 63% in adults [32]. Finally, although finding no difference in immunologic markers, Carreno *et al.* [33] found more frequent vasculitis (44% vs 27%), seizures (18% vs 7.6%), and nephropathy (67% vs 48%) in juvenile versus adult SLE. When taken together, these observations seem to suggest the notion from the 1960s that childhood SLE is more severe than adult SLE, specifically regarding hematologic, renal, and possibly neurologic manifestations of the disease.

Mode of Onset

Arthritis, fever, and rash are the three most common presenting complaints, followed by general systemic symptoms of anorexia, weight loss, fatigue, weakness, and malaise. Perhaps because of this mode of onset, half of the patients described by Meislin and Rothfield [19] in the late 1960s had initially been diagnosed as having diseases other than SLE, including acute rheumatic fever, juvenile rheumatoid arthritis (JRA), discoid lupus, sickle cell anemia, idiopathic thrombocytopenic purpura, and Hodgkin's disease. Similarly, initial diagnoses summarized by Deare *et al*. [28], whose patients were studied between 1947 and 1958, included acute glomerulonephritis, hemolytic anemia, leukemia, allergic rash, epilepsy, infectious mononucleosis, rubella, acute rheumatic fever, and septic arthritis. Only 3 of the 15 children in this ancient series received the diagnosis of SLE at the initial evaluation; the lag in diagnosis averaged 10 months.

Early diagnosis has been aided greatly by laboratory tests for ANA, serum complement and anti-DNA antibodies, the relative infrequency of acute rheumatic fever in modern clinical practice, and increased awareness among pediatricians that SLE occurs in children. Conversely, physicians must interpret a positive ANA in an adolescent female with probable intercurrent illness cautiously because the majority of such patients fulfill fewer than four classification criteria and do not develop SLE [29].

Clinical Manifestations

Fever

The most common presenting sign is fever, which occurs in 70–100% of patients. It is usually insidious and low grade, reaching 102°F daily and returning to normal. High spiking fevers of 104°F have also been reported, with a pattern similar to that observed in systemic onset JRA [30].

Arthritis

Arthritis in childhood SLE is manifested as pain, stiffness, or swelling of the joints and may be difficult to distinguish from other causes of synovitis in children (Table 2). Every series of children with SLE includes some patients whose prominent joint involvement made the clinical picture indistinguishable from that of polyarticular juvenile rheumatoid arthritis, either before other manifestations of SLE became apparent or before laboratory studies established the diagnosis of SLE [30]. The presence of rheumatoid factor in a child with arthritis is more common in SLE than in JRA and can be a source of confusion for the clinician. Unlike JRA, particularly when it is associated with the rheumatoid factor, arthritis in SLE rarely causes significant cartilage destruction, bony erosion, premature epiphyseal fusion, or growth disturbance. When deformities such as swan neck or ulnar deviation of the phalanges are seen, they are usually due to periarticular inflammation and are readily reducible. Intrinsic muscle atrophy may decrease grip strength and affect hand function adversely. Joint aspiration usually reveals a paucicellular fluid with low levels of protein and complement. A distinctive deformity of the hands has been reported in both adults and children with SLE. These hand deformities, described elsewhere in the text, have the eponym of Jaccoud's arthritis and were initially thought to represent chronic rheumatic fever [35]. The association of Jaccoud's deformity and increased occurrence of antibodies to UI RNP has been made [36].

Weight Loss and Anorexia

Weight loss and anorexia have been reported in 35% of patients. Usually weight loss does not exceed 10% of total body weight. Although uncommon, some children may present in an emaciated state, having lost 25–30% of their body mass [30].

Skin Disease

The typical macular or papular erythematous eruption in a butterfly distribution that extends across the bridge of the nose but spares the nasolabial folds is seen in about 50% of children. Crusted scaling lesions that cover the face, neck, upper torso, and arms as well as facial and truncal discoid lesions are also frequent. Nodular, vesicular, and even bullous lesions may occur on the face and extremities in addition to the more common fixed red macular eruptions. Some children have only an erythematous blush or palmar erythema. Subungual hemorrhages and vasculitis are characteristic. These lesions are prone to breakdown and suppuration, cause considerable local problems, and lead to systemic infection [2]. These have been associated frequently with Raynaud's syndrome.

Some children have petechial or purpuric lesions unrelated to thrombocytopenia or trauma, which suggest cutaneous vasculitis. Diffuse hyperpigmentation may appear at sites of previous skin lesions, especially in black children, whereas discoid lesions may resolve with scarring and depigmentation. Sometimes the skin lesions ulcerate and crust. Ulcerated, infarcted lesions are frequent on the hands, especially on the palms. Subcutaneous nodules caused by panniculitis are seen, and nodules that superficially resemble those seen in indi-

TABLE 2 Differential Diagnosis of Childhood Arthritis[a]

- Acute rheumatic conditions
  - Acute rheumatic fever
  - Henoch–Schonlein purpura
  - Kawasaki disease (mucocutaneous lymph node syndrome)
  - Serum sickness and drug reactions
  - Mucha–Haberman disease
  - Sweet's syndrome
- Chronic rheumatic conditions
  - Juvenile chronic polyarthritis
  - Juvenile chronic pauciarthritis
  - Juvenile spondyloarthritis
  - Systemic lupus erythematosus
  - Dermatomyositis/polymyositis
  - Vasculitis
  - Progressive systemic sclerosis
  - Mixed connective tissue disease
  - Neonatal onset lupus
  - Juvenile multisystem disease
- Infectious arthritis
  - Gonnococcal arthritis–dermatitis–tenosynovitis syndrome
  - Bacterial arthritis
  - Syphilis
  - Mycobacterial arthritis
  - Viral arthritis
  - Arthropod-borne arthritis (Rocky Mountain spotted fever, Lyme disease)
  - Acute osteomyelitis with joint effusion
- Inflammatory disorders
  - Inflammatory bowel disease with arthritis
  - Familial Mediterranian fever
  - Relapsing polychondritis
  - Cortical hyperostosis (Caffey's disease) and idiopathic hyperostosis (Goldbloom's syndrome)
  - Sarcoid arthritis
- Hypertrophic osteoarthropathy
  - Secondary to pulmonary, cardiovascular, gastrointestinal or endocrine disorders and neoplasms
  - Cystic fibrosis
  - Biliary atresia
  - Primary hypertrophic osteoarthropathy
- Traumatic arthritis
  - Acute chondrolysis of the hip
  - Child abuse
  - Congenital indifference to pain
  - Frostbite
  - Foreign body synovitis (rose thorn, palm frond)
- Degenerative disorders
  - Avascular necrosis of bone
  - Chrondromalacia patella
  - Osteochondritis dessicans
  - Slipped capital femoral epiphysis
- Metabolic diseases
  - Diabetic cheiroarthropathy
  - Abnormalities of amino acid metabolism (alkaptonuria, homocystinuria, sulfite oxidase deficiency)
  - Abnormalities of lipid metabolism (familial hyperlipoproteinemia, Fabry's disease, Gaucher's disease, Farber's disease)
  - Endocrinopathies (Cushing's syndrome, Addison's disease, thyroid disease, myxedema)
  - Purine/pyrimidine metabolism (gout, Lesh–Nyhan's disease)
  - Heavy metal poisoning
  - Hemochromatosis
  - Hematologic disease (hemogloblinopathies, hemophilia, deficiencies of white cell killing ability)
  - Bone metabolism (rickets, dysplasia, hyperparathyroidism, vitamin A and D poisoning, fluorosis)
- Heritable disorders with joint laxity
  - Marfan's syndrome
  - Ehler–Danlos syndrome
  - Benign hypermobility syndrome
- Neoplasms
  - Benign tumors of bone and cartilage (osteiod osteoma, hemangioma, villonodular synovitis)
  - Malignant tumors of bone (synovial sarcoma, epithelioid sarcoma)
  - Hodgkin's and non-Hodgkin's lymphoma
  - Neuroblastoma
- Immunodeficiency syndromes
  - Selective IgA deficiency
  - Agammaglobulinemia and hypogammaglobulinemia
  - Complement component deficiencies
- Psychiatric disorders
  - Psychogenic rheumatism
  - Reflex sympathetic dystrophy
  - Munchausem's syndrome and factitious fever

[a] Modified from Jacobs [29], with permission.

viduals with rheumatoid arthritis have been described as well. An erythematous blush may be observed on the palms regardless of disease activity. Erythematosus or bullous lesions of mucocutanous areas were present at initial evaluation in 21% of children reported by King *et al.* [2] and 10% of those reported by Jacobs [29]. These shallow ulcers occur on the buccal mucosa, lips, gums, and palate and may be found on the vulva. In some instances the lesions are painless, although patients usually complain of mouth pain or a "sore throat." Both rash and oral lesions reappear from time to time with increased activity of the disease. The skin rash is reportedly photosensitive in about 40% of white females and less often in other patients. In some cases, sun exposure exacerbates or initiates systemic disease. The majority of patients, however, do not exhibit overt cutaneous sun sensitivity. Livedo reticularis is another frequently observed skin manifestation, recently linked to anticardiolipin antibodies in patients with both SLE and the antiphospholipid syndrome [37].

Alopecia

Hair loss as a presenting complaint can be elicited in the history of most children with SLE. Total alopecia does not occur. In many instances the most notable feature is simply a noticeable increase of hair on the pillow after a night's sleep. In addition to generalized hair thinning or patchy loss, the texture of the hair may become altered. Hairs just above the forehead are brittle and prone to breaking off, leaving short "frizzy" hairs at the hairline.

Myalgia

Myalgia is a common manifestation of childhood SLE. Occasionally frank myositis with evident weakness due to muscle inflammation and necrosis with serum enzyme elevation is encountered. Some children with severe myositis who exhibit criteria of mixed connective tissue disease probably represent a subset of SLE; they are discussed later in the section on mixed connective tissue disease (MCTD).

Raynaud's Phenomenon

Raynaud's phenomenon is present in a significant number of children with SLE but its absence does not exclude the diagnosis of the illness. Raynaud's phenomenon may precede the onset of other symptoms of SLE by many years, but it should be emphasized that in children, as in adults, there are many causes of Raynaud's phenomenon and most patients with isolated Raynaud's phenomenon may never develop SLE or any other rheumatic disease [38, 39]. Of 27 children with Raynaud's phenomenon with and without associated symptoms referred to the Pediatric Rheumatology clinic, 14 (52%) had a connective tissue disease, 4 (15%) had a probable rheumatic illness, and 9 (33%) had no underlying disease [40]. Circulating ANA and an abnormal nail-fold capillary pattern correlated strongly with secondary Raynaud's. In our clinics we evaluate children with isolated Raynaud's phenomenon with no serologic abnormalities only if new symptoms appear (Table 3).

TABLE 3 Evaluation of Children with Raynaud's Phenomenon

CBC, differential, ESR	History: systemic symptoms, pain, numbness, drug exposure, sicca symptoms, muscle weakness
BUN	Physical examination: BP, digital pitting scars or tip ulcerations, puffy fingers, sclerodactyly, tight skin, telangiectasias, ocular or oral dryness (Schirmer's), peripheral pulses, arthralgias/arthritis, muscle strength testing,
Creatinine	
Urinalysis	
Liver functions	
Thyroid functions	
ANA, RF	
ENA (Sm/RNP)	
Anticentromere	
SCL-70	
Cryoglobulins	
dsDNA	
CH50, C3, C4	
SSA/Ro, SSB/La	
Nail-fold capillary microscopy	

Hematologic Manifestations

Hematologic abnormalities in children with SLE are frequent and reported to have increased prevalence in pediatric SLE; 38% of pediatric patients versus 16% of adults developed hemolytic anemia and/or thrombocytopenia (<100,000 plts/liter) by 2 years [30]. The leukocyte count in approximately 60% of the patients is reduced below 5000 cells/mm^3; in one series [28], 90% of patients had leukopenia. Leukopenia in lupus is due to lymphopenia and can be differentiated from virally induced leukopenia, which is usually associated with a lymphocytosis. Circulating antilymphocyte antibodies have been documented in 30% of patients [9]. Autoimmune thrombocytopenia occurs in less than 10% of pediatric patients and may be the presenting complaint [41]. Up to 75% of children have a hemoglobin level of <10 g/100 ml [28]. The etiology of the anemia is diverse and may include general causes such as chronic illness, blood loss, nutritional deficiencies, malabsorption, or bone marrow suppression. Hemolysis, classically due to antierythrocyte antibodies manifested as a direct Coombs-positive state, is common and may require folate supplementation.

Thrombotic thrombocytopenic purpura (TTP) in childhood bears a dramatic resemblance to systemic lupus erythematosus. In one retrospective chart and literature review, 9 of 35 (26%) patients diagnosed with TTP fulfilled criteria for SLE and 8 of 35 (23%) were diagnosed with SLE after clinical follow-up [42]. Proteinuria was significantly associated with the development of coexistence of SLE. This stands in contrast to the adult experience. An additional case report of the hemolytic uremic syndrome presenting with juvenile SLE seems supportive of this observation [43].

Elevation of factor VIII-related antigen (FVIIIRAg) has been documented in children with SLE, as well as in children with other rheumatic diseases associated with vasculitis [44]. It is speculated that a high level of circulating FVIIIRAg reflects the presence of vascular endothelial injury and may correlate with increased disease activity.

Lymphadenopathy

Twenty-five to 70% of children develop lymphadenopathy at some time during the course of the illness and 40% have diffuse glandular enlargement at initial evaluation. The classic cases of SLE in young women with striking cervical adenopathy reported by Klemperer *et al*. [45] illustrate this aspect of SLE in the pediatric age group. Biopsy of lymph nodes reveals reactive adenopathy, sometimes with vasculitis. Edema, sinus hyperplasia, and occasionally necrosis with hematoxyllin bodies are found [46]. A disproportionately elevated LDH with fever and adenopathy, usually posterior cervical, should raise the suspicion of necrotizing histiocytic lymphadenitis (NHL), or Kikuchi–Fujimoto disease. Although most patients with Kikuchi syndrome have a self-limiting illness, NHL may complicate SLE and MCTD, as well as herald a life-threatening flare of SLE [47–49] (unpublished observation).

The presence of lymphadenopathy in a young patient with constitutional symptoms raises the possibility of lymphoma and leukemia. While both Hodgkin's lymphoma and acute leukemia have been reported to coexist with or mimic childhood SLE [50–52], this is distinctly uncommon, and in the vast majority of children with lupus, adenopathy represents an integral component of the illness. Of note, children who present with lymphadenopathy often have it again during subsequent flares. A biopsy should be discouraged if there is reasonable likelihood of the diagnosis of SLE because this group of patients frequently requires high-dose prednisone therapy, and notable problems may be encountered with wound healing and infection.

Splenomegaly

Enlargement of the spleen occurs in 25–30% of children with SLE at initial evaluation. The incidence is lower in the older series perhaps because the diagnosis of idiopathic thrombocytopenic purpura or Evan's syndrome excluded SLE. Pathologic findings are the same as in adults and are discussed in Chapter 36. Functional asplenia has been reported in children and represents a potentially serious complication predisposing the patient to overwhelming pneumococcal sepsis [53].

Hepatomegaly

In most series, hepatomegaly is noted at the time of presentation in 25–40% of patients and represents another relatively unique aspect of childhood SLE. Sixty-nine percent of patients described by Meislin and Rothfield [19] had hepatomegaly. Minor abnormalities of liver function are very common, noted in 90% of Cook's patients [54], but clinical jaundice is rare. The absence of significant liver chemistry abnormalities distinguishes SLE from chronic active hepatitis and the special subset of chronic hepatitis affecting young women described by Baehr *et al*. [55] that is associated with arthritis, hypergammaglobulinemia, and striking levels of autoantibodies. Very rarely, both illnesses have been reported to coexist [56].

Gastrointestinal Manifestations

Gastrointestinal manifestations are present in 25–40% of patients; abdominal pain and diarrhea are the most common initial signs. Painful intercurrent abdominal crises may occur frequently in the course of childhood SLE and have diverse causes that are of varying importance. Serositis, vasculitis, pancreatitis, splenic infarcts, lymphadenitis, and peritonitis represent the mechanisms for these episodes [57] and require differentiation from acute appendicitis; "atypical pain," normal bowel sounds, and lack of white blood cell elevation suggest that surgery is not required. Vasculitis of the mesentery and bowel wall may progress to hemorrhage, infarction, and frank perforation with secondary peritonitis [54, 58, 59]. Pancreatitis may be either a presenting sign of SLE [60] or a complication of prednisone therapy [61]. Acute pancreatitis of SLE may rarely be associated with the acute respiratory distress syndrome resembling hyaline membrane disease [61]. Retroperitoneal fibrosis [62], ascites [63], concomitant ulcerative colitis [64], and protein-losing enteropathy [65–67] have all been reported as unusual presenting manifestations of childhood SLE.

Renal Manifestations

Renal involvement can be documented in about 70% of pediatric patients or in as many as 90% reported by Cook *et al*. [54]. Although the older literature associates more severe renal disease with childhood compared with adult-onset SLE [19], studies of children continue to vary in their assessment of this feature of the disease [31–34, 68–70]. Our experience suggests that approximately 50% of children have evidence of renal involvement at the time of diagnosis based on urinalysis and assessment of renal function. Acute renal failure as an isolated initial manifestation of SLE [71], distal renal tubular acidosis preceding lupus glomerulonephritis [72], and nephrotic syndrome preceding SLE [73] are exceedingly rare but have been reported in children. Routine urinalysis should be examined carefully for the presence of red blood cells, white blood cells, cellular casts, and oval fat bodies; the latter suggests the pres-

ence of the nephrotic syndrome. A helpful, early manifestation of renal pathology is the presence of white cells in the urine in the absence of infection [30]. Consideration should be given to the presence of chronic interstitial cystitis as a manifestation of active SLE in the assessment of hematuria [74, 75].

Proteinuria should be quantitated in grams excreted over a 24-h period. This is especially important in children because orthostatic proteinuria is common among pediatric patients [76]. Accurate 24-h collections are understandably difficult for a young patient when it interferes with the school day. In lieu of the 24-h collection analyzed every 6 months for total protein, the spot urine creatinine to protein ratio can be used [77].

Elevation of serum creatinine is a relatively late sign and not useful as an early predictor of outcome. An accurately determined creatinine clearance, however, can be used as a sensitive therapeutic indicator. Decreases in creatinine clearance suggest the need for more vigorous treatment when other guidelines of disease activity are equivocal.

A sudden onset of massive proteinuria in a child with SLE requires an evaluation for renal vein thrombosis [78–80]. Because proteinuria due to renal vein thrombosis does not respond to either steroid or cytotoxic therapy, the physician must distinguish between increased proteinuria as a consequence of renal vein thrombosis and increased proteinuria as a sign of advancing renal disease. Duplex Doppler ultrasound examination may reveal the presence of renal vein thrombosis. If thrombosis is demonstrated, treatment with anticoagulants should be initiated [79].

Evaluation of a child with SLE must include a blood pressure determination during each visit. Elevated blood pressure suggests either vasculitis or diffuse proliferative glomerulonephritis. Hypertension as a presenting sign of childhood SLE is rare but develops in 40–60% of children during the course of the illness [54, 81]. Seizures may be the presenting symptom in children who have developed renal insufficiency or failure and present with hypertensive encephalopathy. In one study, African-American children, particularly boys, had significantly more hypertension than Caucasian patients [81]. The same authors found that chronic renal failure or end-stage renal disease developed more frequently in hypertensive children than those without hypertension, suggesting that early renal involvement with hypertension may be predictive of later renal failure. In addition to improving renal outcome [81, 82], control of hypertension decreases the risk of hypertensive encephalopathy (more common in children than adults), strokes, and premature coronary artery disease.

The frequency of the occurrence of histologic subtypes in children with lupus nephritis is difficult to report. Diffuse proliferative glomerulonephritis is reported in 40–70% of children, possibly due to selection of the most severely affected children. The second most common subtype is focal glomerulonephritis, found in 10–20% of individuals; membranous disease occurs in 5–10% of patients [68]. Baqi *et al*. [83] performed a comprehensive evaluation by biopsy of all children with lupus nephritis to show 42.9% WHO IV, 21.4% WHO III, 19.6% WHO IIB, 5.4% WHO IIA, 3.6% WHO V, and 1.8% WHO I. Although pathologic descriptions may not be useful for predicting transformation or progression of one lesion into another [84, 85], the grading of morphologic changes according to indices of activity and chronicity is considered a sensitive predictor of renal outcome [83, 86–88], particularly in conjunction with persistant hypertension [83, 89]. Those patients progressing to end stage renal disease had persistant hypertension, anemia, nephrotic syndrome, and high chronicity indices [89].

Neuropsychiatric Manifestations

Neuropsychiatric manifestations of childhood SLE reportedly occur in 9–60% of patients [1, 2, 68, 90–92]. The mean age of onset of neurologic disease is approximately 13.3–14 years of age [93, 94] with the majority of patients having excellent recovery and response to treatment and a duration of symptoms of less than 3–4 weeks. Severe neuropsychiatric lupus manifestations in children, such as psychosis, vasculitis, optic neuritis, and transverse myelitis, have responded favorably to intravenous methylprednisolone and cyclophosphamide [95]. Central nervous system (CNS) lupus is discussed in Chapter 26. Comments here are limited to special considerations concerning children.

Neuropsychiatric symptoms in any patient with SLE may be caused by the primary illness, the administered steroids, infectious complications, or an emotional stress. The psychologic impact of SLE is particularly great in adolescence when hormonal and physiologic changes reach their peak. Adolescents, concerned with their body image, become anxious and depressed when their bodies are disfigured by disease or treatment [96]. The fear of peer rejection may lead to feelings of isolation and depression, further compounded by the chronicity of the illness and uncertainty about the future [97].

The diagnosis of neuropsychiatric involvement relies heavily on clinical acumen. Although the presence of elevated serum level of ribosomal P protein may help distinguish between SLE-related psychosis and primary psychosis of childhood, it is not a specific finding in patients with SLE [98]. Some observers reported serum antineuronal antibodies at symptom

onset; these antibodies decline with symptom improvement [99]. Single photon emission-computed tomography (SPECT) analysis appears to be a useful tool for the initial assessment of CNS involvement [100–102]. While SPECT identified the initial extent of CNS damage, it failed to correlate with the patients' subsequent clinical improvement during longitudinal follow-up [103, 104].

Headache is a prominent symptom in children with active SLE and a frequent presenting complaint [58]. The headache usually subsides after other disease manifestations become controlled. Brandt and Lessel [105] reported migrainous phenomena in 10% of their patients with SLE. In children with SLE, headache may be a manifestation of hypertensive encephalopathy, intracranial hemorrhage, meningitis, or pseudotumor cerebri [106–108]; rarely, pseudotumor cerebri is an initial presentation of pediatric SLE [109].

Seizures as a presenting manifestation of SLE are rare. Most cases reported as SLE beginning with seizures represent drug-induced lupus in children receiving anticonvulsant therapy for a primary seizure disorder. The observation that these children had milder disease and a better prognosis prompted the study of anticonvulsant drugs as potential inducers of childhood lupus [30, 110]. A detailed discussion of drug-induced lupus may be found in Chapter 44. When a child known to have SLE has a seizure, as seen in 17% of children, bacterial meningitis must first be presumed to be the cause and the diagnosis of CNS lupus may be made only after infection has been excluded [106]. In one case series of 25 children with neurologic symptoms, all but 1 had an established diagnosis of lupus and 7 children had neurologic symptoms when the diagnosis of SLE was established [111].

Chorea is an unusual symptom of SLE [112–126]. Prior to 1985, chorea was encountered in childhood-onset disease much more frequently than in adults with lupus: 53% of all reported cases of chorea-associated with SLE had been in children [113–120]. In one report of 68 children with SLE, 10% had chorea [116]. Chorea can be a diagnostically important initial manifestation of SLE and rarely develops late in the course of the disease [112–115]. Thirteen children have been described in whom chorea was the sole manifestation of lupus [117–126].

Chorea in SLE likely represents another manifestation of the antiphospholipid antibody (aPL) syndrome [115, 127, 128] and may respond best to anticoagulation. A review of chorea and aPL summarized all cases from 1985 to the present [115]; 40% of patients were less than 18 years old. Similarly, of those patients who had SLE, aPL, and chorea, 37% were children. This is a larger proportion than the reported incidence of SLE in the total population (37% versus 25%, respectively). Of note, all of the children with chorea had circulating lupus anticoagulant.

Other neuropsychiatric manifestations of aPL antibodies in juvenile SLE patients include stroke, cerebral ischemic events [128–130], transverse myelitis [131, 132], cerebral vein thrombosis [133], retinopathy [130, 134], and parkinsonism [135].

Pulmonary Manifestations

Pulmonary manifestations occur in up to 60% of children with SLE [30, 136–138]. Like adults (see Chapter 33), children may develop complications related to the underlying illness, such as pneumonitis, pleuritis, pleural effusion, interstitial fibrosis, vasculitis, diaphragmatic muscle dysfunction, and hemorrhage, as well as complications related to therapy or other organ dysfunctions, including uremia, congestive heart failure, thromboembolism, and infection such as pneumocystis carinii [136–141]. In the past, both acute and chronic interstitial lung diseases were considered minor problems in childhood SLE. Currently, with improved survival, several studies of children with SLE who had no clinical evidence of lung involvement documented pulmonary function abnormalities consistent with a restrictive pattern in up to 67% of patients [136–138, 142]. A longitudinal study of pulmonary function tests (PFT) in 15 children with SLE who were asymptomatic for respiratory disease revealed 40% of patients have at least one abnormality, including significant reductions in FVC and diffusing capacity; on serial reevaluation at 6 and 12 months, there was no change and the patients remained asymptomatic [143]. Our experience, based on annual PFT, suggests that at 2–5 years of follow-up less than 20% of children have had abnormalities. We no longer advocate annual PFTs for children with SLE.

Pulmonary hemorrhage has been recognized as a major cause of death for children with SLE [2, 144, 145] often noted at autopsy [146–148]. Ample evidence suggests that in some cases, acute pulmonary hemorrhage is secondary to pneumonitis caused by the deposition of immune complexes and complement in the interstitium of alveolar walls and along the alveolar capillary walls [149–152]. Clinically, the syndrome of pulmonary hemorrhage is characterized by a sudden onset of dyspnea, with hypoxemia, anemia, and bilateral alveolar infiltrates [153, 154]. Hemoptysis is usually present but is not an invariable feature [146, 155]. Pulmonary vasculitis may be distinguished in children on high-resolution computed tomography from other pathologic states through the presence of a centrilobular pattern, which may obviate the need for biopsy confirmation [156].

Cardiac Manifestations

Cardiovascular abnormalities have been documented in up to 60% of children with SLE [2, 14, 28, 30, 54, 157, 158], an incidence similar to that reported for adults [58, 159–163]. Findings span the gamut of cardiac pathology from nonspecific heart murmurs to acute chronic constrictive pericarditis, arrhythmias, and myocardial infarction. Valvular abnormalities are most common and include Liebman–Sacks vegetations, rigid and thickened valves with stenosis, regurgitation, or both, and miscellaneous valvular abnormalities such as thickening of leaflets, calcification, and mitral valve prolapse [163]. While the presence of carditis seems to be associated with antiphospholipid or anticardiolipin antibodies in adults [164, 165], myocarditis and/or pericarditis is more common in children who have anti-Ro/SS-A and anti-La/SS-B antibodies [166, 167]. Cardiac complications of both systemic and pulmonary hypertension may occur. These are discussed in detail elsewhere in this text; only coronary artery involvement as it affects children is reviewed here.

Unusually early atherosclerotic coronary artery disease and myocardial infarction have been documented in young patients with SLE [168–177]. Contributing factors include associated high levels of serum triglycerides, diabetes, and hypertension [178]. The strongest predictor in these children appears to be nephrotic range proteinuria; ultrasonographic evidence of premature atherosclerosis was evident before clinical hypertension, although blood pressure readings were higher than matched controls [179]. Coronary arteritis has been documented in some of these patients and may represent the initial event [180]. Nonatheromatous coronary vasculopathy without coronary arteritis has been reported to cause fatal myocardial infarction in a child with antiphospholipid antibody syndrome and Raynaud's phenomenon [181], suggesting an additional mechanism of myocardial damage. One prospective study of 26 asymptomatic patients with SLE addressed the issue of prevalence of the coronary artery involvement using exercise thallium-201 cardiac scintigraphy [182]. Eight of the patients were 25 years old or younger and 6 demonstrated myocardial perfusion abnormalities; 5 had abnormal thallium-201 imaging findings and 1 had abnormal M-mode echocardiographic findings. There was no correlation with corticosteroid therapy, particular organ involvement, serologic parameters, or duration of the disease. Dedicated studies in childhood lupus have shown a greater presence of cardiac abnormalities in asymptomatic children using screening electrocardiograms, echocardiograms, and myocardial perfusion studies [183, 184]. These studies also failed to demonstrate a corrolation with corticosteroid dose but suggested a relationship to antiphospholipids and abnormal lipid profiles. Older and earlier studies, however, reported an association with the duration of corticosteroid therapy [168–171, 174] and with the presence of coronary arteritis [172, 180]. Whether alternate-day administration of corticosteroids would lessen the risk of premature ASHD through lipid and glucose metabolism as well as blood pressure regulation is not clear. Abnormal lipid metabolism has been documented in patients with SLE prior to starting steroids [185]. However, diabetes and hypertension, both known risk factors for atherosclerotic heart disease, occur less frequently in patients receiving alternate-day therapy. Until long-term prospective studies answer these questions, it seems prudent to use the alternate-day regimen in children whenever possible to minimize known risk factors.

Endocrinologic Manifestations

Autoimmune thyroid disorders are well recognized in systemic lupus erythematosus. Both thyroid abnormalities and autoantibodies are more common in children with SLE than in healthy children. In a study of 35 children with SLE, 6 (17%) had thyroid abnormalities; 4/6 had overt hypothyroidism. In total, 43% demonstrated antithyroid antibodies, 31% had antimicrosomal antibodies, and 29% had antithyroglobulin antibodies [186]. This finding mimics the adult experience [187]. A second systematic review of thyroid function in 42 patients with juvenile SLE confirmed a higher incidence of thyroid autoantibodies (14%) versus 4.4% in a control population [188]. This study also screened patients with ultrasonography and found found four patients (9.3%), with evidence of thyroiditis. The study only identified one SLE patient with active thyroid disease. Some investigators advocate a high index of suspicion for evolving SLE in pediatric patients with either hypothyroidism or hyperthyroidism and antinuclear antibody positivity based on anecdotal experience of pediatric SLE presenting with thyroid disease [189].

The effect of pituitary and gonadotrophic hormones on the clinical course of SLE is discussed elsewhere (Chapter 7). When FSH and LH physiology in juvenile SLE has been compared to healthy controls, baseline levels of both gonadotrpic hormones were higher than the 51 healthy controls yet the difference did not reach statistical significance [190]. One study suggested improvement in malar rash following oral hormone therapy [191]. This benefit should be considered carefully in the context of the potential risk of a flare of systemic manifestations such as nephritis. Although one dedicated study did not find any evidence of

elevated prolactin levels in juvenile SLE [188], a second suggests a trend toward a baseline elevation that did not reach statistical significance. Elevated prolactin in pediatric SLE has been correlated with CNS disease [192], but the clinical significance has not been determined.

Avascular Necrosis of Bone

A detailed discussion of osteonecrosis can be found in Chapter 37. Several reports suggest that avascular necrosis of bone is more frequent in childhood disease, with up to 40% of patients affected; most patients are symptom free, and the lesion is found during a radiologic survey [193, 194]. Radiographs reveal mottling of the trabecular bone pattern, subchondral demineralization, depression or fragmentation, and irregular areas of lucency with sclerosis [1, 194, 195]. When only symptomatic patients are reported, the incidence of avascular necrosis of bone falls to 4.5% in children, equaling that in adults with SLE [196]. Clinically, osteonecrosis is manifested with severe joint pain and with limitation of motion and ambulation. Some patients progress rapidly to total joint destruction requiring replacement. The risk of collapse appears lower in growing children presumably because of increased blood supply to the growing epiphysis.

Joint replacement and "core decompression" pose special problems in the growing child. Surgery may damage the open physis and alter the growth pattern of the affected bone. Treatment, therefore, is limited to nonsteroidal anti-inflammatory agents approved for children, such as tolmetin, at a dose of 20–30 mg/kg/day or naproxen at a dose of 10–15 mg/kg/day [30, 197]. Limitation of activities and rest help relieve pain but it is unlikely that nonweight bearing alters the natural history of osteonecrosis. Our philosophy, based on experience with chronically ill children, is to allow normal activity limited only by the child.

LABORATORY FINDINGS

Laboratory findings in children with SLE are essentially the same as those found in adults (Chapter 15). As in older patients, circulating antinuclear antibodies detectable by immunofluorescent techniques and elevation of all immunoglobulins, particularly IgG, are found in virtually all children [198]. Various patterns of nuclear staining have been described and correlate with the presence of specific autoantibodies [199]. Reactions with nuclear antigens such as double-stranded (native) DNA and single-stranded (denatured) DNA as well as nonhistone proteins (Sm) and proteins associated with small RNAs (SSA/Ro, SSB/La) have all been documented in children with SLE [199–201]. Antinuclear cytoplasmic antibody staining and ELISA for PR3-IgG, MPO-IgM, lactoferrin-IgG and IgM, cathepsin G-IgG and IgM, and elastase-IgG can also be detected. As ANA and extractable nuclear antigen antibodies patterns can complicate the detection of ANCAs, the pathophysiologic role of ANCA in SLE is unclear [202]. Antibody to double-stranded DNA is the most specific finding and is detected in 78% of children at the time of the diagnosis [203] (Table 1).

A rise in antibody to dsDNA is associated with a concomitant fall in the level of total hemolytic complement; this is observed in 70% of children with SLE [204] (Table 1) and is abnormally low in approximately 90% of patients with renal disease [205, 206]. Low complement can also reflect partial or complete deficiency of the classical pathway [207]. Both of these laboratory tests reflect and may predict changes in disease activity [208–212]. They have become important tools in the management of the disease in children who appear to develop abnormal immune phenomena prior to clinical flares more frequently than adults [213–216]. In our experience, virtually all children with active SLE have circulating antibodies to native (ds)DNA compared with 70% adults reported by Tan [217].

Autoantibodies to ribosomal P proteins (anti-P) in juvenile SLE appear to have a higher prevalence than in adult SLE; one study revealed a prevalence in juvenile SLE of 42% while the adult cohort had a prevalence of 7.7% [218]. Children with SLE with both dsDNA and anti-P had a significant odds ratio for the presence of nephritis as well as the concomitant presence of U1-RNP and Sm autoantibodies. Additionally, titers of anti-P and dsDNA varied in accordance to observed changes in the SLEDAI. Taken together, these observations suggest a critical role of anti-P in juvenile SLE.

The IgM rheumatoid factor can be demonstrated in up to 30% of children with lupus, a frequency exceeding the incidence of rheumatoid factor positivity in juvenile rheumatoid arthritis [30]. The rheumatoid factor in SLE is frequently monoclonal or oligoclonal and, because of its more acidic pH optimum, may not be demonstrated by latex fixation. The rheumatoid factor is often associated with a small cryoglobulin with a cryocrit rarely exceeding 0.5–1%. It should be distinguished from cryofibrinogenemic states or heparin-precipitable material, which is found frequently in SLE plasma [219]. The rheumatoid factor containing cryoprecipitate may be the cause of complement consumption that sometimes occurs during transport of the sample.

CLINICAL COURSE AND PROGNOSIS

SLE in children is a chronic multisystem disease characterized by unpredictable exacerbations and remissions of both systemic symptoms and specific organ involvements. The complexity of clinical manifestations calls for a highly individualized approach to each patient.

Mild SLE in children is rare [1, 2, 19, 30, 68, 90], but with increased awareness among primary care providers, improved diagnostic testing, and early referral, the detection of mild disease may become more common. Assessment of disease severity in 108 children with SLE revealed that 5% of children had mild disease without clinical evidence of major organ involvement, complement consumption, and anti-DNA antibodies. These children were primarily symptom free and did not require steroids to control mild exacerbations of cutaneous or musculoskeletal symptoms. The second group, about 10% of patients, included children exhibiting severe symptoms of generalized SLE at its onset, including biopsy-documented renal disease. These children required high-dose prednisone therapy during the first several years; remission was sustained successfully for many years after the stormy initial course [2]. In our experience this subset accounts for 25–30% of patients. However, the majority of children with SLE have a chronic low-grade disease interrupted by irregular, often intense episodes of exacerbation of function of one major organ or another. These episodes, often described as lupus crises, may result in death and call for immediate and often heroic measures (see later). Most of these children have renal involvement that waxes and wanes concomitant with serologic activity; it may, however, progress slowly and independently of immune phenomena to chronic renal failure [1, 2, 19, 68, 89, 204, 206, 220]. Repeated exacerbations of nephritis and uncontrolled hypertension accelerate this process [222]. As discussed in the section on laboratory findings, the presence of antibody to native DNA and the fall in the level of serum complement may correlate with disease activity and predict exacerbations. These parameters should be followed regularly. If they correlate in a given patient, they are used in the prevention of future exacerbations [197, 205, 209–215, 223]. Regular evaluation of urinalysis, creatinine clearance, and protein excretion may reveal the progression of renal disease and call for more aggressive therapy.

The reported prognosis for children with SLE has improved dramatically over the past two decades. The estimated 5-year mortality of 75–80% in the 1960s [30, 54] became 75–85% survival after 10 years of disease in the 1970s [1, 30, 204, 224]. Increasing age at onset, noncompliance, black race, low socioeconomic status, and thrombocytopenia appear to have a negative impact on survival [225, 226]. During the 1980s, management of compliant children with SLE at major medical centers and under the direction of pediatric rheumatologists achieved 95–100% 5-year survival. The 10-year survival is anticipated to be close to 100% as well [30]. Certainly treatment with high doses of corticosteroids and cytotoxic agents has improved the survival of patients with life-threatening SLE crises and has prevented major organ failure in many patients [227–229, 230]. A favorable course of lupus nephritis has been correlated with the long-term normalization of serum complement [231] and cyclophosphamide therapy. The existence of intensive care units, hemodialysis, and transplantation, the effective treatment of infectious complications (the commonest cause of death in many series), and hypertension, as well as the diagnosis of mild cases, have all resulted in higher survival rates. Recent discovery that acquired hypogammaglobulinemia, treatable with IV gammaglobulin and relieved with increased immunosuppression, is not rare in pediatric patients has also contributed. Aggressive, early treatment of serologically predicted exacerbations enables children with SLE to lead essentially normal lives, attend schools, and stay out of the hospital.

DISCOID LUPUS ERYTHEMATOSUS IN CHILDREN

Although chronic discoid lupus erythematosus is less frequent than SLE in childhood, it is estimated that 2–3% of the reported cases of discoid lupus erythematosus have their onset before 15 years of age [232]. As in adults, cutaneous lesions of discoid lupus consist of well-defined erythematous patches with a frequent occurrence of adherent scales and follicular plugging. Older lesions often show atrophic scarring, although involution of the lesions without scarring can occur. Sun-exposed areas are involved most commonly, and in many patients the lesions disseminate or exacerbate upon exposure to ultraviolet light. Patients with discoid lupus may have mild laboratory abnormalities such as positive ANA, high IgG, and mild leukopenia but they do not have systemic disease [233, 234]. It is not known how many children will progress from discoid lupus erythematosus to SLE, but in our experience this is extremely rare. In studies of all age groups, 7% of patients with discoid lupus erythematosus have progressed to SLE over a period of 5 years from the onset of skin lesions [58]. The patients should therefore be aware of the small risk of progression to a more threatening, systemic illness. A thorough evaluation is recom-

mended once a year or at any time that unusual signs develop.

DRUG-INDUCED LUPUS ERYTHEMATOSUS IN CHILDREN

A syndrome resembling or identical to SLE can be induced by the administration of certain drugs. The illness usually subsides following discontinuation of the offending agent. This subject is discussed in detail in Chapters 12 and 44; the role of anticonvulsant drugs in inducing this syndrome in children is discussed here.

The first report implicating hydantoins as inducers of SLE appeared in 1953 [235]. Many subsequent cases have been reported implicating diphenylhydantoin, methylphenylhydantoin, trimethadione, ethosuximide, and clobazam [236–241]. In 1963, Jacobs reported seven children who developed SLE-like symptoms while receiving anticonvulsants, either alone or in combination [30]. All children had positive LE cells and polyarthritis. More recent reports [242–244] suggest that most children with anticonvulsant-induced lupus have fever, rash, and polyarthritis. Although pleuropericarditis is common and cardiac tamponade is disproportionately frequent and life-threatening [30, 245, 246], CNS disease has not been reported, and most authors emphasize the lack of clinical signs of renal involvement [244, 247].

In the study on the incidence of positive ANAs in children receiving anticonvulsant therapy, Singsen *et al*. [242] found that 14 of 70 children receiving ethosuximide and/or diphenylhydantoin had positive ANAs; 2 of 14 had anti-DNA antibodies. Serum ANA titers in the symptomatic children with drug-induced disease did not differ significantly from those in asymptomatic patients. ANAs were also found in the sera of 5 of 23 children receiving phenobarbital alone. Follow-up studies at 10 months showed no symptoms of SLE in any of the ANA-positive patients, suggesting that asymptomatic children who develop ANAs should be followed carefully but need not discontinue their anticonvulsant therapy [242]. The incidence of antibodies against individual histones and histone complexes is greater than 95% in adults with drug-induced lupus (Chapter 12). This has not been studied systemically in children.

In addition to induction of lupus with anticonvulsant drugs, children, like adults, can develop SLE while receiving hydralazine [248], chlorpromazine [30], oral contraceptives [249, 250], zafirlukast [251], sulfonamides, and sulphasalazine [30]. Due to its use for the treatment of acne, minocycline has become increasing recognized as a cause of drug-induced lupus in adolescents [252]. Unlike other types of drug-induced lupus, these children have been reported to develop hepatitis as well as dsDNA and ANCA serologies [253, 254]. ANCA serologies have been perinuclear patterns associated with antimyeloperoxidase and antielastase autoantibodies [255, 256]; in one study, these patients had either HLA-DR4 or -DR2 and all had an HLA-DQB1 allele encoding for tyrosine at position 30 of the first domain [255].

Exacerbation of SLE has reportedly followed the ingestion of alfalfa tablets [257]. Drug-induced SLE in a 12-year-old boy after long-term and interferon-α and -γ included induction of nephritis and double-stranded DNA [258]; prompt discontinuation of interferon and treatment with prednisone and cyclophosphamide were met with full recovery. This case raises the issue of the role of interferon in the induction of SLE.

Studies of adult patients with drug-induced lupus erythematosus reveal increased susceptibility in individuals with a slow acetylator phenotype [259, 260] and with the presence of the HLA/DR4 antigen [261]. Data are not available on the incidence of the slow acetylator phenotype and the presence of genetic susceptibility in markers in children receiving anticonvulsants who develop lupus erythematosus. It is known, however, that although females are more susceptible to drug-induced lupus than males, drug-induced lupus is the most common form of this disease in adult males [261]. Moreover, patients with a positive family history of rheumatic disease are more prone to develop drug-induced lupus, as are individuals with Klinefelter's syndrome [262, 263].

MIXED CONNECTIVE TISSUE DISEASE IN CHILDHOOD

In 1972, Sharp *et al*. [264] described 3 children and 22 adults with clinical and laboratory features of SLE but with overlapping prominent features of dermatomyositis and scleroderma. Patients' sera contained a newly identified antibody to the extractable nuclear antigen system and the term mixed connective tissue disease was applied to the syndrome. It was later determined that the extractable nuclear antigen (ENA) is composed of two different antigens: Sm and RNP; patients with SLE may have both anti-Sm antibodies and anti-RNP, whereas patients with mixed connective tissue disease are characterized serologically by the presence of anti-ENA antibodies consisting solely of anti-RNP [265, 266]. Although initially children with mixed connective tissue disease were considered to have a worse prognosis than adult patients, this may not be so. Clearly there are children who fulfill the diagnostic criteria for mixed connective tissue disease whose manifestations overlap

various rheumatic conditions and may include arthritis, pericarditis, Raynaud's phenomenon, rash, or sclerodermatous skin changes and whose symptoms can be controlled either with nonsteroidal anti-inflammatory drugs (NSAIDS) alone or with relatively small, alternate-day doses of prednisone [267]. Of the original 43 children with MCTD described in the literature, however, 9 (20%) have died [267–272]. Detailed analysis of all case series (Table 4) [267, 269, 273, 274] revealed that the predominant clinical symptoms were fever, polyarthritis (at times crippling), and rash, either malar and SLE-like or scaling and reminiscent of dermatomyositis. Weight loss and fatigue were common. Raynaud's phenomenon occurred in all patients, and sclerodermatous skin changes with sausage-shaped fingers and myositis occurred in over 75%, a considerably higher rate than in series of childhood lupus [30]. Abnormal esophageal motility was observed in two patients. Pulmonary disease, usually restrictive with or without pulmonary hypertension, occurred in 60% of patients. Salivary gland involvement, manifested by recurrent parotid gland swelling (Sjögren's syndrome), was significant in 30% of children. This is now a recognized, albeit rare, presentation of AIDS in childhood, which may simulate SLE/MCTD [275]. Myocarditis and/or pericarditis was found in 50%. Renal involvement, initially considered rare in mixed connective tissue disease, was present in 30%; when biopsy findings are available, analysis reveals glomerular scarring, membranoproliferative pattern, focal proliferative glomerulonephritis, and mesangial proliferation [270]. CNS disease—rare in adult patients—was seen in 26% of children. All of the children had hypergammaglobulinemia, positive ANA in a speckled pattern, and high titers of anti-RNP antibodies. The total hemolytic complement was usually normal. Severe thrombocytopenia was noted in 6 of 14 children reported by Singsen *et al.* [270]; others did not report this. Two of these 6 children subsequently died, one of hemorrhage and the other of overwhelming sepsis following splenectomy [270]. The initial report that this subset of SLE was mild and not fatal was clearly overly optimistic; when the original cohort of adult and pediatric patients was restudied 5 years later, 36% had died [272]. As time went on, each patient took on more and more of the clinical pattern of one disease, dermatomyositis, scleroderma, or SLE. It was necessary to individualize therapy; initially one of the advantages of separating out patients with mixed connective tissue disease from SLE or dermatomyositis was to identify a group who may require less aggressive therapy. Unfortunately, this approach may have resulted in excess mortality in patients with mixed connective tissue disease who evolved into "ordinary" SLE. We are now on guard for such evolutions and manage children

TABLE 4 Summary of Clinical Features of MCTD in Children

	Incidence (%)					
	Baldassare (1976) (*n* = 5)	**Singsen (1977) (*n* = 14)**	**Oetgen (1981) (*n* = 4)**	**Kotajima (1984) (*n* = 72)**	**Kotajima (1992) (*n* = 70)**	**Mier (1996) (*n* = 11)**
Polyarthritis	100	93	100	72	78	100
Raynaud's phenomenon	60	78	75	97	99	91
Fever	100	71	NR	NR	NR	NR
SLE-like rash	80	57	50	61	69	45
Dermatomyositis-like rash	NR	43	25	10	15	36
Sclerodermatous skin changes	60	78	50	36	55	36
Myositis	80	71	75	63	20	91
Abnormal esophageal motility	66	66	0	6	7	45
Cardiac involvement	20	64	25	16	16	18
Pulmonary involvement	50	43	75	14	12	18
Renal involvement	40	36	0	20	28	NR
CNS disease	20	21	50	7	13	NR
Salivary gland involvement	NR	36	25	NR	25	27
Splenomegaly	NR	64	50	NR	NR	NR
Hepatomegaly	NR	64	50	NR	NR	NR
Lymphadenopathy	NR	50	100	35	43	NR

with MCTD using the same guidelines as those applied to SLE [276, 277]. Interestingly, even with this information available, one study of long-term follow-up of these children documented such varied clinical courses as to question whether MCTD is a distinctive disorder [273].

The largest retrospective study of adult and pediatric patients with MCTD suggested different clinical presentations between these two groups [274]. Seventy-two children and 914 adult patients were evaluated by questionnaire in 1984 and 1992 to follow long-term outcomes. Frequency of pericarditis, renal involvement, and thrombocytopenia were lower than previously reported: 16, 20–28, and 10–19%, respectively. Furthermore, mortality from 1983 to 1990 was 3% for juvenile-onset MCTD versus 8% for adult-onset MCTD. Forty-nine percent of the children versus 29% of the adults fulfilled criteria for SLE, a statistically significant difference between the two groups. In contrast to previous smaller studies, this study suggests that pediatric MCTD has a more favorable outcome with more SLE-like characteristics and fewer cases of systemic sclerosis.

JUVENILE SJÖGREN'S SYNDROME

Sjögren's syndrome is a disorder characterized by kerato-conjunctival sicca in the presence of specific autoantibodies (ANA, SSA, SSB, and RF) or a confirmatory minor salivary gland biopsy [278]. It occurs either as an isolated syndrome, termed primary Sjögren's syndrome, or in association with a defined connective tissue disorder, termed secondary Sjögren's syndrome. Although rare, primary Sjögren's syndrome has been described in children [279–281] and termed juvenile Sjogren's syndrome (JSS). Clinical manifestations are similar to those seen in adults. Serum anti-α-fodrin antibodies have been detected in JSS patients before sicca symptoms [282], as well as before other autoantibody markers [283]. This suggests that α-fodrin antibodies may be an early diagnostic marker for JSS. In adults, secondary Sjögren's syndrome is commonly associated with rheumatoid arthritis and scleroderma [284]; in contrast, secondary Sjögren's syndrome in children has historically been associated more intimately with SLE and MCTD [285, 286]. One study suggests that most children with Sjögren's syndrome will develop SLE or MCTD [286].

The spectrum of JSS is evolving as the area undergoes further study. Earlier reports, reviewed by Deprettere *et al*. [287], revealed that out of 28 children with JSS, 17 had primary JSS and 11 children had secondary JSS; 5/11 children had SLE and 4/11 had MCTD. Parotid gland enlargement, sicca symptoms, ANA > 1:160, and abnormal salivary gland biopsy specimens were the most common findings. In 1995, Anaya *et al*. [281] reported 5 new patients and reviewed 34 cases of primary JSS and 31 cases of secondary JSS. In primary JSS, parotitis was a more common presenting feature in children than in adults (62.5% vs 12.8%, respectively); parotitis and the presence of ANA and RF were the principal findings. In contrast, extraglandular features, sicca complex, occurrence of ANA, and articular symptoms were less common in children than in adults. In secondary JSS, 42% of the patients had JRA, whereas 29 and 26% had SLE and MCTD, respectively. Secondary JSS preceded SLE with a range of 1–9 years in 57%; the diseases appeared concurrently in the remaining children.

With parotitis as a principal presentation of JSS in childhood, an evaluation for all potential infectious etiologies is important prior to the diagnosis of juvenile Sjögren's syndrome. In addition to mumps in young children, the diffuse infiltrative lymphocytosis syndrome can mimic both Sjögren's syndrome and SLE [288] and should be considered in the differential diagnosis of atypical kerato-conjunctival sicca or parotitis [289]. Children with HIV disease can present with parotitis [290], but the increased prevalence of pulmonary involvement and infrequent occurrence of autoantibodies should suggest this infectious etiology [275]. The presence of SLE does not rule out parotitis from HIV [291].

Therapy is largely supportive [285, 286] with artificial tears and saliva substitutes to palliate sicca symptoms. Parotitis has been treated effectively with short courses of corticosteroids [281]. Trials of oral pilocarpine, topical cyclosporine, and SNI-2011 are ongoing in adults [292]. Treatment of extraglandular symptoms is covered in appropriate sections.

ANTIPHOSPHOLIPID ANTIBODY SYNDROME

The relatively new syndrome of antiphospholipid antibodies and thrombotic phenomena has long been observed in children [293–296]. The lupus anticoagulant (LA), an antibody that acts as an inhibitor of the thromboxinase complex and *in vitro* prolongs both intrinsic and extrinsic clotting pathways, does not cause bleeding unless there is a coexistent prothrombin deficiency or thrombocytopenia [296–298]. Rather, the presence of the circulating anticoagulant is associated with a tendency to thrombosis presumably due to initial endothelial injury [299–305]. The LA has been demonstrated in children by the presence of a prolonged partial thromboplastin time (PTT) that fails to correct with 1:1

dilution, the kaolin cephalin clotting time (KCT), Russell viper venom time (RVVT), and the platelet neutralization time. The LA has been demonstrated by KCT in the absence of a prolonged PTT in pediatric SLE, however, suggesting that KCT may be a more sensitive screening test [306]. Antiphospholipid antibodies may also present as IgM and IgG anticardiolipin antibodies (aCL) as well as a false-positive syphilis serology.

The presence of antiphospholipid antibodies alone may neither suggest the diagnosis of SLE nor correlate with the risk of thrombosis in children. Children who fulfill the criteria for primary aPL syndrome may not fulfill the ACR criteria for SLE [307]. Further, aPL in children with other autoimmune disorders tend to occur in lower titers and may not be associated with thrombosis [306]. Children with SLE and their first-degree relatives both have increased incidence of aPL; 38% of young SLE patients had at least one aPL and 19% of the first-degree relatives had at least one aPL [308]. Although the aPL positive proband had a 21% incidence of thrombotic events, no thrombotic events occurred in the first-degree relatives [309].

The antiphospholipid antibody syndrome is defined by specific laboratory parameters and clinical manifestations (Chapter 43); criteria have been applied successfully to children. Unfortunately, studies of aPL in children with SLE use different combinations of aCL and LA tests with and without the false-positive syphilis serology to evaluate the incidence and clinical manifestations of aPL syndrome. Thus, the reported incidence of aPL ranges from 9 to 87% [127, 306, 310, 312]. Associated clinical manifestations mirror the adult experience and include thrombocytopenia, Coombs positive hemolytic anemia, vascular thrombosis or digital ischemia, stroke syndromes, chorea, pulmonary vasoocclusive disease, Addison's disease, partial seizures, movement disorders, and hemidystonias [307, 312–316]. Antiphospholipid antibodies apparently have little to no role in the evaluation or sequelae of lupus nephritis [317]. A catastrophic antiphospholipid antibody syndrome has also been described [318]. Hemorrhage can occur if a patient with aPL syndrome develops factor II deficiency reported in the presence of the LA [319, 320] (personal communication). The mechanism of this association is not clear but may be secondary to rapid hepatic clearance of the antigen/antibody factor II complex and usually responds to corticosteroids with or without vitamin K and factor replenishment.

Neurologic events correlate with elevated IgG-aCL in most studies [321] but not all [312]. Although the LA is likely a better marker for thrombotic risk than aCL [321], there are no longitudinal randomized studies for prophylaxis; many pediatric rheumatologists, however, support the use of low-dose aspirin for children with SLE and serologic evidence of IgG aCL antibodies [322]. The current standard of care supports the use of pharmacotherapeutics only after a thrombotic event. Although no randomized trials are available for treatment guidelines, Silverman [323] reported treatment of thrombotic events with heparin followed by coumadin for 6 months to maintain the INR at 2.5. Thereafter, his patients were committed to indefinite coumadin to maintain the INR at 1.5–2.0. Neither recurrent thrombus nor adverse bleeding was reported [323]. Carbamazepine has been reported to treat this type of chorea [324].

GENERAL APPROACH TO MANAGEMENT OF CHILDREN WITH SLE

The treatment plan for children with SLE must be based on a careful assessment of the extent and severity of the disease versus the risk of treatment, while keeping in mind the need for growth and normal function at home and in school. Symptoms of children with mild disease can be managed with NSAIDS: tolmetin (20–30 mg/kg/day) or naproxen (10–15 mg/kg/day) [30, 325, 277] to control fever and reduce joint inflammation. Regular monitoring of liver enzymes is indicated, although the frequency of drug-induced hepatotoxicity is much less with NSAIDs than that associated with the use of salicylates, which occurred frequently in children with SLE [327]. Rash and minor constitutional symptoms, with or without arthritis, may respond to hydroxychloroquine (<6.5 mg/kg/day) [2, 277, 328, 329]. To prevent worsening of the rash, the use of sunscreens, visors, and parasols, as well as avoidance of direct sun exposure, cannot be overemphasized. If minor constitutional symptoms cannot be controlled, low-dose, alternate-day prednisone can be used.

As stated earlier, many children with SLE present with active nephritis, are seriously ill, and develop evidence of advancing disease while treated conservatively. For those children, prednisone, up to 2 mg/kg/day in divided daily doses, is used to alter or abolish the specific mechanism of disease responsible for the life- or organ-threatening process. In general, high-dose prednisone therapy (2 mg/kg/day in divided doses) should be reserved for acute life-threatening episodes and maintained for the briefest possible time, using both clinical and laboratory parameters of disease activity, specifically elevation of antibodies to double-stranded DNA and fall in the serum complement levels [276, 277, 330, 331]. Usually, significant clinical improvement occurs within 2 to 6 weeks, and the dose is consolidated to a single morning regimen. Once normalization of immune abnormalities is achieved, the dose may be adjusted to

an alternate-morning regimen by doubling the daily dose and adding another 20–40% [28]. While maintaining control, the drug may be tapered slowly over 2–3 years to the minimum level required for the control of symptoms and serologic parameters. Whenever possible after the acute period, administration of hydroxychloroquine and nonsteroidal anti-inflammatory drugs may allow for further reduction in the steroid. Hydroxychloroquine at a dose not exceeding 6 mg/kg/day is well tolerated for many years with nominal risk of retinal toxicity [332] and has been associated with fewer flares of SLE in adulthood [329].

If the disease cannot be controlled adequately with alternate-day prednisone, the use of azathioprine (2–3 mg/kg/day) or pulse intravenous cyclophosphamide (500–1000 mg/m^2) may be indicated for individual patients [30, 276, 277, 333–335] as steroid-sparing agents. A gradual reduction in prednisone can usually be accomplished after control has been achieved with the addition of the cytotoxic agent. Methotrexate has been used successfully as a steroid-sparing agent for pediatric patients failing either prednisone or prednisone–cyclophosphamide; in both cases, improvement in laboratory and clinical end points, such as nephritis, was seen [336]. Cyclosporine A has been used successfully in adults and children [324, 337, 338]. Autologous stem cell transplantation (ASCT) for pediatric rheumatic diseases continues to receive increasing attention as protocols are developed and indications established [339]. Children with SLE have been the successful recipients of ASCT, with the first two patients reported to have complete remissions and increases in growth curve velocity [340]. The role of ASCT in the treatment of rheumatic diseases has yet to be completely defined.

Glucocorticoid-induced osteoporosis requires special attention and can be stabilized with appropriate nutritional support with at least 1 of calcium carbonate and 400 IU vitamin D daily [340a].

TREATMENT OF SPECIFIC PROBLEMS AND LONG-TERM COMPLICATIONS

Children who are in an emergent situation, are comatose, hypertensive, or hemorrhaging require vigorous intervention, such as pulse steroid therapy (30 mg/kg of methylprednisolone daily for 3 days) and intravenous cyclophosphamide (750 mg/m^2) or lymphoplasmapheresis three times a week, concomitant with a cytotoxic agent. Specifically, the treatment of pulmonary hemorrhage, an often fatal early complication of childhood SLE [2], should include intravenous steroid, cyclophosphamide, and plasma- or lymphoplasmapheresis [154, 341–343]. This approach, although successful only anecdotally, appears to have promise [344, 345].

Indications for the use of cytotoxic therapy in the treatment of SLE in children neither immediately threatened with a fatal complication nor exhibiting active lupus nephritis have not been firmly established. Patients with certain neurologic manifestations of SLE, as well as several with gastrointestinal vasculitis and pneumatosis intestinalis, have been treated successfully with intravenous boluses of cyclophosphamide [346, 347]. Patients with severe thrombocytopenia or myositis may be candidates for such treatment [348], although intravenous immunoglobulins have also been successful in controlling SLE complicated by thrombocytopenia in both children and adults [349, 350]. Other authors report successful management of CNS crises, including transverse myelitis, with massive doses of methylprednisolone (30 mg/kg/day) for 6 days [351–353]. A lack of prospective placebo-controlled trials makes the decision-making process extremely difficult and underscores the need for randomized studies of different treatment modalities in the management of SLE in children.

Abdominal Crises

A sudden onset of even mild abdominal pain and low-grade fever in a patient whose SLE is well controlled with corticosteroids or cytotoxic therapy may represent sepsis with peritonitis [354–356]. Immediate treatment with broad-spectrum intravenous antimicrobials is indicated while defining the nature of the problem. The organisms involved are most frequently pneumococci, staphylococci, or streptococci followed by gram-negative bacteria, *Salmonella*, and fungi [30, 356, 357]. General supportive measures—maintenance of fluid and electrolyte balance, blood pressure, and ventilation, as well as stress levels of steroids—are essential to the patient's survival. Early surgery may be life saving in cases of perforation [358], but may significantly worsen the course in a child with sepsis and peritonitis or acute SLE. Once careful evaluation has ruled out perforation and sepsis, prednisone (1–2 mg/kg/day) may be used until symptoms are brought under control.

Hypertension and Renal Disease

The control of hypertension should be vigorously pursued whenever this complication occurs [81, 359]. In addition to antihypertensive agents, azathioprine or cyclophosphamide may be added to the steroid regimen. This permits modification of steroid therapy to alternate mornings, a regimen resulting in less blood pressure elevation [360]. The management of renal

disease should be highly individualized [361, 362]. High-dose prednisone should be used whenever renal biopsy reveals active glomerular involvement. Because patients with mesangial or focal glomerulonephritis have a relatively good prognosis in terms of potential for renal failure, low-dose, alternate-day therapy is probably adequate [363]. If this principle is not strictly adhered to, deaths from treatment of patients with insignificant renal involvement may exceed fatalities from the disease [364]. Acute deterioration in renal function may be reversed with immediate pulse methylprednisolone therapy, 30 mg/kg/day for 3–6 days [365–367] or monthly cyclophosphamide boluses [361, 368]. The potential exists, however, for life-threatening sepsis disseminating from an occult site with these aggressive therapies [369]. Since the recognition of the potential for histologic change [85, 87, 370], the index of activity and chronicity has been used to estimate prognosis for individual patients. As expected, the higher the activity index, the more likely the reversal of the inflammatory lesion. Patients with high-activity and low-chronicity indices, therefore, reportedly have a good outcome if they are treated with any one of the various regimens: oral corticosteroids, cyclophosphamide, or azathioprine; high-dose intravenous cyclophosphamide plus steroids; high-dose steroids; or even low-dose steroids [361, 368, 371]. Refractory lupus nephropathy remains a clinical challenge, which has been managed successfully with either cyclosporine [372] or mycophenolate mofetil [373, 374]. It is prudent to choose the least toxic regimen for such patients but one that will ultimately eliminate the activity without resulting in scarring. Prednisone alone may be enough to accomplish this.

Patients with a moderate degree of scarring seem to be appropriate candidates for cytotoxic therapy, either alone or in combination with corticosteroids [334, 361, 368, 375]. Long-term prospective studies designed without consideration for the activity and chronicity index rating have shown that cytotoxic therapy is superior in the treatment of diffuse proliferative glomerulonephritis [163, 376–385]. Patients with a high chronicity index already destined for renal failure and dialysis or transplantation may benefit little from immunosuppressive therapy and only suffer from its toxicity. For these patients, a less vigorous therapy should be considered [361]. Nevertheless, studies currently in progress suggest that intravenous boluses of cyclophosphamide may have beneficial effects on some renal lesions with a high degree of chronicity [361]. While the pharmacokinetics and side effects of bolus oral cyclophosphamide appear similar to those of intravenous cyclophosphamide, neither the degree of potential benefit nor toxicity has been fully evaluated [378].

Chronic Renal Failure

Eventually, children with renal failure secondary to SLE require dialysis, renal transplantation, or both [386]. Retrospective analysis has revealed that DPGN (WHO class IV) and hypertension are significant predictors of progression to ESRD [387, 388]. Baqi *et al.* [388] suggest that a creatinine level >1.6 mg/dl associated with a C3 level <63 U/ml at presentation are also predictive of progression to ESRD [388]. The ultimate prognosis for those patients who do not have significant active extralegal disease is similar to that for patients with idiopathic renal failure [389]. The overall function and survival of children treated with chronic hemodialysis were considered favorable; one study reported a 5-year actuarial patient survival of 95% [388]. More recently, however, McCurdy *et al.* [387] reported only 50% survival of children with SLE receiving chronic dialysis.

In addition, chronic dialysis is associated with retarded linear growth [391–394], osteodystrophy [393], hypertension, chronic anemia, neurologic dysfunction, hepatitis, and psychosocial disturbances [396–398]. Although children may be able to attend school regularly, the great amount of time and effort required to give dialysis treatments precludes full rehabilitation. Vitamin D analogs have been used successfully to improve intestinal calcium absorption, reverse secondary hyperparathyroidism, and support and/or improve bone mineral density, as well as improve linear growth in some patients [340a, 399, 400]. Either calcium carbonate or calcium lactate provides both calcium and a source of alkali [401–404].

The optimal treatment for all children with renal failure is renal transplantation; a successful transplant provides the best opportunity for normal growth and development, as well as a better acceptance of living with chronic disease [405]. Renal transplantation has been successfully undertaken in young children [406]. Thus far, few children have been reported to develop glomerulonephritis in the donor kidney [407]. A less aggressive disease course that often accompanies renal failure and the posttransplantation regimen of immunosuppression most likely accounts for the low frequency of disease in the donor kidney.

SPECIAL CONSIDERATIONS IN THE ADOLESCENT WITH SLE

Adolescence is a stage of growth and development marked by “great emotional upset with wide fluctuations in behavior” [408]. Body image is fragile and the need for acceptance by peers is at its peak. The

emotional strain of chronic disease is intensified by the disfiguring stigmata of both disease and, more importantly, its treatment [409]. Among the manifestations most disturbing to the teenager are dermatitis, hair loss, and vasculitic lesions frequently involving the face. Control of the disease usually results in improvement; to control the disease, however, the young patient often exchanges disease-related symptoms for even more upsetting cushingoid stigmata of mood facies, obesity, striae, and fine tremors [410]. Because peer acceptance is so highly valued, the young patient may not comply with therapy in order to avoid cushingoid appearance, acne, hair loss, and weight gain. One study revealed noncompliance in 59% of adolescent patients required to take steroids [411]. Alternate-day steroid regimen should be used as early as possible to lessen the risk of infection, to avoid grotesque cushingoid appearance, and to avoid growth retardation, diabetes, and hypertension [412].

The young patient with SLE quickly learns that his or her chronic disease is punctuated by exacerbations and must be monitored every 1–3 months with blood and urine studies. During the initial active stage, weekly follow-up may be required, significantly interfering with school and social activities. It is important to ensure that the patient does not become estranged from his or her peer group; it has been documented that patients who isolate themselves from their peers tend to have exacerbations of disease twice as frequently as those who remain socially active [413, 414], although cause and effect here are difficult to determine.

As with any chronic illness, the adolescent may exhibit a wide range of psychological reactions [408]. The intensity of these responses will depend on the degree of illness, particular symptoms, and the psychological makeup of the individual patient [415]. The fear of inability to meet goals, such as working and raising a family, and the fear of death need to be brought out and discussed openly. Education about the nature and course of the disease often leads to acceptance and alleviation of unfounded fears. Structured psychiatric intervention is advisable if the patient develops depression or has suicidal tendencies. With proper emotional support in the form of group or individual discussion with professionals trained to care for children with chronic disease, adolescents with SLE can make a successful adjustment to their illness [416].

Finally, physicians caring for adolescent patients must address the issues of contraception and pregnancy. Estrogen-containing oral contraceptives have been associated with exacerbation of SLE, whereas progesterone, equally effective in contraception, does not induce flares [249, 250, 417]. The inability to bear children often concerns young women with SLE and those adolescents who require prolonged treatment with cyclophosphamide. Although pregnancy is fully covered elsewhere in this book, it is important to mention here that when the disease is controlled, the chances of conceiving and bearing a child without inducing a flare approach those for healthy women [30, 418–421]. While cyclophosphamide therapy may lead to oligomenorrhea [422] and ovarian and testicular failure [423], ovarian failure is much less common in younger women than those in their middle age. Monthly intramuscular GnRH analogs have been shown to be protective in eight of eight female SLE patients receiving cyclophosphamide, whereas five of nine control patients developed premature ovarian failure [424]. The youngest SLE patients in this study were ages 20–23 years; however, the same group has administered GnRH analogs to preserve ovarian function in children as young as 15 years who were treated for malignancies with cytotoxic agents [425]. Sperm should be stored in sperm banks for the males, who are at high risk of sterility. In addition, bimonthly intramuscular testosterone begun 1 month prior to and continued during treatment with cyclophosphamide has been shown to preserve sperm count in young adults [426] and could be considered for adolescent males with documented skeletal maturation.

References

1. Fish, A. J., Blau, E. B., Westberg, N. G., *et al*. (1977). SLE within the first two decades of life. *Am. J. Med*. **62**, 99.
2. King, K. K., Kornreich, H. K., Bernstein, B. H., *et al*. (1977). The clinical spectrum of SLE in childhood. *Arthritis Rheum*. **20**, 287.
3. Lehman, J. A., McCurdy, D. K., Bernstein, B. H., *et al*. (1989). Systemic lupus erythematosus in the first decade of life. *Pediatrics* **83**, 235.
4. Hochberg, M. C. (1985). The incidence of SLE in Baltimore, Maryland, 1970–1977. *Arthritis Rheum*. **28**, 20.
5. Michet, C. J., McKenna, C. H., Elveback, L. R., *et al*. (1985). Epidemiology of systemic lupus erythematosus and other connective tissue diseases in Rochester, Minnesota, 1950 through 1979. *Mayo Clin. Proc*. **60**, 105.
6. Fesssel, W. J. (1988). Epidemiology of systemic lupus erythematosus. *Rheum. Dis. Clin. North Am*. **14**, 15.
7. Shoenfield, Y., Segol, G., Segol, B., *et al*. (1987). Detection of antibodies to total histones and their subfractions in systemic lupus erythematosus patients and their asymptomatic relatives. *Arthritis Rheum*. **30**, 169.
8. Lehman, T. J. A., Curd, J. G., and Zvaifler, N. J. (1982). The association of ANAs, anti-lymphocyte antibodies, and C4 activation among the relatives of children with SLE. *Arthritis Rheum*. **25**, 556.
9. Lehman, T. J. A., Hanson, J., Zvaifler, N., *et al*. (1984). Antibodies to nonhistone nuclear antigens and anti-lymphocyte antibodies among children and adults with SLE and their relatives. *J. Rheumatol*. **11**, 644.

10. Jones, J. V., *et al*. (1987). Familiar systemic lupus erythematosus: Evidence for separate loci controlling C4 deficiency and formation of antibodies to DNA, nRNP, Ro and La. *J. Rheumatol*. **14**, 263.
11. Tsuyoshi, S., *et al*. (1989). Familiar occurrence of impaired interleukin-2 activity and increased peripheral blood B Cells actively secreting immunoglobulins in systemic lupus erythematosus. *Am. J. Med*. **86**, 385.
12. Block, S. R., Winfield, J. B., and Lockshin, M. D. (1975). Studies of twins with SLE: A review of the literature and presentation of 12 additional sets. *Am. J. Med*. **59**, 533.
13. Lieberman, E., Heuser, E., Hanson, V., *et al*. (1968). Identical 3-year-old twins with disseminated LE: One with nephrosis and one with nephritis. *Arthritis Rheum*. **11**, 22.
14. Brunjes, S., Zike, S., and Julian, R. (1961). Familial SLE: A review of literature with a report of 10 additional cases in four families. *Am. J. Med*. **30**, 529.
15. Block, S. R., Lockshin, M. D., Winfield, J. B., *et al*. (1976). Immunologic observations on 9 sets of twins either concordant or discordant for SLE. *Arthritis Rheum*. **19**, 545.
16. Kaplan, D. (1984). The onset of disease in twins and siblings with systemic lupus erythematosus. *J. Rheumatol*. **11**, 5.
17. James, J. A., Kaufman, K. M., Farris, A. D., *et al*. (1997). An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J. Clin. Invest*. **100**, 3019.
18. Moore, T. L., Bandlamudi, R., Alam, S. M., *et al*. (1999). Parvovirus infection mimicking systemic lupus erythematosus in a pediatric population. *Semin. Arthritis Rheum*. **28**, 314.
19. Meislin, A. G., and Rothfield, N. (1968). Systemic lupus in childhood. *Pediatrics* **42**, 37.
20. Emery, H. (1986). Clinical aspects of SLE in childhood. *Ped. Clin. North Am*. **33**, 1177.
21. Lee, L. A., and Weston, W. L. (1986). Lupus erythematosus in childhood. *Dermatol Clin*. **4**, 151.
22. Kaufman, D. B., Laxer, R. M., Silverman, E. D., *et al*. (1986). SLE in childhood and adolescence: The problem, epidemiology, incidence, susceptibility, genetics and prognosis. *Curr. Probl. Pediatr*. **16**, 545.
23. Ansell, B. M. (1987). Perspectives in pediatric SLE. *J. Rheumatol*. **13**, 177.
24. Chen, J. H., Lin, C. Y., Chen, W. P., *et al*. (1987). Systemic lupus erythematosus in children. *Chin. J. Microbiol. Immunol*. **20**, 23.
25. Lee, B. W., Yap, H. K., Yip, W. C., *et al*. (1987). A 10 year review of systemic lupus erythematosus in Singapore children. *Aust. Paediatr. J*. **23**, 163.
26. Cassidy, J. T., and Petty, R. E. (1990). SLE In "Textbook of Pediatric Rheumatology, " 2nd Ed. Churchill Livingstone, New York.
27. Gribetz, D., and Henley, W. L. (1959). SLE in childhood. *J. Mount Sinai Hosp*. **26**, 289.
28. Deane, P. M. G., Liard, G., Siegal, D. M., and Baum, J. (1995). The outcome of children referred to a Pediatric Rheumatology Clinic with a positive antinuclear antibody test but without an autoimmune disease. *Pediatrics* **95**(6), 892.
29. Jacobs, J. C. (1982). "Pediatric Rheumatology for the Practitioner." Springer-Verlag, New York.
30. Tucker, L. B., Menon, S., Schaller, J. G., *et al*. (1995). Adult and childhood-onset systemic lupus erythematosus: A comparison of onset, clinical features, serology and outcome. *Br. J. Rheumatol*. **34**, 866.
31. Font, J., Cervera, R., Espinosa, G., *et al*. (1998). Systemic lupus erythematosus in childhood: Analysis of clinical and immunological findings in 34 patients and comparison with SLE characteristics in adults. *Ann. Rheum. Dis*. **57**, 456.
32. Rood, M. J., Ten Cate, R., Lisette, W. A., *et al*. (1999). Childhood-onset systemic lupus erythematosus: Clinical presentation and prognosis in 31 patients. *Scand. J. Rheumatol*. **28**, 222.
33. Carreno, L., Lopez-Longo, F. J., Monteagudo, I., *et al*. (1999). Immunological and clinical differences between juvenile and adult onset of systemic lupus erythematosus. **8**, 287.
34. Iqbal, S., Sher, M. R., Good, R. A., *et al*. (1999). Diversity in presenting manifestations of systemic lupus erythematosus in children. *J. Pediatr*. **135**, 500.
35. Manthorpe, R., Bendixen, G., Schiller, H., *et al*. (1980). Jaccoud's syndrome: A nosographic entity associated with SLE. *J. Rheumatol*. **7**, 169.
36. Reilly, P. A., Evison, G., McHugh, N. J., *et al*. (1990). Arthropathy of hands and feet in systemic lupus erythematosus. *J. Rheumatol*. **17**, 777.
37. Englert, H. J., Loizou, S., Derue, G. G. M., *et al*. (1989). Clinical and immunologic features of livedo reticularis in lupus: A case-control study. *Am. J. Med*. **87**, 408.
38. Gerbracht, D. D., Steen, V. D., Ziegler, G. L., *et al*. (1985). Evolution of primary Raynaud's phenomenon (Raynaud's disease) to connective tissue disease. *Arthritis Rheum*. **28**, 87.
39. Fitzgerald, O., Hess, E. V., O'Connor, G. T., *et al*. (1988). Prospective study of the evolution of Raynaud's phenomenon. *Am. J. Med*. **84**, 718.
40. Duffy, C. M., Laxer, R. M., Lee, P., *et al*. (1989). Raynaud syndrome in childhood. *J. Pediatr*. **114**, 73.
41. Lipnick, R. N., Tsokos, G. C., Bray, G. L., *et al*. (1990). Autoimmune thrombocytopenia in pediatric systemic lupus erythematosus: Alternative therapeutic modalities. *Clin. Exp. Rheumatol*. **8**, 315.
42. Brunner, H. I., Freedman, M., and Silverman, E. D. (1999). Close relationship between systemic lupus erythematosus and thrombotic thrombocytopenic purpura in childhood. *Arthritis Rheum*. **42**(11), 2346.
43. Ogawa, T., Arai, H., Maruyama, K., *et al*. (2000). Association of systemic lupus erythematosus and hemolytic uremic syndrome in a child. *Pediatr. Nephrol*. **14**, 62.
44. Bowyer, S. L., Ragsdale, C. G., and Sullivan, D. B. (1989). Factor VII related antigen and childhood rheumatic diseases. *J. Rheumatol*. **16**, 1093.
45. Klemperer, P., Pollack, A. D., and Baehr, G. (1942). Diffuse collagen disease: Acute disseminated lupus erythematosus and diffuse scleroderma. *JAMA* **119**, 331.
46. Case records of the Massachusetts General Hospital (1979). Case No. 42-1979. *N. Engl. J. Med*. **301**, 881.

47. Dorfman, R. F., and Berry, G. J. (1988). Kikuchi's histiocytic necrotizing lymphadenitis: An analysis of 108 cases with emphasis on differential diagnosis. *Semin. Diagn. Pathol.* **5**, 329.
48. Eisner, M. D., Amory, J., Mullaney, B., *et al.* (1996). Necrotizing lymphadenitis associated with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **26**, 1.
49. Lin, S. H., Ko, W. S., Lee, H. S., and Hwang, W. S. (1992). *J. Rheumatol.* **2**(19), 1995.
50. Tsumatsu, Y., Koide, R., Sasaki, M., and Takahashi, H. (1984). Acute myeloid leukemia with preceding SLE and autoimmune hemolytic anemia. *Jpn. J. Clin. Oncol.* **14**, 107.
51. Saulsbury, F. T., Sabio, H., Conrad, D., *et al.* (1984). Acute leukemia with features of systemic lupus erythematosus. *J. Pediatr.* **105**, 57.
52. Efremidis, A., Eiser, A. R., Grishman, E., and Rosenberg, V. (1984). Hodgkin's lymphoma in an adolescent with SLE. *Cancer* **53**, 142.
53. Malleson, P. N., Petty, R. E., Nodel. H., *et al.* (1988). Functional asplenia in childhood onset SLE. *J. Rheumatol.* **15**, 1648.
54. Cook, C. D., Wedgwood, R. J. P., Craig, J. M., *et al.* (1960). SLE: Description of 37 cases in children and a discussion of endocrine therapy in 32 of the cases. *Pediatrics* **26**, 570.
55. Baehr, A. G., Kunkel, H. G., *et al.* (1956). The problem of chronic liver disease in young women. *Am. J. Med.* **21**, 3.
56. Runyon, B. A., Labrecque, D. R., and Arenas, S. (1980). The spectrum of liver disease in SLE. *Am. J. Med.* **69**, 187.
57. Nadorras, R. L., Nakazato, Y., and Landing, B. H. (1987). Pathologic features of gastrointestinal tract lesions in childhood-onset SLE: Study of 26 patients, with review of the literature. *Pediatr. Pathol.* **7**, 245.
58. Dubois, E. L. (1974). "Lupus Erythematosus," 2nd Ed. University of California Press, Los Angeles.
59. Ropes, M. W. (1976). "Systemic Lupus Erythematosus." Harvard University Press, Cambridge, MA.
60. Alverson, D. C., and Chase, H. P. (1977). SLE in childhood presenting as hyperlipoproteinemia. *J. Pediatr.* **91**, 72.
61. Goldberg, B. H., and Bergstein, J. M. (1978). Acute respiratory distress in a child after steroid induced pancreatitis. *Pediatrics* **61**, 317.
62. Lloyd, D. D., Balfe, J. W., and Barkin, M. (1974). SLE with signs of retroperitoneal fibrosis. *J. Pediatr.* **85**, 226.
63. Bitran, J., McShane, D., and Ellman, M. H. (1976). Ascites as major manifestation of SLE. *Arthritis Rheum.* **19**, 782.
64. Stevens, H. P., Ostlere, L. S., and Rustin, M. H. A. (1994). Systemic lupus erythematosus in association with ulcerative colitis: Related autoimmune diseases. *Br. J. Dermatol.* **130**, 385.
65. Molina, J. F., Brown, R. F., Gedalia, A., and Espinoza, L. R. (1996). Protein losing enteropathy as the initial manifestation of childhood systemic lupus erythematosus. *J. Rheumatol.* **23**, 1269.
66. Tsukahana, M., Matsuo, K., and Kojima, H. (1980). Protein-losing enteropathy in a boy with SLE. *J. Pediatr.* **97**, 778.
67. Chase, G. J., O'Shea, P. A., Collins, E., and Brein, A. S. (1982). Protein-losing enteropathy in SLE. *Hum. Pathol.* **13**, 1053.
68. Cassidy, J. T., Sullivan, D. B., Petty, R. E., and Ragsdale, C. (1977). Lupus nephritis and encephalopathy: Prognosis in 58 children. *Arthritis Rheum.* **20**, 315.
69. Woolf, A., Crocker, B., Osofsky, S. G., and Kredich, D. W. (1979). Nephritis in children and young adults with SLE and normal urinary sediment. *Pediatrics* **64**, 678.
70. Garin, E. H., Donnelly, W. H., Fennell, R. S., III, and Richard, G. A. (1976). Nephritis in SLE in children. *J. Pediatr.* **89**, 366.
71. Phadke, K., Trachtman, H., Nicastri, A., *et al.* (1984). Acute renal failure as the initial manifestation of systemic lupus erythematosus in children. *J. Pediatr.* **105**, 38.
72. Bagga, A., Jain, Y., Srivastava, R. N., and Bhuyan, U. N. (1993). Renal tubular acidosis preceding systemic lupus erythematosus. *Pediatr. Nephrol.* **7**, 735.
73. Dudley, J., Fentou, T., Unsworth, J., *et al.* (1996). Systemic lupus erythematosus presenting as congenital nephrotic syndrome. *Pediatr. Nephrol.* **10**, 752.
74. La Manna, A., Polito, C., Papale, M. R., *et al.* (1998). Chronic interstitial cystitis and systemic lupus erythematosus in an 8 year old girl. *Pediatr. Nephrol.* **12**, 139.
75. Tanaka, H., Waga, S., Tateyama, T., *et al.* (2000). Interstitial cystitis and ileus in pediatric-onset systemic lupus erythematosus. *Pediatr. Nephrol.* **14**, 859.
76. West, CD. (1976). Asymptomatic hematuria and proteinuria in children: Causes and appropriate diagnostic studies. *J. Pediatr.* **89**, 111.
77. Yoshimoto, M., Tsukahari, H., Syito, H., Hayashi, S., Haruki, S., Fujiswa, S., and Sudo, M. (1990). Evaluation of variability of proteinuria indices. *Pediatr. Nephrol.* **4**, 136.
78. Appel, G. B., Williams, G. S., Meltzer, J. I., *et al.* (1976). Renal vein thrombosis, nephrotic syndrome, and SLE: An association in four cases. *Ann. Intern. Med.* **85**, 310.
79. Ross, D. I., and Lubowitz, R. (1978). Anticoagulation in renal vein thrombosis. *Arch. Intern. Med.* **138**, 1349.
80. Mailloux, L. U., Susin, M., Stein, H. L., *et al.* (1978). Nephrotic syndrome, membranous nephropathy, and renal vein thrombosis: Long-term follow-up. *N.Y. State. J. Med.* **78**, 1873.
81. Ostrov, B. E., Min, W., Eichenfield, A. H., *et al.* (1989). Hypertension in children with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **19**, 90.
82. Laragh, J. H. (1980). Hypertension. *Drug Ther.* **10**, 71.
83. Baqi, N., Moazami, S., Singh, A., *et al.* (1996). Lupus nephritis in children: A longitudinal study of prognostic factors and therapy. *J. Am. Soc. Nephrol.* **7**, 924.
84. Fries, J. F., Porta, J., and Liang, H. H. (1978). Marginal benefit of renal biopsy in SLE. *Arch. Intern. Med.* **138**, 1386.
85. Lee, H. S., Mujais, S. K., Kasinath, B. S., *et al.* (1984). Course of renal pathology in patients with systemic lupus erythematosus. *Am. J. Med.* **77**, 612.
86. Austin, H. A., III, Munz, L. R., Joyce, K. M., *et al.* (1984). Diffuse proliferative lupus nephritis: Identification of

specific pathologic features affecting renal outcome. *Kidney Int*. **25**, 689.
87. Nossent, H. C., Henzen-Logmans, S. C., Vroom, T. M., *et al*. (1990). Contribution of renal biopsy data in predicting outcome in lupus nephritis: Analysis of 116 patients. *Arthritis Rheum*. **33**, 970.
88. Rush, P. J., Baumal, R., Shore, A., *et al*. (1986). Correlation of renal histology with outcome in children with lupus nephritis. *Kidney Int*. **29**, 1066.
89. Emre, S., Bilge, I., Sirin, A., *et al*. (2001). Lupus nephritis in children: Prognostic significance of clinicopathological findings. *Nephron* **87**, 118.
90. Norris, D. G., Colon, A. R., and Stickler, G. B. (1977). SLE in children: Complex problems of diagnosis and treatment encountered in 101 such patients at the Mayo Clinic. *Clin. Pediatr*. **16**, 774.
91. Yancey, C. L., Doughty, R. A., and Arthreya, B. H. (1981). CNS involvement in childhood SLE. *Arthritis Rheum*. **24**, 1389.
92. Silber, T. J., Chatoor, I., and White, P. H. (1984). Psychiatric manifestations of SLE in children and adolescents. *Clin. Pediatr*. **23**, 331.
93. Parikh, S., Swaiman, K. F., and Kim, Y. (1995). Neurologic characteristics of childhood lupus erythematosus. *Pediatr. Neurol*. **13**, 198.
94. Steinlein, M. I., Blaser, S. I., Gilday, D. L., *et al*. (1995). Neurologic manifestations of pediatric systemic lupus erythematosus. *Pediatr. Neurol*. **13**, 191.
95. Baca, V., Lavalle, C., Garcia, R., *et al*. (1999). Favorable response to intravenous methylprednisolone and cyclophosphamide in children with severe neuropsychiatric lupus. *J. Rheumatol*. **26**, 432.
96. Gallagher, R., Herald, F., and Garrell, A. (1976). "The Medical Care of the Adolescent Patient." Appleton-Century-Crofts, New York.
97. Blumenfield, M. (1978). Psychological aspects of SLE. *Prim. Care*. **5**, 159.
98. Press, J., Palayew, K., Laxer, R. M., *et al*. (1996). Antiribosomal P antibodies in pediatric patients with systemic lupus erythematosus and psychosis. *Arthritis Rheum*. **39**(4), 671.
99. Beckwitt Turkel, S., Miller, J. H., and Reiff, A. (2001). Case series: neuropsychiatric symptoms with pediatric systemic lupus erythematosus. *J. Am. Acad. Child. Adolesc. Psychiatr*. **40**, 482.
100. Rubbert, A., Marienhagen, J., Pirner, K., *et al*. (1993). Single-photon-emission computed tomography analysis of cerebral blood flow in the evaluation of central nervous system involvement in patients with systemic lupus erythematosus. *Arthritis Rheum*. **36**(9), 1253.
101. Russo, R., Gilday, D., Laxer, R. M., *et al*. (1998). Single photon emission computed tomography (SPECT) scanning in childhood systemic lupus erythematosus. *J. Rheumatol*. **25**, 576.
102. Falcini, F., De Cristofara, T. R., Ermini, M., *et al*. (1997). Regional cerebral blood flow in juvenile systemic lupus erythematosus: A prospective study. *J. Rheumatol*.
103. Szer, I. S., Miller, J. H., Rawlings, D., *et al*. (1993). Cerebral perfusion abnormalities in children with central nervous system manifestation of lupus detected by single-photon-emission computed tomography. *J. Rheumatol*. **20**, 2143.
104. Reiff, A., Miller, J., Shaham, B., *et al*. (1997). Childhood CNS lupus; longitudinal assessment using single-photon-emission computed tomography (SPECT). *J. Rheum*. **24**, 2461.
105. Brandt, K. D., and Lessel, S. (1978). Migrainous phenomena in SLE. *Arthritis Rheum*. **21**, 7.
106. Lehman, T. J., Bernstein, B., Hanson, V., *et al*. (1981). Meningococcal infection complicating systemic lupus erythematosus. *J. Pediatr*. **99**, 94.
107. Carlow, T. J., and Glaser, J. S. (1974). Pseudotumor cerebri in SLE. *JAMA* **228**, 197.
108. DelGuidice, G. C., Scher, C. A., Athreya, B. H., *et al*. (1986). Pseudotumor cerebri and childhood SLE. *J. Rheumatol*. **13**, 748.
109. Padeh, S., and Passwell, J. H. (1996). Systemic lupus erythematosus presenting as idiopathic intracranial hypertension. *J. Rheumatol*. **23**, 1266.
110. Beernick, D. H., and Miller, J. J., III. (1973). Anticonvulsant-induced antinuclear antibodies and lupuslike disease in children. *J. Pediatr*. **82**, 113.
111. Quintero, A. L., and Miller, V. (2000). Neurologic symptoms in children with systemic lupus erythematosus. *J. Child. Neurol*. **15**, 803.
112. Donaldson, I. M., and Espiner, E. A. (1971). Disseminated LE presenting as chorea gravidarum. *Arch. Neurol*. **25**, 240.
113. Lusins, J. O., and Szilagyi, P. A. (1975). Clinical features of chorea associated with SLE. *Am. J. Med*. **558**, 857.
114. Groothius, J. R., Groothius, D. R., Mukhopadhyay, D., *et al*. (1977). Lupus-associated chorea in childhood. *Am. J. Dis. Child*. **131**, 1131.
115. Cervera, R., Asherson, R. A., Font, J., *et al*. (1997). Chorea in the antiphospholipid syndrome: Clinical, radiographic and immunologic characteristic of 50 patients from our clinics and the recent literature. *Medicine* **76**(3), 203.
116. Dressner, I. G., Dysart, N. K., and Michael, A. F. (1978). CNS lupus erythematosus in children. *Pediatr. Res*. **12**, 551. [Abstract]
117. Commarata, R. J., Raodnan, G. P., and Grittenden, J. O. (1963). SLE with chorea. *JAMA* **184**, 149.
118. Bauer, F. K., Riley, W. C., and Cohen, E. B. (1950). Disseminated LE with Sydenham's chorea and rheumatic heart disease: Report of a case with autopsy. *Ann. Intern. Med*. **33**, 1042.
119. Lusins, J. O., and Szilagyi, P. A. (1973). Clinical features and histological data of chorea associated with SLE. *Trans. Ann. Neurol. Assoc*. **98**, 279.
120. Paradise, J. L. (1960). Sydenham's chorea without evidence of rheumatic fever. *N. Engl. J. Med*. **263**, 625.
121. Heilman, K. M., Kohler, W. C., and LeMaster, P. C. (1971). Haloperidol treatment of chorea associated with SLE. *Neurology* **21**, 963.
122. Fermaglich, J., Streib, E., and Auth, T. (1973). Chorea associated with SLE: Treatment with haloperidol. *Arch. Neural*. **28**, 276.

123. Olsen, J. E. (1968). Chorea minor associated with SLE. *Acta. Med. Scand.* **183**, 127.
124. Greenhouse, A. H. (1966). On chorea, SLE, and cerebral arteritis. *Arch. Intern. Med.* **117**, 389.
125. Rowe, P. B. (1963). Disseminated LE with Sydenham's chorea: Report of a case with a review of the literature. *Med. J. Aust.* **2**, 586.
126. Arisaka, O., Obinata, K., Sasaki, H., *et al.* (1984). Chorea as initial manifestation of SLE: A case report of a 10-year-old girl. *Clin. Pediatr.* **23**, 298.
127. Shergy, W. J., Kredich, D. W., and Pisetsky, D. S. (1988). The relationship of anticardiolipin antibodies to disease manifestations in pediatric SLE. *J. Rheum.* **15**, 1389.
128. Steinlein, M. I., Blaser, S. I., Gilday, D. L., *et al.* (1995). Neurologic manifestations of pediatric systemic lupus erythematosus. *Pediatr. Neurol.* **13**, 191.
129. Peck, B., Hoffman, G. S., and Franck, W. A. (1978). Thrombophlebitis in SLE. *JAMA* **240**, 1728.
130. Pope, J. M., Canny, C. L., and Bell, D. A. (1991). Cerebral ischemic events associated with endocarditis, retinal vascular disease, and lupus anticoagulant. *Am. J. Med.* **90**, 299.
131. Lavalle, C., Pizarro, S., Drenkard, C., *et al.* (1990). Transverse myelitis: A manifestation of systemic lupus erythematosus strongly associated with antiphospholipid antibodies. *J. Rheumatol.* **17**, 34.
132. Al-Mayouf, S. M., and Bahabri, S. (1999). Spinal cord involvement in pediatric systemic lupus erythematosus: Case report and literature review. *Clin. Exp. Rheumatol.* **17**, 505.
133. Uziel, Y., Laxer, R. M., Blaser, S., *et al.* (1995). Cerebral vein thrombosis in childhood systemic lupus erythematosus. *J. Pediatr.* **126**, 722.
134. Ravelli, A., Di Fuccia, G., Caporali, R., *et al.* (1993). Severe retinopathy in systemic lupus erythematosus associated with IgG anticardiolipin antibodies. *Acta. Paediatr.* **82**, 624.
135. Kwong, K. L., Chu, R., and Wong, S. N. (2000). Parkinsonism as unusual neurological complication in childhood systemic lupus erythematosus. *Lupus* **9**, 474.
136. Delgado, E. A., Malleson, P. N., Carter, J., *et al.* (1987). Pulmonary manifestations of childhood systemic lupus erythematosus. *Arthritis Rheum.* **30**, 527.
137. DeJongste, J. C., Neijens, H. J., Duiverman, E. J., *et al.* (1986). Respiratory tract disease in SLE. *Arch. Dis. Child.* **61**, 478.
138. Nadorra, R. L., Landing, B. H. (1987). Pulmonary lesions in childhood-onset SLE: Analysis of 26 cases, and summary of literature. *Pediatr. Pathol.* **7**, 1.
139. Jay, M. S., Jerath, R., VanDerzalm, T., *et al.* (1984). Pneumothorax in an adolescent with fulminant SLE. *J. Adolesc. Health Care* **5**, 142.
140. Case records of the Massachusetts General Hospital (1986). Case No. 48-1986. *N. Engl. J. Med.* **315**, 1469.
141. Foster, H., Malleson, P. N., Petty, R. E., *et al.* (1996). Pneumocystis carinii pneumonia in childhood systemic lupus erythematosus. *J. Rheumatol.* **23**, 753.
142. Miller, R. W., Magilavy, D. B., Bock, G. H., *et al.* (1988). Pulmonary function abnormalities in pediatric patients with systemic lupus erythematosus. *Immunol. Allergy Pract.* **10**, 17.
143. Trapani, S., Camiciottoli, G., Ermini, M., *et al.* (1998). Pulmonary involvement in juvenile systemic lupus erythematosus: A study on lung function in patients asymptomatic for respiratory disease. *Lupus* **7**, 545.
144. Rajani, K. B., Achbacher, L. V., and Kinney, T. R. (1978). Pulmonary hemorrhage and SLE. *J. Pediatr.* **93**, 810.
145. Loughlin, G. M., Taussig, L. M., Murphy, S. A., *et al.* (1978). Immune-complex mediated GN and pulmonary hemorrhage simulating Goodpasture's syndrome. *J. Pediatr.* **93**, 181.
146. Marino, C. T., and Petschuk, L. P. (1981). Pulmonary hemorrhage in SLE. *Arch. Intern. Med.* **141**, 201.
147. Matthay, R. A., Schwartz, M. I., Perry, T. L., *et al.* (1974). Pulmonary manifestations of SLE: Review of 12 cases of acute pneumonitis. *Medicine* **54**, 397.
148. Haupt, H. M., Moore, G. W., and Hutchins, G. M. (1981). The lung in SLE: Analysis of the pathologic changes in 120 patients. *Am. J. Med.* **71**, 791.
149. Takatoshi, I., Kanayama, Y., Ohe, A., *et al.* (1979). Immunopathologic studies of pneumonitis in SLE. *Ann. Intern. Med.* **91**, 30.
150. Lewis, E. J., Schur, P. H., and Busch, G. J. (1973). Immunopathologic features of a patient with GN and pulmonary hemorrhage. *Am. J. Med.* **554**, 507.
151. Churg, A., Franklin, W., Chan, K. L., *et al.* (1980). Pulmonary hemorrhage and immune complex deposition in the lung. *Arch. Pathol. Lab. Med.* **104**, 388.
152. Willoughby, W. F., and Dixon, F. J. (1920). Experimental hemorrhagic pneumonitis produced by heterologous antilung antibody. *J. Immunol.* **104**, 28.
153. Ramirez, R. E., Glasier, D., Kirks, D., *et al.* (1984). Pulmonary hemorrhage associated with SLE in children. *Radiology* **152**, 409.
154. Miller, R. W., Salcedo, J. R., Fink, R. J., *et al.* (1986). Pulmonary hemorrhage in pediatric patients with systemic lupus erythematosus. *J. Pediatr.* **108**, 576.
155. Carrette, S., Macher, A. M., and Nussbaum, A. (1984). Severe acute pulmonary disease in patients with SLE: Ten years experience at the NIH. *Semin. Arthritis Rheum.* **14**, 52.
156. Connolly, B., Manson, D., Eberhard, A., *et al.* (1996). CT appearance of pulmonary vasculitis in children. *Am. J. Roentgenogr.* **167**, 901.
157. Peterson, R. D. A., Jerneier, R. L., and Good, R. A. (1963). Lupus erythematosus. *Pediatr. Clin. North Am.* **10**, 941.
158. Englund, J. A., and Lucas, R. V., Jr. (1983). Cardiac complications in children with SLE. *Pediatrics* **72**, 724.
159. Strauer, B. E., Brune, I., Schenk, H., *et al.* (1976). Lupus cardiomyopathy: Cardiac mechanics, hemodynamics, and coronary blood flow in uncomplicated SLE. *Am. Heart J.* **92**, 715.
160. Brigden, W., Bywaters, G. L., Lessef, M. H., *et al.* (1960). The heart in SLE. *Br. Heart J.* **22**, 1.
161. Shearn, M. A. (1959). The heart in SLE: Review. *Am. Heart J.* **558**, 452.

162. Hejtmancik, M. R., Wright, J. C., Quint, R., *et al*. (1964). The cardiovascular manifestations of SLE. *Am. Heart J.* **68**, 119.
163. Galve, E., Candell-Riera, J., Pigrau, C., *et al*. (1988). Prevalence, morphologic types, and evolution of cardiac valvular disease in systemic lupus erythematosus. *N. Eng. J. Med.* **319**, 817.
164. Nihoyannopoulos, P., Gomez, P. M., Joshi, J., *et al*. (1990). Cardiac abnormalities in systemic lupus erythematosus. *Circulation* **82**, 369.
165. Leung, W. H., Wong, K. L., Lau, C. P., *et al*. (1990). Association between antiphospholipid antibodies and cardiac abnormalities in patients with systemic lupus erythematosus. *Am. J. Med.* **89**, 411.
166. Logar, D., Kveder, T., Rozman, B., and Dobovisek, J. (1990). Possible association between anti-Ro antibodies and myocarditis or cardiac conduction defects in adults with systemic lupus erythematosus. *Ann. Rhuem. Dis.* **49**, 627.
167. Oshiro, A. C., Derbes, S. J., Stopa, A. R., and Gedalia, A. (1997). Anti-Ro/SS-A and Anti-La/SS-B antibodies associated with cardiac involvement in childhood systemic lupus erythematosus. *Ann. Rheum. Dis.* **56**, 272.
168. Ishikawa, S., Segar, W. E., Gilbert, E., *et al*. (1978). Myocardial infarct in a child with SLE. *Am. J. Dis. Child.* **132**, 696.
169. Heincy, C. J., Liberthson, R. R., Fallen, I. T., *et al.* (1982). Ischemic heart disease in SLE in the young patient: Report of 6 cases. *Am. J. Cardiol.* **49**, 478.
170. Tsakraklides, V. G., Blieden, L. C., and Edwards, J. E. (1974). Coronary atherosclerosis and myocardial infarction associated with SLE. *Am. Heart J.* **87**, 637.
171. Meller, J., Conde, C. A., Deppische, L. M., *et al*. (1975). Myocardial infarction due to coronary atherosclerosis in three young adults with SLE. *Am. J. Cardiol.* **35**, 309.
172. Bonfiglio, T. A., Botti, R. E., and Hagstrom, J. W. C. (1972). Coronary arteritis, occlusion and myocardial infarction due to LE. *Am. Heart J.* **83**, 153.
173. Spiera, H., and Rothenberg, R. R. (1983). Myocardial infarction in four young people with SLE. *J. Rheumatol.* **10**, 464.
174. Haider, Y. S., and Roberts, W. C. (1981). Coronary artery disease in SLE: Quantification of degrees of narrowing in 22 necropsy patients (21 women) aged 16 to 37 years. *Am. J. Med.* **70**, 775.
175. Englund, J. A., and Lucase, R. V., Jr. (1983). Cardiac complications in children with SLE. *Pediatrics* **72**, 724.
176. Friedman, D. M., Lazaras, H. M., and Fierman, A. H. (1990). Acute myocardial infarction in pediatric systemic lupus erythematosus. *J. Pediatr.* **117**, 263.
177. Clinicopathologic Conference (1987). Sudden death in a 22-year-old woman with lupus erythematosus. *Am. J. Med.* **82**, 65.
178. Nashel, D. J. (1986). Is atherosclerosis a complication of long-term corticosteroid treatment? *Am. J. Med.* **80**, 925.
179. Falaschi, F., Ravelli, A., Martignoni, A., *et al*. (2000). Nephrotic-range proteinuria, the major risk factor for early atherosclerosis in juvenil-onset systemic lupus erythematosus. *Arthritis Rheum.* **43**(6), 1405.
180. Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1984). Immune complex deposition and coronary vasculitis in SLE. *Am. J. Med.* **77**, 141.
181. Miller, D. J., Maisch, S. A., Perez, M. D., *et al*. (1995). Fatal myocardial infarction in an 8-year-old girl with systemic lupus erythematosus, Raynaud's phenomenon and secondary antiphospholipid antibody syndrome. *J. Rheumatol.* **22**, 768.
182. Hosenpud, J. D., Montanara, A., Hart, M. V., *et al*. (1984). Myocardial perfusion abnormalities in asymptomatic patients with SLE. *Am. J. Med.* **77**, 286.
183. Gazarian, M., Feldman, B. M., Benson, L. N., *et al*. (1998). Assessment of myocardial perfusion and function in childhood systemic lupus erythematosus. *J. Pediatr.* **132**, 109.
184. Guervara, J. P., Clark, B. J., and Athreya, B. H. (2001). Point prevalence of cardiac abnormalities in children with systemic lupus erythematosus. *J. Rheumatol.* **28**, 854.
185. Ilowite, N. T., Lamuel, P., Ginzler, E., *et al*. (1988). Dyslipoproteinemia in pediatric SLE. *Arthritis Rheum.* **31**, 859.
186. Eberhard, A. B., Laxer, R. M., Eddy, A. A., and Silverman, E. D. (1991). Presence of thyroid abnormalities in children with systemic lupus erythematosus. *J. Pediatr.* **19**(2), 277.
187. Miller, F. W., Moore, G. F., Weintraub, B. D., and Steinberg, A. D. (1987). Prevalence of thyroid disease and abnormal thyroid function test results in patients with systemic lupus erythematosus. *Arthritis Rheum.* **30**(10), 1124.
188. Ronchezel, M. V., Len, C. A., Spinola e Castro, A., *et al*. (2001). Thyroid function and serum prolactin levels in patients with juvenile systemic lupus erythematosus. *J. Pediatr. Endocrinol Metab.* **14**, 165.
189. Lehman, T. J. A. (1995). A practical guide to systemic lupus erythematosus. *Pediatr. Clin. North Am.* **42**(5), 1223.
190. Athreya, B. H., Rafferty, J. H., Sehgal, G. S., and Lahita, R. G. (1993). Adenohyophyseal and sex hormones in pediatric rheumatic disease. *J. Rheumatol.* **20**, 725.
191. Pando, J. A., Gourley, M. F., Wilder, R. L., and Crofford, L. J. (1995). Hormonal supplementation as treatment of cyclical rashes in patients with systemic lupus erythematosus. *J. Rheumatol.* **22**, 2159.
192. El-Garf, A., Salah, S., Shaarawy, M., *et al*. (1996). Prolactin hormone in juvenile systemic lupus erythematosus: A possible relationship to disease activity and CNS manifestations. *J. Rheumatol.* **23**, 374.
193. Hurley, R. M., Steinberg, R. H., Patriquin, H., *et al*. (1974). Avascular necrosis of the femoral head in childhood SLE. *Can. Med. Assoc. J.* **111**, 781.
194. Smith, F. E., Sweet, D. E., Brunner, C. M., *et al*. (1976). Avascular necrosis in SLE: An apparent predilection for young patients. *Ann. Rheum. Dis.* **35**, 227.
195. Bergstein, H. M., Wiens, C., and Fish, A. F. (1974). Avascular necrosis of bone in SLE. *J. Pediatr.* **85**, 31.
196. Abeles, M., Urman, J. D., and Rothfield, N. F. (1978). Aseptic necrosis of bone in SLE: Relationship to corticosteroid therapy. *Arch. Intern. Med.* **138**, 750.

197. Szer, I. S. (1990). Systemic lupus erythematosus and mixed connective tissue disease. *In* "Current Pediatric Therapy" (S. S. Gellis and B. M. Kagan, eds.), 13th Ed., p. 338. Saunders, Philadelphia.
198. Schaller, J. G., and Szer, I. S. (1989). SLE in childhood. *In* "Textbook of Rheumatology" (W. N. Kelley, E. D. Harris, Jr., S. Ruddy, *et al*., eds.), 3rd Ed. Saunders, Philadelphia.
199. Cassidy, J. T., Walker, S. E., Soderstrom, S. J., *et al*. (1978). Diagnostic significance of antibody to native DNA in children with JRA and other connective tissue disease. *J. Pediatr.* **93**, 416.
200. Weinstein, A., Bordwell, B., Stone, B., *et al*. (1983). Antibodies to native DNA and serum complement levels: Application to diagnosis and classification of SLE. *Am. J. Med.* **74**, 201.
201. Miller, M. L., Magilavy, D. B., and Warren, R. W. (1986). The immunologic basis of lupus. *Pediatr. Clin. North Am.* **33**, 1191.
202. Wong, S. N., Shah, V., and Dillon, M. J. (1995). Antineutrophil cytoplasmic antibodies in childhood systemic lupus erythematosus. *Eur. J. Pediatr.* **154**, 43.
203. Gidden, R. S., Mantzouranis, E. C., and Borel, Y. (1983). SLE in childhood: Clinical manifestations and improved survival in fifty-five patients. *Clin. Immunol. Immunopathol.* **29**, 196.
204. Platt, J. L., Burke, B. A., and Fish, A. J. (1982). SLE in the first two decades of life. *Am. J. Kidney Dis.* **2**(Suppl. 1), 212.
205. Schur, P. H., and Sandson, J. (1968). Immunological factors and clinical activity in SLE. *N. Engl. J. Med.* **178**, 533.
206. Rush, P. J., Baumal, R., Shore, A., *et al*. (1986). Correlation of renal histology with outcome of children with lupus nephritis. *Kidney Int.* **29**, 1066.
207. Bakkaloglu, A., Pascual, M., Schifferli, *et al*. (1995). Partial C4 deficiency in two children with systemic lupus erythematosus. *Turkish J. Ped.* **37**, 147.
208. Singsen, B. H., Bernstein, B. H., King, K. K., *et al*. (1976). SLE in childhood: Correlations between changes in disease activity and serum complement level. *J. Pediatr.* **89**, 358.
209. Pincus, T., Hughes, G. R. V., Pincus, D., *et al*. (1971). Antibodies to DNA in childhood SLE. *J. Pediatr.* **78**, 981.
210. Weinstein, A., Bordwell, B., Stone, B., *et al*. (1983). Antibodies to native DNA and serum complement (C3) levels. *Am. J. Med.* **74**, 206.
211. Pillemer, S. R., Austin, H. A., Tsokos, G. C., *et al*. (1988). Lupus nephritis: Association between serology and renal biopsy measures. *J. Rheumatol.* **15**, 284.
212. Kohler, P. F., and Ten Besel, R. (1969). Serial complement component alterations in acute GN and SLE. *Clin. Exp. Immunol.* **4**, 191.
213. Appel, A. E., Sablay, L. B., Golden, R. A., *et al*. (1978). The effect of normalization of serum complement and anti-DNA antibody on the course of lupus nephritis. *Am. J. Med.* **64**, 274.
214. Ginzler, E. M., Bollet, A. J., and Friedman, E. A. (1980). The natural history and response to therapy in lupus nephritis. *Annu. Rev. Med.* **31**, 463.
215. Ter Borg, E. J., Horst, E., Hummel, E. J., *et al*. (1990). Measurement of increases in anti-double stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. *Arthritis Rheum.* **33**, 634.
216. Ward, M. M., and Studenski, S. (1990). Age associated clinical manifestations of systemic lupus erythematosus: A multivariate regression analysis. *J. Rheumatol.* **17**, 476.
217. Tan, E. M. (1989). Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* **44**, 93.
218. Reichlin, M., Faulkner Broyles, T., Hubscher, O., *et al*. (1999). Prevalence of autoantibodies to ribosomal P proteins in juvenile-onset systemic lupus erythematosus compared with the adult disease. *Arthritis Rheuma.* **42**(1), 69.
219. Yang, L. C., Norman, M. E., and Doughty, R. A. (1980). A micro method for the analysis of cryoglobulins via laser nephelometry: Evaluation and comparison to Clq binding activity in autoimmune diseases in pediatrics. *Pediatr. Res.* **14**, 858.
220. Wallace, D. J., Podell, T., Weiner, J., *et al*. (1981). SLE—Survival patterns: Experience with 609 patients. *JAMA* **245**, 934.
221. Brenner, B. M., Meyer, T. W., and Hostetter, T. H. (1982). Dietary protein intake and the progressive nature of kidney disease: The role of hemodynamically-mediated glomerular injury in the pathogenesis of progressive glomerulosclerosis in aging, renal ablation and intrinsic renal disease. *N. Engl. J. Med.* **307**, 652.
222. Donadio, J. V., Holley, K. E., Ferguson, R. H., *et al*. (1978). Treatment of diffuse proliferative lupus nephritis with prednisone and combined prednisone and cyclophosphamide. *N. Engl. J. Med.* **299**, 1151.
223. Hale, G. M., Highton, J., Kalmakoff, J., *et al*. (1986). Changes in anti-DNA antibody affinity during exacerbations of SLE. *Scand. J. Rheumatol.* **15**, 243.
224. Waldavens, P. A., and Chase, P. (1976). The prognosis of childhood SLE. *Am. J. Dis. Child.* **130**, 929.
225. Reveille, J. D., Bartolucci, A., and Alarcon, G. S. (1990). Prognosis in systemic lupus erythematosus: Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. *Arthritis Rheum.* **33**, 37.
226. Studenski, S., Allen, N. B., Caldwell, D. S., *et al*. (1987). Survival in systemic lupus erythematosus: A multivariate analysis of demographic factors. *Arthritis Rheum.* **30**, 1327.
227. Jacobs, J. C. (1977). Childhood onset SLE: Modern management and improved prognosis. *N.Y. State J. Med.* **77**, 2231.
228. Jacobs, J. C. (1977). Treatment of SLE in childhood. *Arthritis Rheum.* **20**(Suppl.), 304.
229. Felson, D. T., and Anderson, J. (1984). Evidence for the superiority of immunosuppressive drugs and prednisone over prednisone alone in lupus nephritis. *N. Engl. J. Med.* **311**, 1528.
230. Szer, I. S. (1998). Clinical developments in the management of lupus in the neonate, child, and adolescent. *Curr. Opin. Rheum.* **10**, 431.

231. Laitman, R. S., Glicklich, D., and Sablay, L. B. (1989). Effect of long-term normalization of serum complement levels on the course of lupus nephritis. *Am. J. Med.* **87**, 132.
232. Prystowsky, S. D., Herndon, J. H., and Gilliam, J. N. (1975). Chronic cutaneous lupus erythematosus (DLE). *Medicine* **55**, 183.
233. Epstein, J. H., and Tuffanelli, D. L. (1974). Discoid lupus erythematosus. *In* "Lupus Erythematosus" (E. L. Dubois, ed.), 2nd Ed. University of California Press, Los Angeles.
234. Rothfield, N., March, C. H., Miescher, P., *et al*. (1963). Chronic discoid LE: A study of 65 patients and 65 controls. *N. Engl. J. Med.* **269**, 1155.
235. Miescher, P., and Delacrataz, J. (1953). Demonstration d'un phenomene "L.E." positif dans deux cas d'hypersensibilite medicamenteuse. *Schweiz Med. Woch* **83**, 536.
236. Lindquist, T. (1957). Lupus erythematosus disseminates after administration of mesantoin. *Acta Med. Scan.* **158**, 131.
237. Shulman, L. E., and Harvey, A. McG (1960). The nature of drug-induced SLE. *Arch. Rheum.* **3**, 464.
238. Rallison, M. L., *et al*. (1961). Lupus erythematosus and Stevens-Johnson syndrome. *Am. J. Dis. Child.* **101**, 725.
239. Benton, J. W., *et al*. (1962). SLE occurring during anticonvulsive therapy. *JAMA* **180**, 115.
240. Ansell, B. M. (1993). Drug-induced systemic lupus erythematosus in a nine-year-old boy. *Lupus* **2**, 193.
241. Caramaschi, P., Biasi, D., Carletto, A., Manzo, T., and Bambara, L. M. (1995). Clobazam-induced systemic lupus erythematosus. *Clin. Rheumatol.* **14**(1), 116.
242. Singsen, B. H., Fishman, L., and Hanson, V. (1976). ANA's and lupuslike syndromes in children receiving anticonvulsants. *Pediatrics* **57**, 529.
243. Beernick, D. H., and Miller, J. J. III. (1973). Anticonvulsant-induced antinuclear antibodies and lupuslike disease in children. *J. Pediatr.* **82**, 113.
244. Miller, J. J., III. (1977). Drug-induced lupus-like syndromes in children. *Arthritis Rheum.* **20**, 308.
245. Greenberg, J. H., and Lutcher, C. L. (1972). Drug induced SLE: A case with life-threatening pericardial tamponade. *JAMA* **222**, 191.
246. Carey, R. M., Coleman, M., and Feder, A. (1983). Pericardial tamponade: A major presenting manifestation of hydralazine-induced lupus syndrome. *Am. J. Med.* **85**, 554.
247. Lee, S. L., and Chase, P. H. (1975). Drug-induced SLE: A critical review. *Semin. Arthritis Rheum.* **5**, 83.
248. Irias, J. J. (1995). Hydralazine-induced lupus erythematosus-like syndrome. *Am. J. Dis. Child.* **129**, 862.
249. Chapel, T. A., and Burns, P. E. (1971). Oral contraceptives and exacerbations of lupus erythematosus. *Am. J. Obstet. Gynecol.* **110**, 366.
250. Garovich, M., Agudelo, C., and Pisko, E. (1980). Oral contraceptives and SLE. *Arthritis Rheum.* **23**, 1396.
251. Finkel, T. H., Hunter, D. J., Paisley, J. E., *et al*. (1999). Drug-induced lupus in a child after treatment with zafirlukast (Accolate). *J. Allergy Clin. Immunol.* **103**, 533.
252. Akin, E., Miller, L. C., and Tucker, L. B. (1999). Minocycline-induced lupus in adolescents. *Pediatrics* **101**, 926.
253. Sturkenboom, M. C. J., Meier, C. R., Jick, H., *et al*. (1999). Minocycline and lupuslike syndrome in acne patients. *Arch. Intern. Med.* **159**, 493.
254. Lawson, T. M., Amos, N., Bulgen, D., *et al*. (2001). Minocycline-induced lupus: Clinical features and response to rechallenge. *Rheumatology* **40**, 329.
255. Dunphy, J., Oliver, M., Rands, A. L., *et al*. (2000). Antineutrophil cytoplasmic antibodies and HLA class II alleles in minocycline-induced lupus-like syndrome. *Br. J. Dermatol.* **142**, 461.
256. Tournigand, C., Genereau, T., Prudent, M., *et al*. (1999). Minocycline-induced clinical and biological lupus-like disease. *Lupus* **8**, 773.
257. Robert, J. L., and Hayashi, J. A. (1983). Exacerbation of SLE associated with alfalfa ingestion. *N. Engl. J. Med.* **308**, 1361.
258. Tolaymat, A., Leventhal, B., Sakarcan, A., *et al*. (1992). Systemic lupus in a child receiving long-term interferon therapy. *J. Pediatr.* **120**, 429.
259. Woodsley, R. L., Dryer, E. D., Reidenberg, M. M., *et al*. (1978). Effect of acetylator phenotype on the rate at which procainamides induce ANA and the lupus syndrome. *N. Engl. J. Med.* **298**, 1157.
260. Baer, A. N., Woosley, R. L., and Pincus, T. (1986). Further evidence for the lack of association between acetylator phenotype and systemic lupus erythematosus. *Arthritis Rheum.* **29**, 508.
261. Batchelor, J. R., Welsh, K. I., Tinoco, R. M., *et al*. (1980). Hydralazine-induced SLE: Influence of HLA-DR and sex on susceptibility. *Lancet* **1**, 1107.
262. Fam, A. G., Izsak, M., and Saiphoo, C. (1980). SLE and Klinefelter syndrome. *Arthritis Rheum.* **23**, 124.
263. Schattner, A., and Berrebi, A. (1989). Klinefelter's syndrome associated with autoimmune disease. *J. R. Soc. Med.* **82**, 560.
264. Sharp, G. C., Irvin, W. S., Tan, E. M., *et al*. (1972). MCTD: An apparently distinct rheumatic disease syndrome associated with a specific antibody to extractable nuclear antigen (ENA). *Am. J. Med.* **52**, 148.
265. Sharp, G. C., Irvin, W. S., May, C. M., *et al*. (1976). Association of antibodies to ribunocleoprotein and Sm antigens with MCTD, SLE, and other rheumatic diseases. *N. Engl. J. Med.* **295**, 1149.
266. Muneus, E. F., and Schur, P. H. (1983). Antibodies to Sm and RNP: Prognosticators of disease involvement. *Arthritis Rheum.* **26**, 848.
267. Singsen, B. H., Bernstein, B. H., Kernreich, H. K., *et al*. (1977). MCTD in childhood. *J. Pediatr.* **90**, 893.
268. Oetgen, W. J., Boice, J. A., and Lawless, O. J. (1981). MCTD in children and adolescents. *Pediatrics* **67**, 333.
269. Baldassare, A., Weiss, T., and Auclair, R. (1976). MCTD in children. *Arthritis Rheum.* **19**, 788. [Abstract]
270. Singsen, B. H., Swanson, J. L., Bernstein, B. H., *et al*. (1980). A histologic evaluation of MCTD in childhood. *Am. J. Med.* **68**, 710.
271. Fraga, A., Gudino, J., Ramos-Niembro, F., *et al*. (1978). MCTD in childhood: Relationship with Sjögren's syndrome. *Am. J. Dis. Child.* **132**, 263.

272. Nimelstein, S. H., Brody, S., McShane, D., *et al*. (1980). MCTD: A subsequent evaluation of the original 25 patients. *Medicine* **59**, 293.
273. Mier, R., Ansell, B., Hall, M. A., *et al*. (1996). Long term follow-up of children with mixed connective tissue disease. *Lupus* **5**, 221.
274. Kotajima, L., Aotsuka, S., Sumiya, M., *et al*. (1996). Clinical features of patients with juvenile onset mixed connective tissue disease: Analysis of data collected in a nationwide collaborative study in Japan. *J. Rheum.* **23**, 1088.
275. Itescu, S. (1992). Diffuse infiltrative lymphocytosis syndrome in children and adults infected with HIV-1: A model of rheumatic illness caused by acquired viral infection. *Am. J. Reprod. Immunol.* **28**, 247.
276. Szer, I. S. (1986). The diagnosis and management of systemic lupus erythematosus in childhood. *Pediatr. Ann.* **15**, 596.
277. Szer, I. S., and Athreya, B. H. (2002). Treatment of systemic lupus erythematosus . *In* "Gellis and Kagan's Current Pediatric Therapy", 17th Ed. Saunders, Philadelphia.
278. Fox, R. I., Robinson, C. A., Curd, J. C., Kozin, F., and Howell, F. V. (1986). Sjögren's syndrome: Proposed criteria for classification. *Arthritis Rheum.* **29**, 577.
279. Kraus, A., and Alarcón-Segovia, D. (1988). Primary juvenile Sjögren's syndrome. *J. Rheumatol.* **15**, 803.
280. Kobayashi, I., Furuta, H., Tame, A., *et al*. (1996). Complications of childhood Sjögren's syndrome. *Eur. J. Pediatr.* **155**, 890.
281. Anaya, J. M., Ogawa, N., and Talal, N. (1995). Sjögren's syndrome in childhood. *J. Rheumatol.* **22**, 1152.
282. Maeno, N., Takei, S., Imamaka, H., *et al*. (2001). Anti-alpha-fodrin antibodies in Sjogren's syndrome. *J. Rheumatol.* **28**, 860.
283. Kobayashi, I., Kawamura, N., Okano, M., *et al*. (2001). Anti-alpha-fodrin autoantibody is an early diagnostic marker for childhood primary Sjogren's syndrome. *J. Rheumatol.* **28**, 363.
284. Moutsopoulos, H. M., and Tzioufas, A. G. (1994). Sjögren's syndrome. *In* "Rheumatology" (J. H. Klippel and P. A. Dieppe, eds.), pp 6.27.1–27. Mosby-Yearbook Europe Limited, London.
285. Cassidy, J. T., and Petty, R. E. (eds.) (1995). Systemic lupus erythematosus. *In* "Textbook of Pediatric Rheumatology," 3rd Ed., pp. 306–307. Saunders, Philadelphia.
286. Jacobs, J. C. (ed.) (1993). Systemic lupus erythematosus. *In* "Pediatric Rheumatology for the Practitioner," 2nd Ed., pp. 467–469. Springer-Verlag, New York.
287. Deprettere, A. J., Van Acker, K. J., De Clerck, L. S., *et al*. (1988). Diagnosis of Sjögren's syndrome in children. *Am. J. Dis. Child* **142**, 1185.
288. De Clerck, L. S., Couttenye, M. M., Broe, M. E., and Stevens, W. J. (1988). Aquired immunodeficiency syndrome mimicking Sjögren's syndrome and systemic lupus erythematosus. *Arthritis Rheum.* **31**(2), 272.
289. Itescu, S. (1991). Diffuse infiltrative lymphocytosis syndrome in human immunodeficiency virus infection: A Sjögren's-like disease. *Rheum. Dis. Clin. North Am.* **17**(1), 99.
290. Pahwa, S., Kaplan, M., Fikrig, S., *et al*. (1986). Spectrum of human T-cell lymphotrophic virus type III infection in children: Recognition of symptomatic, asymptomatic and seronegative patients. *JAMA* **255**(17), 2299.
291. Yeh, C. K., Fox, P. C., Goto, Y., *et al*. (1992). Human immunodeficiency virus (HIV) and HIV infected cells in saliva and salivary glands of a patient with systemic lupus erythematosus. *J. Rheumatol.* **19**, 1810.
292. Fox, R. I., and Maruyama, T. (1997). Pathogenesis and treatment of Sjögren's syndrome. *Curr. Opin. Rheum.* **9**(5), 393.
293. Mackay, R. J., Menahem, S., and Ekert, H. (1982). Deep vein thrombosis in association with a circulating endogenous anticoagulant. *J. Pediatr.* **101**, 75.
294. Bernstein, M. L., Salusinsky-Sternbach, M., Bellefleur, M., *et al*. (1984). Thrombotic and hemorrhagic complications in children with the lupus anticoagulant. *AJDC* **138**, 1132.
295. Kaplan, R. E., Springate, J. E., Feld, L. G., *et al*. (1985). Pseudotumor cerebri associated with cerebral venous sinus thrombosis, internal jugular vein thrombosis, and systemic lupus erythematosus. *J. Pediatr.* **107**, 266.
296. Appan, S., Boey, M. L., and Lim, K. W. (1987). Multiple thrombosis in SLE. *Arch. Dis. Child* **62**, 739.
297. Scheleider, M. A., Nachman, R. L., Jaffe, E. A., *et al*. (1976). A clinical study of the lupus anticoagulant. *Blood* **48**, 499.
298. Colaco, C. B., and Elken, K. B. (1985). The lupus anticoagulant. *Arthritis Rheum.* **28**, 67.
299. Watson, K. V., and Schorer, A. E. (1991). Lupus anticoagulant inhibition of in vitro prostacyclin release is associated with a thrombosis-prone subset of patients. *Am. J. Med.* **90**, 47.
300. Trimble, M., Bell, D. A., and Brien, W. (1990). The antiphospholipid syndrome: Prevalence among patients with stroke and transient ischemic attacks. *Am. J. Med.* **88**, 593.
301. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. *Ann. Intern. Med.* **119**, 683.
302. Alving, B. M., Barr, C. F., and Tang, D. B. (1990). Correlation between lupus anticoagulants and anticardiolipin antibodies in patients with prolonged activated partial thromboplastin times. *Am. J. Med.* **88**, 112.
303. Firkin, B. G., Howard, M. A., and Rodford, N. (1980). Possible relationship between lupus inhibitor and recurrent abortion in young women. *Lancet* **2**, 366.
304. Mueh, J. R., Herbst, K. D., and Rapaport, S. I. (1980). Thrombosis in patients with the lupus anticoagulant. *Ann. Intern. Med.* **92**, 156.
305. Boey, M. L., Colaco, C. B., Gharavi, A. E., *et al*. (1983). Thrombosis in SLE: Striking association with the presence of circulating lupus anticoagulant. *Br. Med. J.* **287**, 1021.
306. Gattorno, M., Buoncompagni, A., Molinari, A. C., *et al*. (1995). Antiphospholipid antibodies in paediatric systemic lupus erythematosus, juvenile chronic arthritis and overlap syndromes: SLE patients with both lupus anticoagulant and high titer anticardiolipin antibodies are at

risk for clinical manifestations related to the antiphospholipid syndrome. *Br. J. Rheum.* **34**, 873.
307. von Scheuen, E., Athreya, B. H., Rose, C. D., *et al*. (1996). Clinical Characteristics of aPL syndrome in children. *J. Pediatr.* **129**, 339.
308. Levy, M., Molta, C., Meyer, O., *et al*. (1992). Antiphospholipid antibodies in patients with childhood onset systemic lupus erythematosus and their relatives. *Contrib. Nephrol.* **99**, 26.
309. Molta, C., Meyer, O., Dosquet, C., *et al*. (1993). Childhood-onset systemic lupus erythematosus: Antiphospholipid antibodies in 37 patients and their first degree relatives. *Pediatrics* **92**, 849.
310. Montes de Oca, M. A., Babron, M. C., Bletry, O., *et al*. (1991). Thrombosis in systemic lupus erythematosus: A French collaborative study. *Arch. Dis. Child.* **66**, 713.
311. Ravelli, A., Caporal, R., Di Fuccia, G., *et al*. (1994). Anticardiolipin antibodies in pediatric systemic lupus erythematosus. *Arch. Pediatr. Adolesc. Med.* **148**, 398.
312. Seaman, D. E., Londino, V., Kwoh, C. K., *et al*. (1995). Antiphospholipid antibodies in pediatric systemic lupus erythematosus. *Pediatric* **96**, 1040.
313. Baca, V., Garcia-Ramirez, R., Ramirez-Lacayo, M., *et al*. (1996). Cerebral infarction and antiphospholipid syndrome in children. *J. Rheumatol.* **23**, 1428.
314. Angelini, L., Zibordi, F., Zorzi, G., *et al*. (1999). Neurologic disorders other than stroke associated with antiphospholipid antibodies in childhood. *Neuropediatrics* **27**, 149.
315. Manco-Johnson, M. J. (1998). Antiphosphospholipid antibodies in children. *Semin. Thromb. Hemost.* **24**(6), 591.
316. Angelini, L., Granata, T., Zibordi, F., *et al*. (1998). Partial seizures associated with antiphospholipid antibodies in childhood. *Neuropediatrics* **29**, 249.
317. Massengill, S. F., Hedrick, C., Ayoub, E. M., *et al*. (1997). Antiphospholipid antibodies in pediatric lupus nephritis. *Am. J. Kidney Dis.* **29**(3), 355.
318. Falcini, F., Taccetti, G., Ermini, M., *et al*. (1997). Catastrophic antiphospholipid antibody syndrome in pediatric systemic lupus erythematosus. *J. Rheumatol.* **24**, 389.
319. Eberhard, A., Sparling, C., Sudbury, S., *et al*. (1994). Hypoprothrombinemia in childhood systemic lupus erythematosus. *Semin. Arthritis Rheum.* **24**(1), 12.
320. Hudson, N., Duffy, C. M., Rauch, J., *et al*. (1997). Catastrophic haemorrhage in a case of paediatric primary antiphospholipid ayndrome and factor II deficiency. *Lupus* **6**, 68.
321. Ravelli, A., Martini, A., and Burgio, G. R. (1994). Antiphospholipid antibodies in pediatrics. *Eur. J. Pediatr.* **153**, 472.
322. Ravelli, A., and Martini, A. (1997). Antiphospholipid antibody syndrome in pediatric patients. *Rheum. Dis. Clin. North Am.* **23**(3), 657.
323. Silverman, E. (1996). What's new in the treatment of pediatric SLE. *J. Rheumatol.* **23**, 1657.
324. Harel, L., Zecharia, A., Staussberg, R., *et al*. (2000). Successful treatment of rheumatic chorea with carbamazepine. *Pediatr. Neurol.* **23**(2), 147.
325. Szer, I. S. (1990). Rheumatic disease of childhood. *In* "Current Pediatric Therapy" (S. S. Gellis and B. M. Kagan, eds.), 13th Ed., p. 341. Saunders, Philadelphia.
326. Lehman, T. J. A., McCurdy, D. K., Bernstein, B. H., *et al*. (1989). Systemic lupus erythematosus in the first decade of life. *Pediatrics* **83**, 235.
327. Hanse, J. R., McCray, P. B., Bale, J. F., *et al*. (1985). Reye syndrome association with aspirin therapy for systemic lupus erythematosus. *Pediatrics* **76**, 202.
328. Barron, K. S., Silverman, E. D., Gonzales, J., and Revaille, J. (1993). Clinical, serologic, and immunogenetic studies in childhood-onset systemic lupus erythematosus. *Arthritis Rheum.* **36**(3), 348.
329. The Canadian Hydroxychloroquine Study Group (1991). A randomized study of the effect of withdrawing hydroxychloroquine sulfate in systemic lupus erythematosus. *N. Engl. J. Med.* **324**, 150.
330. Lehman, T. J., Hanson, V., Singsen, G. H., *et al*. (1980). The role of antibodies directed against double-stranded DNA in the manifestation of SLE in childhood. *J. Pediatr.* **96**, 657.
331. Barada, F. A., Jr., Andrews, B. S., Davis, J. S., IV, *et al*. (1981). Antibodies to Sm in patients with SLE; correlation of Sm antibody titers with disease activity and other laboratory parameters. *Arthritis Rheum.* **24**, 1236.
332. Levy, G. D., Munz, S. J., Paschal, J., *et al*. (1997). Incidence of hydroxychloroquine retinopathy in 1,207 patients in a large multicenter outpatient practice. *Arthritis Rheum.* **40**, 1482.
333. Editorial (1991). Therapy for systemic lupus erythematosus. *N. Engl. J. Med.* **324**, 189.
334. Lehman, T. J., Sherry, D. D., and Wagner-Weiner, L. (1989). Intermittent intravenous cyclophosphamide therapy for lupus nephritis. *J. Pediatr.* **114**, 1055.
335. McCune, W. J., Golbus, J., and Zeldes, W. (1988). Clinical and immunologic effects of monthly administration of intravenous cyclophosphamide in severe systemic lupus erythematosus. *N. Engl. J. Med.* **318**, 1423.
336. Abud-Mendoza, C., Sturbaum, A. K., Vazquez-Compean, R., *et al*. (1993). Methotrexate therapy in childhood systemic lupus erythematosus. *J. Rheumatol.* **30**, 731.
337. Favre, H., Miescher, P. A., Huang, Y. P., Chatelanat, F., and Mihatsch, M. J. (1989). Cyclosporine in the treatment of lupus nephritis. *Am. J. Nephrol.* **9**(Suppl. 1), 57.
338. Caccavo, D., Lagana, B., Mitterhoffer, A. P., *et al*. (1997). Long-term treatment of systemic lupus erythematosus with cyclosporine A. *Arthritis Rheum.* **40**, 27.
339. Barron, K. S., and Wallace, C. (2001). Autologous stem cell transplantation for pediatic rheumatic diseases. *J. Rheumatol.* **28**, 2337.
340. Wulffrat, N. M., Sanders, E. A. M., Kamphuis, S. S. M., *et al*. (2001). Prolonged remission without treatment after autologous stem cell transplantation for refractory childhood systemic lupus erythematosus. *Arthritis Rheum.* **44**(3), 728.
340a. Langman, C. G., Mazur, A. T., Baron, R., and Norman, M. E. (1982). 25-Hydroxyvitamin D3 (calciferol) therapy for juvenile renal osteodystrophy: Beneficial effect on linear growth velocity. *J. Pediatr.* **100**, 815.

341. Millman, R. P., Cohen, T. B., and Levinson, A. I. (1981). SLE complicated by pulmonary hemorrhage: Recovery following plasmapheresis and cytotoxic therapy. *J. Rheumatol.* **8**, 1021.
342. Rossen, R. D., Hersh, E. M., and Sharp, J. T. (1977). Effect of plasma exchange on circulating immune complexes and antibody formation in patients treated with cyclophosphamide and prednisone. *Am. J. Med.* **63**, 674.
343. Jones, J. V. (1982). Plasmapheresis in SLE. *Clin. Rheum. Dis.* **8**, 245.
344. Mintz, G., Galindo, L. F., Fernandez-Diaz, J., *et al*. (1978). Acute massive pulmonary hemorrhage in SLE. *J. Rheumatol.* **5**, 39.
345. Turner-Stokes, L., and Turner-Warwick, M. (1982). Intrathoracic manifestation of SLE. *Clin. Rheum. Dis.* **8**, 229.
346. Ropper, A. H., and Poskanzer, D. C. (1978). The prognosis of acute and subacute transverse myelopathy based on early signs and symptoms. *Ann. Neurol.* **4**, 51.
347. Laing, T. J. (1988). Gastrointestinal vasculitis and pneumatosis intestinalis due to systemic lupus erythematosus: Successful treatment with pulse intravenous cyclophosphamide. *Am. J. Med.* **85**, 55.
348. Boumpas, D. T., Barez, S., Klippel, J. H., *et al*. (1990). Intermittent cyclophosphamide for the treatment of autoimmune thrombocytopenia in systemic lupus erythematosus. *Ann. Intern. Med.* **112**, 674.
349. Gaedicke, G., Teller, W. M., Kohne, E., *et al*. (1984). IgG therapy in systemic lupus erythematosus: Two case reports. *Blut* **48**, 387.
350. Akashi, K., Nagasawa, K., Mayumi, T., *et al*. (1990). Successful treatment of refractory systemic lupus erythematosus with intravenous immunoglobulins. *J. Rheumatol.* **17**, 375.
351. Dowling, P. C., Bosch, V. V., and Cook, S. D. (1980). Possible beneficial effect of high-dose intravenous steroid therapy in acute demyelinating disease and transverse myelitis. *Neurology* **30**, 33.
352. Warren, R. W., and Kredich, D. W. (1984). Transverse myelitis and acute CNS manifestations of SLE. *Arthritis Rheum.* **27**, 1058.
353. Yancey, C. L., Doughty, R. A., and Athreya, B. H. (1981). CNS involvement in childhood SLE. *Arthritis Rheum.* **24**, 1389.
354. Case records of the Massachusetts General Hospital (1979). Case No. 25-1078. *N. Engl. J. Med.* **301**, 881.
355. Lipsky, P. E., Hardin, J. A., and Schour, L. (1975). Spontaneous peritonitis and SLE. *JAMA* **232**, 929.
356. Lacks, S., and White, P. (1990). Morbidity associated with childhood systemic lupus erythematosus. *J. Rheumatol.* **17**, 941.
357. Abramson, S., Kramer, S. B., Radin, A., *et al*. (1985). Salmonella bacteremia in SLE. *Arthritis Rheum.* **28**, 75.
358. Zizic, T. M., Shulman, L. E., and Stevens, M. B. (1975). Colonic perforations in SLE. *Medicine* **54**, 411.
359. Budman, D., and Steinberg, A. D. (1976). Relationship between hypertension and renal disease in SLE. *Arch. Intern. Med.* **136**, 1003.
360. Axelrod, L. (1976). Glucocorticoid therapy. *Medicine* **55**, 39.
361. Nephrology Forum (1986). The treatment of lupus nephritis. *Kidney Int.* **30**, 769.
362. Dumas, R. (1985). Lupus nephritis: Collaborative study by the French Society of Pediatric Nephrology. *Arch. Dis. Child.* **60**, 126.
363. Rush, P. J., Baumal, R., Shore, A., *et al*. (1986). Correlation of renal histology with outcome in children with lupus nephritis. *Kidney Int.* **29**, 1066.
364. Appel, G. B., Silva, F. G., Pirany, C. L., *et al*. (1978). Renal involvement in SLE. *Medicine* **57**, 371.
365. Cathcart, E. S., Idelson, B. A., Scheinberg, M. A., *et al*. (1976). Beneficial effects of methyl prednisone "pulse" therapy in diffuse proliferative lupus nephritis. *Lancet* **1**, 163.
366. Cole, B. R., Brocklebank, J. T., Kienstra, R. A., *et al*. (1976). "Pulse" methyl prednisone therapy in the treatment of severe glomerulonephritis. *J. Pediatr.* **88**, 307.
367. Barron, J. E., Person, D. A., and Brewer, E. J. (1982). Pulse methyl prednisone therapy in DPLN. *J. Pediatr.* **101**, 137.
368. Austin, H. A., Klippel, J. H., Balow, J. E., *et al*. (1986). Therapy of lupus nephritis: Controlled trial of prednisone and cytotoxic drugs. *N. Engl. J. Med.* **314**, 614.
369. Garret, R., and Paulus, H. (1980). Complications of intravenous methyl prednisone pulse therapy. *Arthritis Rheum.* **23**, 677. [Abstract]
370. Balow, J. E., Austin, H. A., III, and Muenz, L. R. (1984). Effect of treatment on the evolution of renal abnormalities in lupus nephritis. *N. Engl. J. Med.* **311**, 491.
371. Lehman, T. J. A., and Onel, K. (2000). Intermittent intravenous cyclophosphamide arrests progression of the renal chronicity index in childhood systemic lupus erythematosus. *J. Pediatr.* **136**, 243.
372. Singh, A., Tejani, C., and Tejani A. (1999). One-center experience with cyclosporine in refractory nephrotic syndrome in children. *Pediatr. Nephrol.* **13**, 26.
373. Fu, Y. F., and Lui, G. L. (2001). Mycophenolate mofetil therapy for children with lupus nephritis refractory to both intravenous cyclophosphamide and cyclosporine. *Clin. Nephrol.* **55**, 318.
374. Buratti, S., Szer, I. S., Spencer, C., Bartosh, S., and Reiff, A. (2002). Mycophenylate Mofetil (MMF, CellCept) treatment of severe renal disease in peditric onset SLE. *J. Rheumatol.*
375. Klippel, J. H., Austin, H. A., III, Balow, J. E., *et al*. (1987). Studies of immunosuppressive drugs in the treatment of lupus nephritis. *Rheum. Clin. North Am.* **13**, 47.
376. Felson, D. T., and Anderson, J. (1984). Evidence for the superiority of immunosuppressive drugs and prednisone over prednisone alone in lupus nephritis. *N. Engl. J. Med.* **311**, 1528.
377. Carette, S., Klippel, J. H., Decker, J. L., *et al*. (1983). Controlled studies of oral immunosuppressive drugs in lupus nephritis: A long-term follow-up. *Ann. Intern. Med.* **99**, 1.
378. Steinberg, A. D., Kaltreider, B., Staples, P. J., *et al*. (1971). Cyclophosphamide in lupus nephritis: A controlled trial. *Ann. Intern. Med.* **75**, 165.

379. Dinant, H. J., Decker, J. L., Klippel, J. H., *et al*. (1982). Alternative modes of cyclophosphamide and azathioprine therapy in lupus nephritis. *Ann. Intern. Med.* **96**, 728.
380. Donadio, J. V., Holley, K. E., Wagoner, R. D., *et al*. (1972). Treatment of lupus nephritis with prednisone and combined prednisone and azathioprine. *Ann. Intern. Med.* **77**, 829.
381. Donadio, J. V., Holly, K. E., Wagoner, R. D., *et al*. (1974). Further observations on the treatment of lupus nephritis with prednisone and combined prednisone and azathioprine. *Arthritis Rheum.* **17**, 573.
382. Steinberg, A. D., and Decker, J. L. (1974). A double-blind controlled trial comparing cyclophosphamide, azathioprine, and placebo in the treatment of lupus glomerulonephritis. *Arthritis Rheum.* **17**, 923.
383. Sztejnbok, M., Stewart, A., Diamond, H., and Kaplan, D. (1971). Azathioprine in the treatment of systemic lupus erythematosus: A controlled study. *Arthritis Rheum.* **14**, 639.
384. Cade, R., Spooner, G., Schlein, E., *et al*. (1973). Comparison of azathioprine, prednisone, and heparin alone or combined in treating lupus nephritis. *Nephron* **10**, 37.
385. Hahn, B. H., Kantor, O. S., and Osterland, C. K. (1975). Azathioprine plus prednisone compared with prednisone alone in the treatment of systemic lupus erythematosus: Report of a prospective controlled trial in 24 patients. *Ann. Intern. Med.* **83**, 597.
386. Pollack, C. A., and Ibels, L. S. (1987). Dialysis and transplantation in patients with renal failure due to SLE: The Australian and New Zealand experience. *Aust. N. Z. J. Med.* **17**, 321.
387. McCurdy, D. K., Lehman, T. J. A., Bernstein, B., *et al*. (1992). Lupus nephritis: Prognostic factors in children. *Pediatrics* **89**, 240.
388. Baqi, N., Moazami, S., Singh, A., *et al*. (1996). Lupus nephritis in children: A longitudinal study of prognostic factors and therapy. *J. Am. Soc. Nephrol.* **7**, 924.
389. Fries, D. F., Powers, R., and Kepson, R. L. (1974). Late stage lupus nephropathy. *J. Rheumatol.* **1**, 166.
390. Avner, E. D., Harmon, W. E., Grupe, W. E., *et al*. (1981). Mortality of chronic hemodialysis and renal transplantation in pediatric end-stage disease. *Pediatrics* **67**, 412.
391. Potter, D. E., and Greifer, I. (1978). Statural growth of children with renal disease. *Kidney Int.* **14**, 334.
392. Chantler, C., and Holliday, M. A. (1983). Growth in children with renal disease with particular reference to the effects of caloric malnutrition: A review. *Clin. Nephrol.* **1**, 230.
393. Holliday, M. A., and Chantler, C. (1978). Metabolic and nutritional factors in children with renal insufficiency. *Kidney Int.* **14**, 306.
394. Broyer, M., Kleinkrecht, C., Loirat, C., *et al*. (1974). Growth in children treated with long-term hemodialysis. *J. Pediatr.* **84**, 642.
395. Norman, M. E., Mazur, A. T., Borden, S., *et al*. (1981). Early diagnosis of juvenile renal osteodystrophy. *J. Pediatr.* **97**, 226.
396. Lazarus, J. M., and Kjellstrand, C. M. (1981). Dialysis: Medical aspects. *In* "The Kidney" (B. M. Brenner and F. C. Rector, eds.), 2nd Ed. Saunders, Philadelphia.
397. Wolters, W. H. G. (1979). Psycho social care at a center for hemodialysis and renal transplantation in children and adolescents. *Acta Paedopsychiatr.* **44**, 85.
398. Wolters, W. H. G., Daniels-Wegdam, T., and Donckerwolcke, R. A. M. G. (1980). Family reactions to the hemodialysis of a child. *Acta Paedopsychiatr.* **45**, 345.
399. Chesney, R. W. (1983). Treatment of calcium and phosphorus abnormalities in childhood renal osteodystrophy. *Dialysis Transplant.* **12**, 270.
400. Henderson, R. G., Russell, R. G. G., Ledingham, J. G. G., *et al*. (1975). Effects of 1,25-dihydroxycholecalciferol on calcium absorption, muscle weakness, and bone disease in chronic renal failure. *Lancet* **1**, 379.
401. Warady, B. D., Lindsley, C. B., Robinson, R. G., and Lukert, B. P. (1994). Effect of nutritional supplementation on bone mineral status of children with rheumatic diseases receiving corticosteroid therapy. *J. Rheumatol.* **21**, 530.
402. Dabbagh, S., and Chesney, R. W. (1980). Treatment of renal osteodystrophy during childhood. *In* "Treatment of End-stage Renal Disease in Children" (R. N. Fine and A. Gruskin, eds.). Saunders, Philadelphia.
403. Wassner, S. J. (1982). The role of nutrition in the care of children with renal insufficiency. *Pediatr. Clin. North Am.* **29**, 973.
404. Chantler, C., Bishti, M., and Counohan, R. (1980). Nutritional therapy in children with chronic renal failure. *Am. J. Clin. Nutri.* **33**, 1682.
405. Baum, M., Powell, D., Calvin, S., McDavid, T., *et al*. (1982). Continuous ambulatory peritoneal dialysis in children: Comparison with hemodialysis. *N. Engl. J. Med.* **307**, 1537.
406. Miller, L. C., Bock, G. H., Lum, C. T., *et al*. (1982). Transplantation of adult kidney into the very small child: Long-term outcome. *J. Pediatr.* **100**, 675.
407. Amend, W., Vincenti, F., Covey, C., *et al*. (1977). Renal transplantation in SLE. *Proc. Eur. Dial. Transplant. Assoc.* **7**, 18.
408. Masterson, J. R., Jr. (1967). "The Psychiatric Dilemma of Adolescence." Little Brown, Boston.
409. Nashel, D. J., and Ulmer, C. C. (1982). SLE: Important considerations in the adolescent. *J. Adolesc. Health Care* **2**, 273.
410. Urowitz, M. B., and Gladman, D. D. (1980). Late mortality in SLE: "The price we pay for control." *J. Rheumatol.* **7**, 412.
411. Smith, S. D., Rosen, D., Trueworthy, R. C., *et al*. (1979). A reliable method for evaluating drug compliance in children with cancer. *Cancer* **43**, 168.
412. Thorn, G. W. (1973). The adrenal cortex: Reflections, progress and speculations. *Trans. Assoc. Am. Phys.* **86**, 65.
413. Hall, R. C. W., Stickney, S. K., and Gardner, E. R. (1981). Psychiatric symptoms in patients with SLE. *Psychosomatics* **22**, 15.

414. Orr, D. P., Weller, S. C., Satterwhite, B., *et al*. (1984). Psycho social implications of chronic illness in adolescence. *J. Pediatr.* **104**, 151.
415. Bloomfield, M. (1984). Psychological aspects of SLE. *Primary Care* **5**, 159.
416. Silber, T. J., Chatoor, I., and White, P. H. (1984). Psychiatric manifestations of SLE in children and adolescents: A review. *Clin. Pediatr.* **23**, 331.
417. Jungers, P., Dougados, M., Pelissier, C., *et al*. (1982). Influence of oral contraceptive therapy on the activity of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 618.
418. Meehan, R. T., and Dorsey, K. J. (1987). Pregnancy among patients with systemic lupus erythematosus receiving immunosuppressive therapy. *J. Rheumatol.* **14**, 252.
419. Lockshin, M. D., Reinitz, E., Druzin, M., *et al*. (1984). Lupus pregnancy: Case-control prospective study demonstrating absence of lupus exacerbation during or after pregnancy. *Am. J. Med.* **77**, 893.
420. Nossent, H. C., and Swaak, T. J. G. (1990). Systemic lupus erythematosus. VI. Analysis of the interrelationship with pregnancy. *J. Rheumatol.* **17**, 771.
421. Mintz, G., Miz, J., Gutierrez, G., *et al*. (1986). Prospective study of pregnancy in SLE: Results of a multidisciplinary approach. *J. Rheumatol.* **13**, 732.
422. Gonzalez-Crespo, M. R., Gomez-Reino, J. J., Merino, R., *et al*. (1995). Menstrual disorders in girls with systemic lupus erythematosus treated with cyclophosphamide. *B. J. Rheumatol.* **34**, 737.
423. Warne, G. L., Fairley, K. F., Hobbs, J. B., *et al*. (1973). Cyclophosphamide-induced ovarian failure. *N. Engl. J. Med.* **289**, 1159.
424. Blumenfeld, Z., Shapiro, D., Shteinberg, M., Avivi, I., and Nahir, M. (2000). Preservation of fertility and ovarvian function and minimizing gonadotoxicity in young women with systemic lupus erythematosus treated by chemotherapy. *Lupus* **9**, 401.
425. Blumenfeld, Z. (2001). Ovarian rescue/protection from chemotherapeutic agents. *J. Soc. Gynecol. Invest.* **8**, S60.

19

IMAGING OF SYSTEMIC LUPUS ERYTHEMATOSUS: STATE OF THE ART[1]

Lorraine G. Shapeero

Systemic lupus erythematosus (SLE) is a relatively common chronic inflammatory disease that affects bones and joints, skin, lungs, serous membranes (pleura, pericardium, and peritoneum), kidneys, gastrointestinal tract, cardiovascular and nervous systems, and a variety of other organs. Since the mid-1970s, the author has evaluated the various imaging modalities for diagnosing SLE from conventional radiography, sonography, and scintigraphy to the more advanced techniques such as magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positive emission tomography (PET), computed tomography (CT), and power Doppler. This chapter describes and illustrates the current role of imaging for SLE and its complications in each organ system.

IMAGING OF MUSCULOSKELETAL SLE

General Radiographic Features

The radiographic manifestations of SLE vary with the organs affected and with the duration of the disease. The most frequent presenting symptom is joint involvement [1, 2]. The symmetric polyarthritis of lupus, often nondeforming and nonerosive, affects particularly the small joints of the hand and often the wrists, the knees, and the shoulders [3]. In a review of 62 hands of patients with established SLE who fulfilled the American Rheumatism Association criteria, nondeforming arthritis was seen in 74% of patients. Five patients (16%) with severe disease had deformities of the hands and wrists; of these, four had flexible or reducible changes. Deforming arthritis was mentioned frequently in the early descriptions of SLE [1]; however, when more specific criteria for the classification of SLE were established, the prevalence of this type of arthritis was considerably less (5%), although occasionally it could range from 5 to 50% [1, 3]. Even the most disfiguring arthritis of the hands can usually be reduced when the hands are pressed firmly against an X-ray cassette (Fig. 1) [3]. In some instances, the joint deformities are not correctable (Fig. 2).

The common types of hand involvement are swelling at the proximal interphalangeal (PIP) joints and metacarpophalangeal (MCP) joint of the thumb (hitchhiker's thumb), swan neck deformities (Fig. 3), subluxations of the MCP joints, and ulnar deviation of the fingers [1, 2].

Fusiform swelling of the fingers is a manifestation of inflammatory changes in the pericapsular soft tissues rather than a joint effusion, which is usually unimpressive in this disease. Juxta-articular osteoporosis of the hands has been reported in 50% of lupus patients [2]. In the authors' experience, it was found less frequently, in only 16% of patients. Atrophy of the thenar and

[1]The opinions or assertions contained herein are the private views of the author and are not to be construed as official nor as reflecting the views of the Department of Defense, the Uniformed Services University of the Health Sciences, the Department of the Army, or the United States Military Cancer Institute.

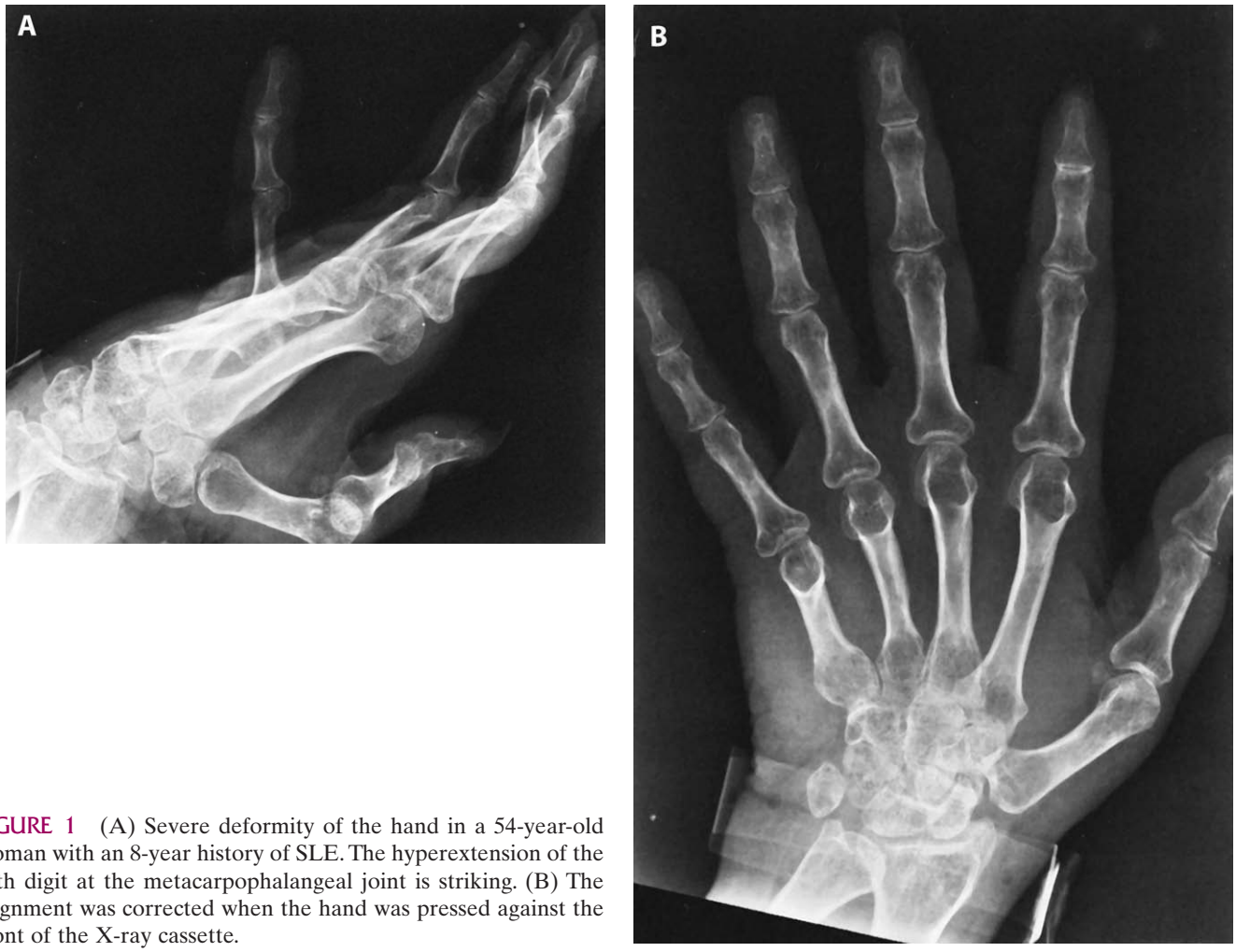


FIGURE 1 (A) Severe deformity of the hand in a 54-year-old woman with an 8-year history of SLE. The hyperextension of the fifth digit at the metacarpophalangeal joint is striking. (B) The alignment was corrected when the hand was pressed against the front of the X-ray cassette.

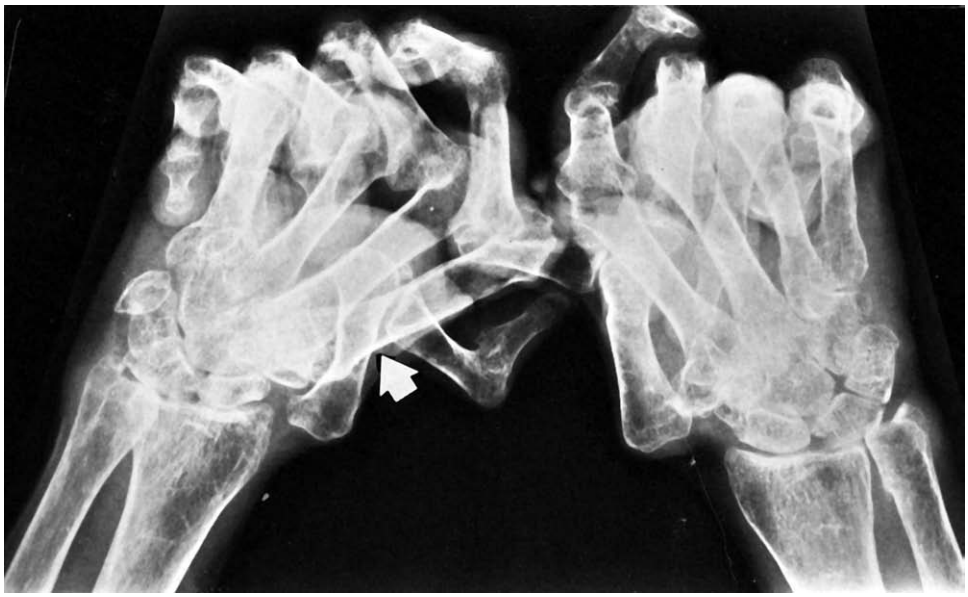


FIGURE 2 This 52-year-old woman with SLE has severe irreducible hand deformities simulating a mutilating arthritis. The profound osteoporosis, the "hitch-hiker's" thumb (*arrow*) and the metacarpal subluxations are pronounced.

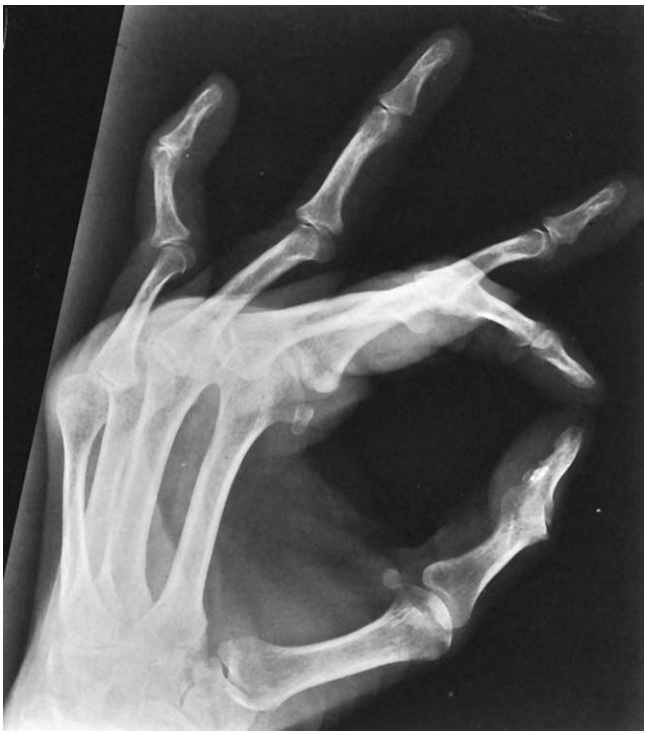


FIGURE 3 This 37-year-old woman with SLE since childhood has subluxations at the metacarpophalangeal joint and a pronounced swan neck malalignment of the fifth finger. Despite the severity, the deformities are reducible.

hypothenar muscles is rare but occasionally can be a prominent feature of SLE [2]. Even when deforming arthritis is present, loss of articular cartilage and erosive changes are unusual [3–5]. Age appears to have no significant influence on the frequency of this finding [4].

Advanced Arthropathy (Jaccoud's Arthritis)

Bywaters [6] noted a striking similarity between the arthritic deformities of SLE and Jaccoud's arthritis—an entity originally thought to result from rheumatic fever. Clinically, Jaccoud-like arthritis may simulate rheumatoid arthritis [3–6] but patients show little functional disability despite the severity of the deformities. Although Jaccoud-like arthropathy may have a variable appearance, the more characteristic features are symmetric arthritis, ulnar deviation of the fingers, MCP subluxation, swan neck deformities of the digits [hyperextension at the PIP joints and flexion at the distal interphalangeal (DIP) joints], and boutonnière deformities (flexion at the PIP joints and hyperextension at the DIP joints). As mentioned earlier, pressure on the hands by firm placement on a radiographic cassette can correct the malalignment of the fingers on radiographs even when patients clinically present with severe deformities [1] (Fig. 1). Rarely, radiographs demonstrate cyst-like lesions and hook defects in the metacarpal heads.

The prevalence of Jaccoud's syndrome is reported as 2.4–7% [1, 7–10]. Perhaps as the survival rate of patients with SLE increases, this prevalence will also increase. Jaccoud's disease appears to be a long-standing arthritis (average duration of 22 years), whereas the classical arthritis of SLE is a nondeforming arthropathy of short duration (average of 7.2 years).

Occasionally, Jaccoud's arthritis may affect the knees and elbows. De la Sota *et al*. [7] reported a Jaccoud's arthritis of the knees with an unusual severe functional impairment accompanying the nonerosive deformity. In a few of the patients in their series, Esdaile *et al*. [1] described a fixed flexion contracture of the elbows as an atypical finding in Jaccoud's arthritis. This subtype is distinctive from classical Jaccoud's disease and probably represents a variant.

The pathogenesis of Jaccoud's arthritis is not clearly understood, but fibrosis is a prevalent feature in the reparative response. In addition, the frequent association of mitral stenosis in Jaccoud's arthritis suggests that fibrosis is more generalized than suspected. The association with rheumatoid arthritis is rare [2, 3, 10, 11].

Subchondral Cysts and Erosions

Cystic bone lesions in SLE are typically located in the subchondral region in the small joints of the hands and feet. In one series of 141 patients [12], 41% of lupus patients had subchondral cysts predominantly in the metacarpal heads and carpal bones, although most authors state that it occurs less frequently. Cysts are usually multiple and show progressive development over the course of the disease. No correlation is found with other manifestations of SLE, such as pleuritis, pericarditis, and joint and central nervous system (CNS) disease involvement. These subchondral cysts may simulate the "punched-out" lesions of gouty arthritis (Fig. 4) and occasionally may be accompanied by erosions and overhanging edges. Several investigators have discussed the coexistence of gout and SLE [13–15]. The clinical picture is that of classic gout, particularly affecting the great toe. The diagnosis should not be confused with a flare-up of SLE or with cellulitis of the foot. Radiographs reveal the typical gouty erosions with an "overhanging edge" of the interphalangeal joint of the great toe. Aspiration of the MCP joint shows sodium urate crystals in the fluid and elevated serum uric acid levels of 10–11 mg/100 ml. Investigators have also discussed an association among SLE, nephritis, and gout [14, 15], and one author mentioned the coexistence of SLE, gout, and Paget's disease [15]. These reports stress the problem of

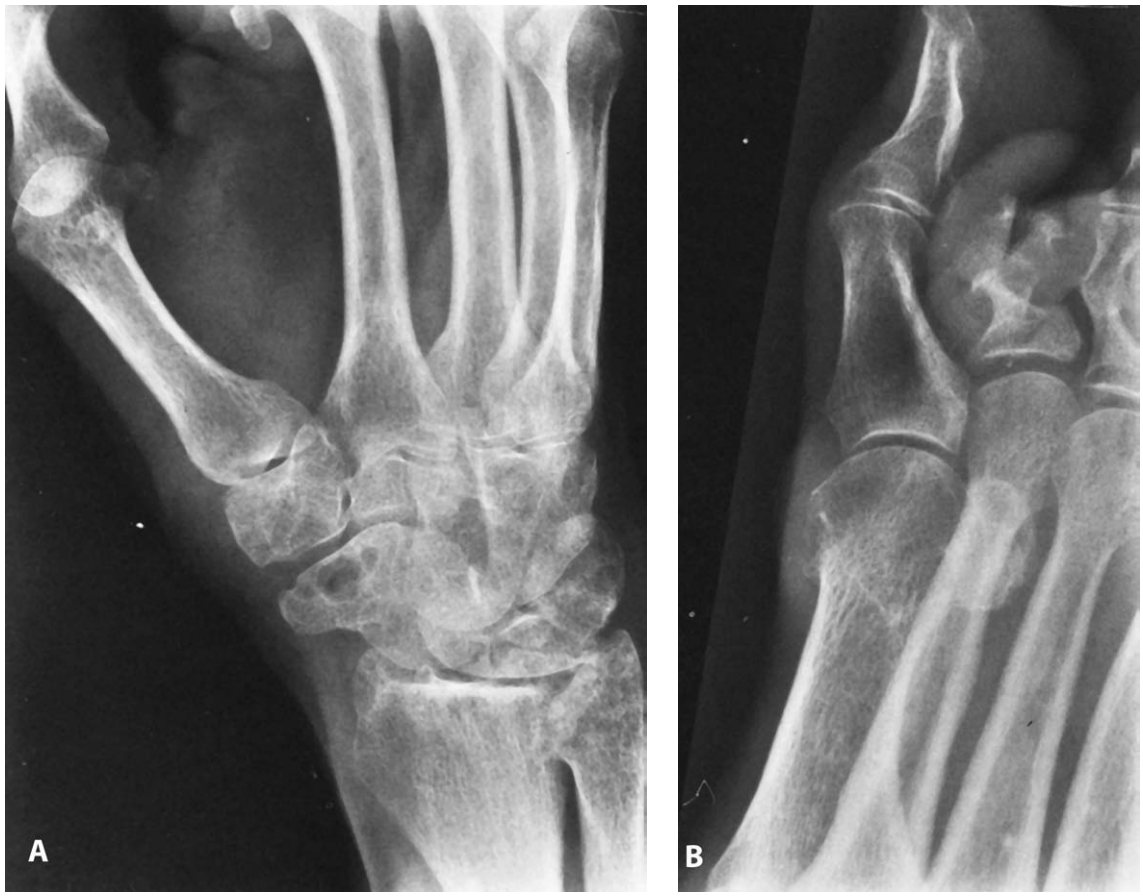


FIGURE 4 (A) A well-circumscribed cyst-like defect of the scaphoid simulates tophaceous gout in this patient with SLE. The absence of joint space narrowing is frequently seen with intraosseous tophi. (B) This patient has SLE with erosions of the metatarsal head, soft tissue masses, and a mild loss of joint space, features typical of gout. The uric acid levels, however, were normal.

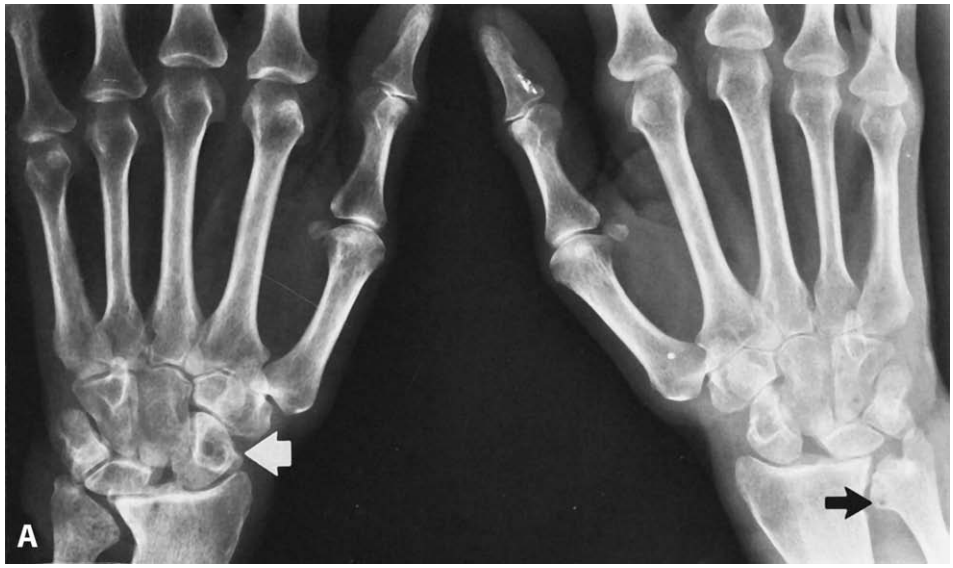
diagnosis when coexisting arthritic or osseous conditions are present.

Hook-like erosions, an unusual finding in SLE [2, 16, 17], develop in the wrists (Fig. 5A), shoulders (Fig. 5B), metacarpal heads, and, occasionally, in the metatarsal heads. Rarely, erosions have been reported in the temporomandibular joints [17a]. Erosions may be unilateral or bilateral. The defects are characteristically on the volar-radial surface of the bone and are difficult to distinguish from the erosions of rheumatoid arthritis, pseudogout, osteoarthritis, gout, and hemosiderosis [1–4, 6, 10].

Acral Sclerosis–Tuft Resorption and Periarticular Calcifications

In one series, radiographs showed acral sclerosis in 17% of lupus patients. Focal sclerotic changes, however, are not specific for SLE; 8% of normal patients have this finding. Tuftal sclerosis is also associated with rheumatoid arthritis, psoriatic arthropathy, sarcoidosis, scleroderma, and dermatomyositis [2, 18, 19]. Because radiographs show acral sclerosis and osteoporosis in lupus patients with and without Raynaud's phenomenon, it is not possible to separate patients with Raynaud's phenomenon by radiographic features alone. However, in one study, all lupus patients who had radiographic findings of terminal tuft resorption or periarticular calcification had clinical Raynaud's phenomenon [2].

Soft tissue calcifications (calcinosis universalis and calcinosis cutis) [20, 21] are discussed later in the section on dermatological imaging of SLE. When radiographs show the triad of periarticular calcification, acral sclerosis, and joint deformity without erosions, SLE should be considered, and patients should be evaluated by the American Rheumatism Association criteria. One should also consider the possibility of an overlap syndrome, namely SLE and scleroderma or mixed connective tissue disease. A diagnostic feature of mixed connective tissue disease is the elevation of antinuclear



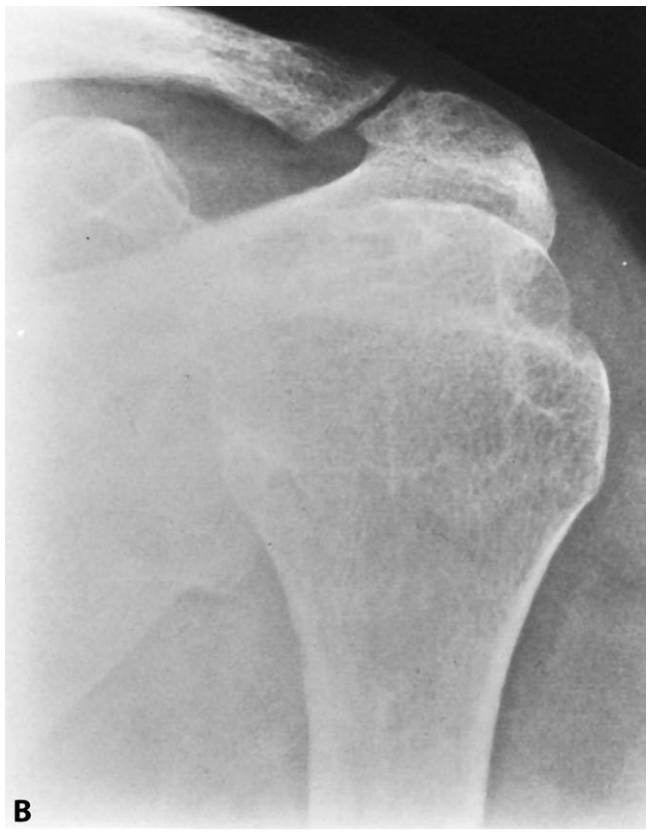


FIGURE 5 (A) Erosions in the wrists of a 32-year-old patient with SLE involve the radioulnar joints (*arrow*), the distal ulna, and the articular ends of the thumb metacarpals. Note the widening of the scapholunate articulation consistent with bilateral scapholunate ligamentous tear. (B) A large, scooped-out erosion is present in the greater tubercle of the humerus.

antibody titers with specificity for nuclear ribonucleoprotein (extractable nuclear antigen) [2].

Sacroiliitis

Sacroiliitis is an infrequent manifestation of SLE [22–24, 24a]. Usually, radiographs show early changes such as loss of the cortical "white line" of the iliac side of the joint and small erosions. The late findings of sclerosis and joint fusion that are found in the spondyloarthropathies are distinctly unusual in SLE. Tc-99m methylene diphosphonate (^{99m}Tc MDP) bone scans show elevated radiopharmaceutical uptake ratios of the affected sacroiliac joint(s) in lupus patients [25]. Currently, MRI, especially with contrast enhancement and fat suppression, is considered to be the most sensitive

method for detecting sacroiliitis and is superior to CT for identifying active bone erosions and cartilaginous abnormalities.

Abnormalities of Tendons and Ligaments

Tendinous or ligamentous interstitial, partial or complete tears, tenosynovitis, and tendonitis are not unusual in rheumatic disorders, including SLE, and occasionally herald the onset of the disease [27, 27b]. For example, the incidence of rotator cuff tears in patients attending a rheumatic disease clinic can be as high as 50% (Fig. 6).

When available, MRI is the modality of choice for defining globally the extent of tendinous and ligamentous inflammation or tear and abnormalities of the adjacent bones, labra and other soft tissues [27c–30].

MRI creates images based on the response of atomic nuclei (protons) to radiofrequency pulses in a strong magnetic field with absorption of energy for resonance and subsequent emission of energy for relaxation. Each nucleus has a nuclear spin that is proportional to its angular momentum. For spin-echo sequences (T1- and T2-weighted sequences), the radiofrequency pulse flips the vectors of the nuclei 90° to create the conditions for excitation, resonance, and spin coherence (spins are in phase). The T1–relaxation or spin–lattice relaxation reflects the process whereby excited nuclei give off energy to their environment, the lattice, and thereby relax back to their original stable state. T2–relaxation or spin–spin relaxation is the process whereby the spins lose their phase coherence and become out of phase.

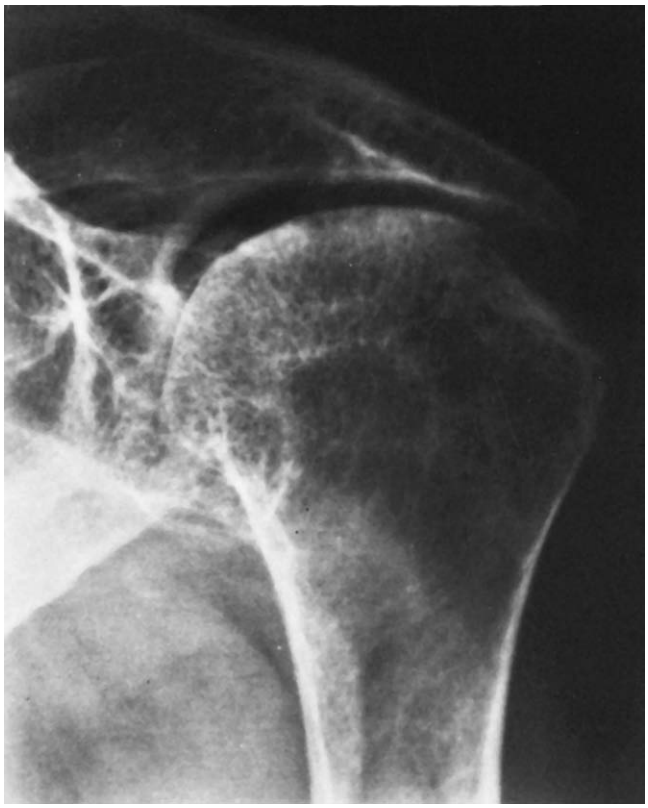


FIGURE 6 The marked 3-mm narrowing of the acromiohumeral space signifies a complete tear of the rotator cuff in this patient with SLE. Note the erosion of the greater tubercle. The similarity to rheumatoid arthritis is striking.

The ability to recognize abnormalities on MR images depends on tissue contrast. T2-weighted images can show excellent contrast between *high-signal-intensity (hyperintense) pathologic tissue* such as inflammation, cysts, edema, cysts, or tumor and *low-signal-intensity (hypointense) normal muscle and fibrous tissue*. On T2-weighted sequences, fat in marrow and subcutaneous tissue will appear medium or high signal intensity depending on the standard or fat sequence. Therefore, differentiation between fat and pathologic processes may be difficult. To improve lesion conspicuity, fat suppression (saturation) is added with the result that fat-containing tissue becomes hypointense and separable from hyperintense pathologic lesions.

T1-weighted images are used to demonstrate anatomic relationships, but not for delineating pathologic lesions because there is low tissue contrast within and among tissues on this sequence. Only fat, proteinacious material (e.g., abscesses), and subacute hemorrhage are hyperintense on T1-weighted sequences. Other tissues (e.g., muscle and fibrous tissue) and pathologic processes display low signal intensity and therefore may not be differentiated easily. The addition of contrast medium and fat suppression to the T1-weighted sequence can improve lesion identification because pathologic tissue often displays intense enhancement and is therefore separable from the nonenhancing normal fat and fibrous tissue and mildly enhancing muscle.

With musculoskeletal MRI, the normal tendon or ligament shows low signal intensity on T1- and T2-weighted images. In contrast, tendonitis or intrasubstance tear produces a focal area of high signal intensity within the tendon, and tenosynovitis is associated with peritendinous hyperintense fluid (Fig. 7). With complete rupture, hyperintense fluid separates the disrupted ligament (Fig. 8). In the lupus patient on corticosteroid or immunosuppressive therapy, MRI cannot distinguish between the infected tenosynovitis (Fig. 9) and the noninfected inflammation. Sonographic localization of fluid and sonographically-guided aspiration enhance rapid diagnosis and appropriate treatment [31].

Duplex sonography, when performed by experienced musculoskeletal sonologists or sonographers, is useful for studying individual tendons (e.g., Achilles tendon,

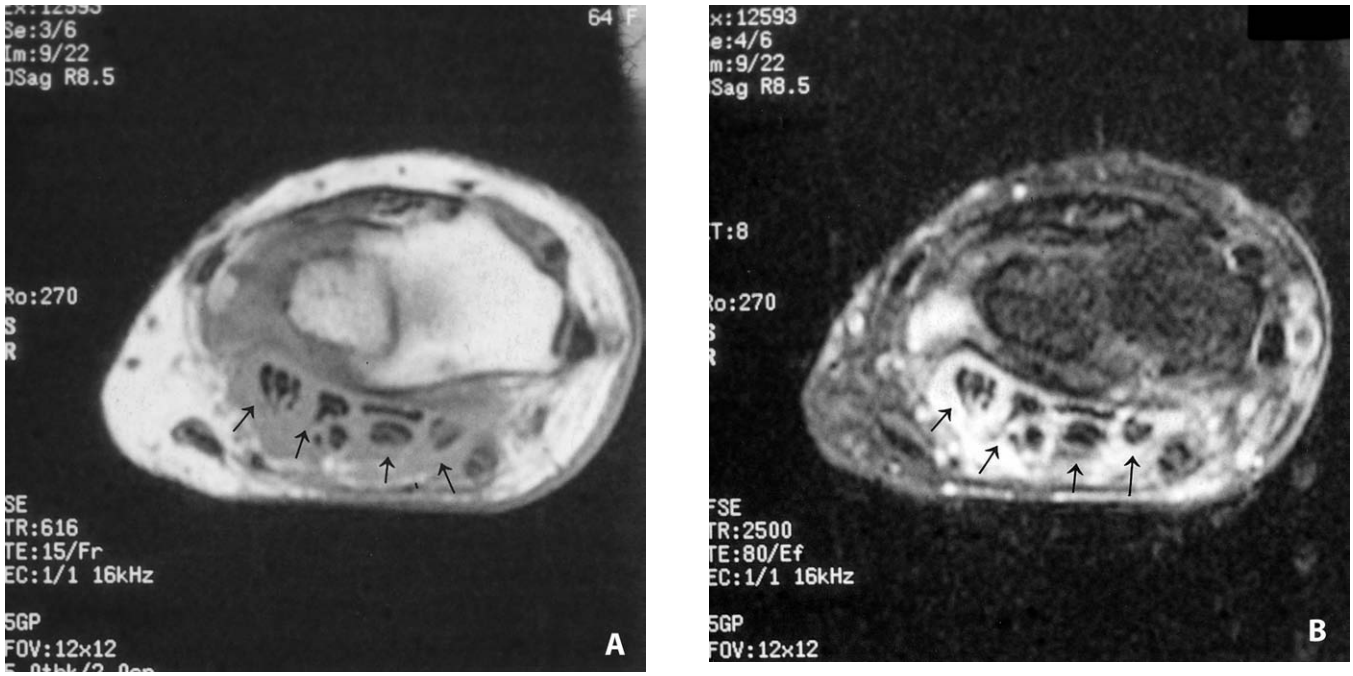


FIGURE 7 (A) Axial T1-weighted MR image (616/15) of the distal ulna and radius shows low-signal-intensity fluid (*black arrows*) surrounding the flexor tendons in a 46-year-old woman with SLE. (B) T2-weighted MR image with fat saturation (2500/80) shows high-signal-intensity fluid (*black arrows*) surrounding the flexor tendons consistent with a tenosynovitis and some high signal intensity within the tendons consistent with tendonitis.

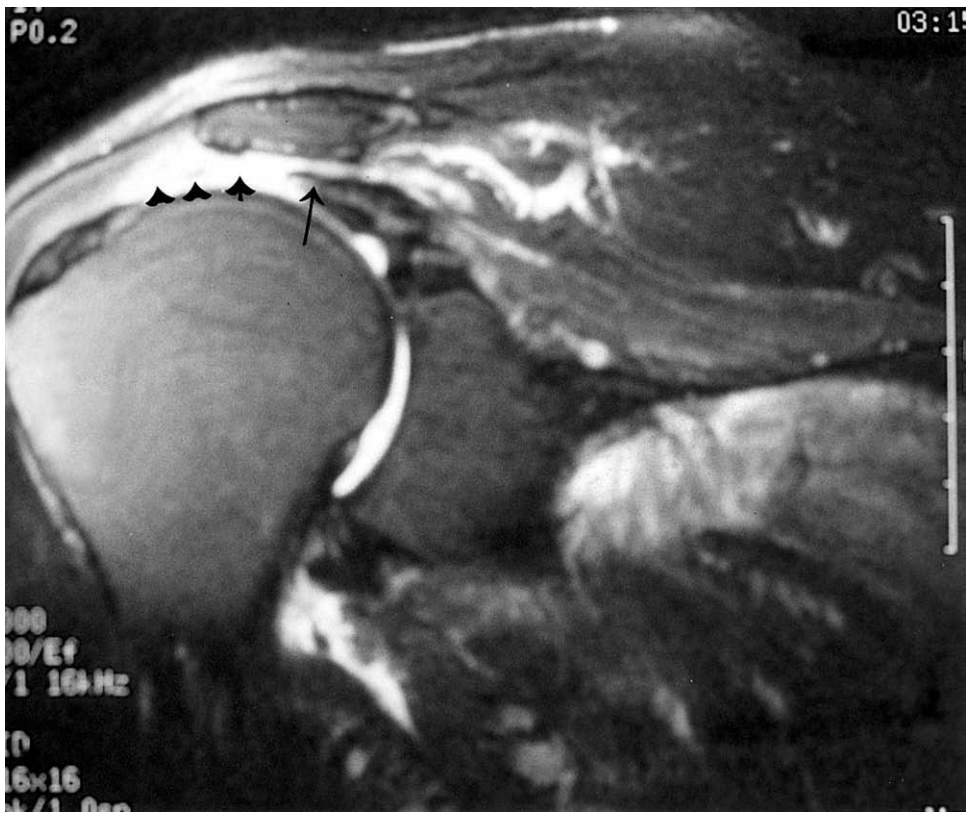


FIGURE 8 Coronal oblique T2-weighted MR image with fat saturation (3000/100) of the right shoulder of a 54-year-old woman with SLE who developed shoulder pain while on long-term steroids. High-signal-intensity fluid communicates between the subacromial bursa and the shoulder joint and defines the point of tear (*arrowheads*) and the retracted rotator cuff (*arrow*).

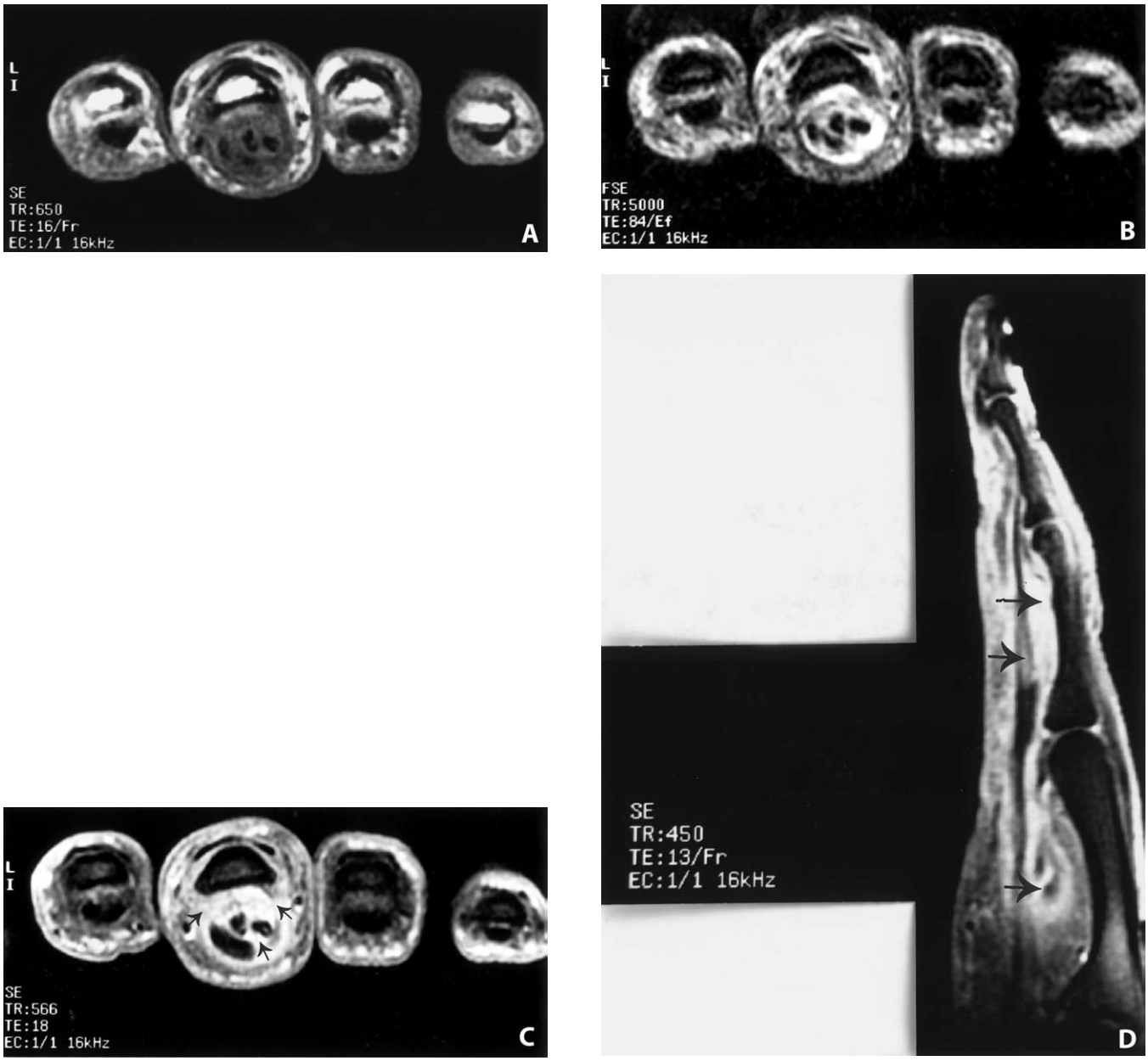


FIGURE 9 (A) Axial T1-weighted MR image (650/16) of the fingers shows low signal intensity around the deep and superficial flexor tendons of the third digit and the adjacent soft tissues, which becomes high signal intensity on (B) axial T2-weighted with fat saturation MR images (5000/84) This 67-year-old woman with SLE, who had received high-dose corticosteroids, presented with fever and a swollen finger. Axial (C) and sagittal (D) contrast-enhanced T1-weighted with fat saturation MR images (586/18) show enhancement (*arrows*) of the tendons and soft tissues. The tenosynovitis was biopsied, and the culture grew slow-growing mycobacterium.

biceps tendon) or groups of tendons (e.g., rotator cuff), although adjacent osseous structures cannot be evaluated [32, 33] More recently, the addition of tissue harmonic sonographic imaging, extended field-of-view imaging, and power Doppler to standard sonography have improved the detection of tears and inflammation [33a–33c]. Tissue harmonic sonographic imaging shows better spatial and contrast resolution of normal and pathologic tissues than standard sonography [33a]. Extended field-of-view imaging shows the full extent of a particular tendon or ligament or focus of inflammation or abscess on one image [33b]. Power Doppler

sonography is more sensitive than conventional Doppler for depicting increased blood flow in areas of inflammation in and around tendons, ligaments, bursae, and muscles [33c].

SLE is not the only etiologic factor predisposing to ligamentous or tendinous tears. Corticosteroids, even in the absence of ligamentous disease, may cause detritic structural changes that result in rupture. Histologic examination of the ligament shows features of collagen damage. Resnick [3] reported spontaneous rupture of tendons in SLE patients and indicated that these patients typically had received corticosteroids. Attrition of the periarticular capsular structures may be an explanation for laxity and joint deformities.

Carpal instability in SLE is not uncommon and occurred in 14.5% of our series and was associated with bilateral scapholunate dissociation in 9.7% of the patients. At radiography, a 3-mm or greater widening of the scapholunate joint is a sign of this instability and has been named the "Terry Thomas" or, more recently, the "David Letterman" sign (Fig. 10) [34]. Radiographs of the hand in the neutral position may be normal. However, when the patient is examined at fluoroscopy, transient carpal subluxation or "popping" of a carpal bone may be seen during ulnar–radial and flexion–extension movement of the wrist. Radiographs taken in ulnar deviation are a useful static method for showing instability. Intraarticular contrast-enhanced wrist arthrography can delineate the ligamentous tears by showing communication between wrist compartments [35]. More recently, high-resolution MRI, especially with intraarticular contrast enhancement, has been useful for delineating tears of the ligaments and tendons of the wrist [36] and may replace conventional arthrography in the future. The newer modalities of ultrafast electron beam CT and fast (echoplanar) MRI have potential application for dynamic cross-sectional imaging of joint kinematics and stability [37, 38].

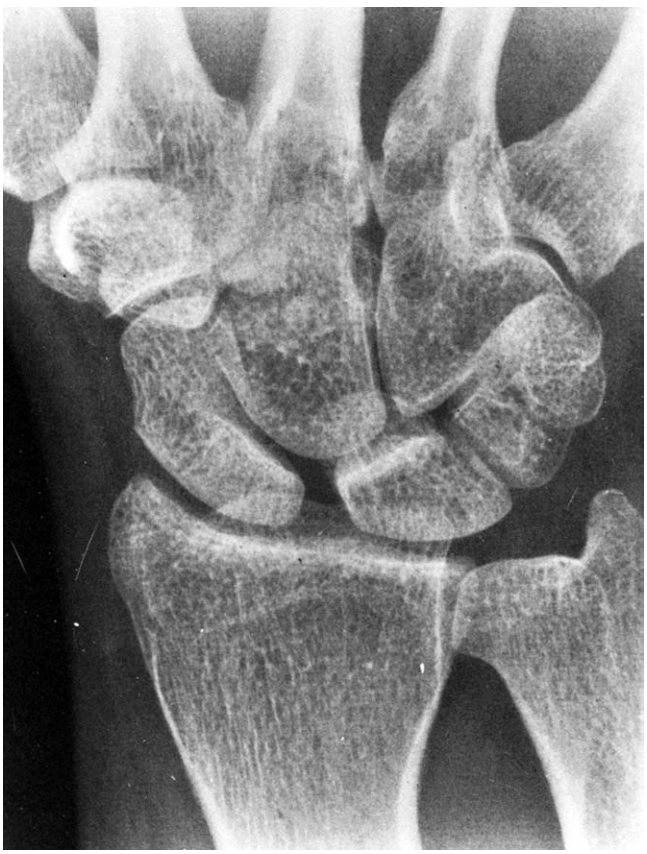


FIGURE 10 This patient with SLE presented with clicking and instability of the wrist. Anteroposterior radiograph of the wrist shows widening of the scapholunate joint consistent with rupture of the scapholunate ligament.

Complications of Treatment of SLE

Osteonecrosis

One of the more serious complications of SLE is osteonecrosis of the articular ends of the bones followed by secondary joint degeneration (osteoarthritis). In 1960, Dubois and Cozen [39] described the association of "avascular (aseptic) bone necrosis" and SLE. They reported a series of 400 patients with SLE; 10 patients (2.5%) developed osteonecrosis. All except 1 patient had received corticosteroids before the appearance of ischemic necrosis. Osteonecrosis may involve three or more anatomic sites and may be unifocal or multifocal. In a retrospective study of patients with osteonecrosis of various etiologies, 3% (32 of 1032 patients) had multifocal disease. Thirteen of the 32 patients were lupus patients treated with chronic corticosteroid therapy [40]. The spontaneous onset of osteonecrosis in SLE is rare [40a].

Since the initial report of Pietrogrande and Mastromarino in 1957 [41], corticosteroid-induced osteonecrosis has become a well-established entity. The prevalence of osteonecrosis in adult patients with SLE varies from 5 to 6% [3, 39, 42] to 32% (our series). All of our patients were receiving prednisone. However, the prevalence of osteonecrosis in children with lupus appears to be higher at 40% [43–45]. MRI [46–49] is the most sensitive modality for detecting early osteonecrosis and infarcts and also should be used for following response to medical or surgical therapy for osteonecrosis (e.g., changes with nonweight-bearing, core decompression, osteotomy, fibular graft placement).

Klipper *et al.* [50] reported Raynaud's phenomenon in 61% of patients with SLE and osteonecrosis, especially those who had received high or sustained dosages

of corticosteroids. However, Griffiths *et al*. [51] found no significant difference in the prevalence of Raynaud's disease when osteonecrosis complicated SLE.

Osteonecrosis has a predilection for the femoral head (Fig. 11) but also involves the humeral head, the femoral and tibial condyles, the talus, and the smaller tubular bones of the hands and feet. Weight-bearing joints are typically more affected than nonload-bearing joints of the upper extremities. In the lower extremity, the most commonly affected site is the head of the femur and the least frequently are the bones of the foot and ankle. Similarly, in the upper extremity, the head of the humerus is involved most commonly and, considerably less frequently, the smaller bones of the wrist and hand. Diaphyseal infarctions are less common than osteonecrosis at the articular ends of the bone. The clinical onset of osteonecrosis may be insidious. The onset of pain and limitation of motion in patients with chronic osteonecrosis may alert the clinician to the development of detritic synovitis, secondary osteoarthritis, or both [52]. However, osteonecrosis in SLE may be occult.

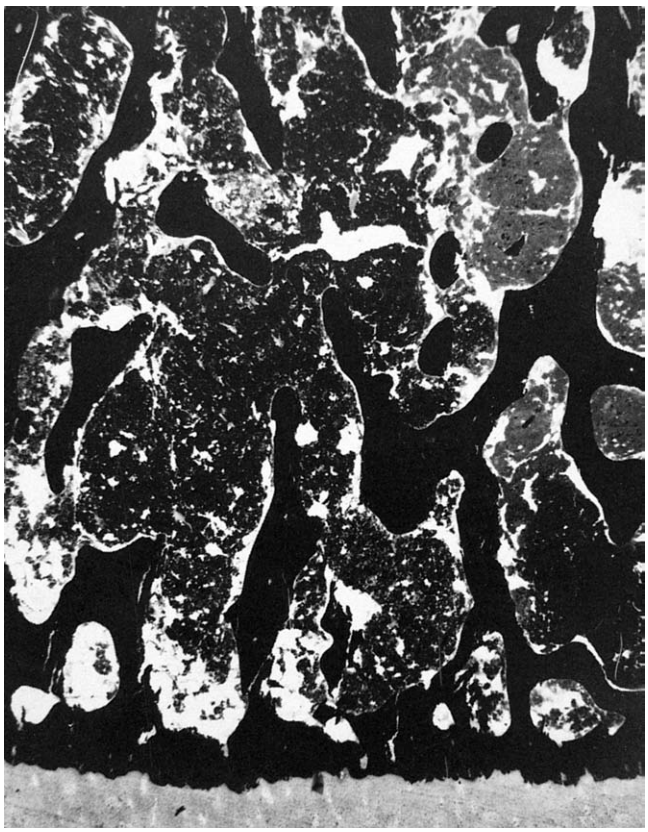


FIGURE 11 A photomicrograph of the femoral head from a 34-year-old man with SLE shows a corticosteroid-induced osteonecrosis. The increased density in the subchondral region results from extensive calcification in the marrow (undecalcified bone, von Kossa's stain) (magnification ×26).

In a study of 66 lupus patients who had received at least 5 mg/day prednisone for longer than 6 months, 8% of patients had asymptomatic osteonecrosis of the hip documented at MRI, which was metabolically inactive as evidenced by normal radiopharmaceutical uptake on ^{99m}Tc MDP bone scans. The lesions did not show progression or regression on clinical or imaging examination during a 1-year interval. The authors suggested that the stability of the lesions may also be related to the stability of prednisone dosages and disease activity during the time period studied [53a].

The radiographic features of osteonecrosis in SLE are identical to those seen in other conditions (e.g., trauma, corticosteroids, Gaucher's disease, sickle cell disease, and decompression bone disease). Regardless of the underlying cause, the radiographic findings and pathogenesis are the same. Histologic examination of osteonecrosis shows that, after the vascular insult, the subarticular bone and fatty marrow become necrotic and that calcifications are deposited in the nonviable marrow (saponification of fat). In the early stage, when the calcification is deposited minimally in the marrow, radiographic findings are often absent because the foci of calcification cannot produce adequate density. However, at this stage, MRI demonstrates early high-signal-intensity edema and hemorrhage. When calcification of the marrow is more extensive, the medullary bone will appear denser at radiography. Once the reparative process begins and vascular granulation tissue erodes the subchondral zone (e.g., femoral head), bone is resorbed and the articular cortex becomes structurally weakened and can collapse under the strain of weight-bearing. At radiography, the subchondral fracture is a crescent of radiolucency that parallels the articular cortex of the bone (Fig. 12). This crescent sign is pathognomonic of osteonecrosis [53]. The radiolucent crescent subtends the necrotic zone like an umbrella covering the area of infarct. Ultimately, the dead cancellous bone in the involved area undergoes microfractures, with the result that osteochondral fractures of the articular surface are detected radiographically. Appositional new bone scaffolding on necrotic trabeculae is a significant process that contributes to the increased density of the ischemic portion of the bone (Fig. 13) [3, 53]. This process of repair is what Pfemister called *creeping substitution*. The infarcted area is composed of three zones: (1) a central "yellow zone" of necrotic trabeculae and detritic marrow, (2) a "red zone" of fibrovascular granulation tissue delimiting the necrotic tissue, and (3) the "white zone" of reparative sclerosis around the periphery of the ischemic infarction. The greater the repair response in the infarcted bone, the denser it appears. In order of importance, the following factors contribute to the radiodensity of the bone: (1) the appo-

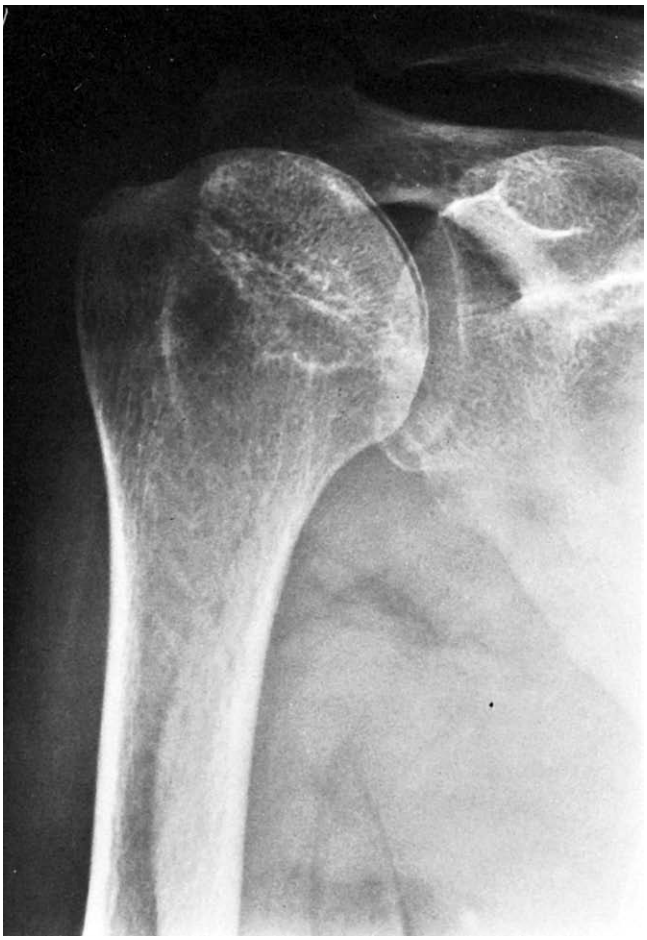


FIGURE 12 The radiolucent crescent of subchondral bone is the hallmark of osteonecrosis on this radiograph of a shoulder of a patient with SLE. Initially, the joint space is unaffected.

sition of new bone on nonviable trabeculae, (2) the calcification of necrotic fatty marrow (see Fig. 11), (3) microfractures and compression of the dead trabeculae, and (4) the apparent increased bony density because of the surrounding osteoporotic viable bone. In addition to epiphyseal osteonecrosis, medullary infarcts may develop in lupus. Often, however, the infarcts may not be visible on radiographs but are well defined at MRI (Fig. 14). Laminar periostitis is another radiographic finding occasionally associated with vascular complications of SLE [54, 55].

Prior to MR imaging, ^{99m}Tc MDP bone scans were the preferred modality for identifying osteonecrosis. When the blood flow to the necrotic zone is deficient (ischemic), the bone scan focally becomes photopenic because of an absent uptake of the radiopharmaceutical. When the reparative process begins, scintigraphy shows increased radiopharmaceutical uptake.

MRI is the most sensitive test for early recognition of osteonecrosis (Fig. 15) [47–49]. Different stages of osteonecrosis show different characteristics. With early osteonecrosis, 85% of patients may have no symptoms or only mild symptoms, radiographs are normal, and bone scans are false negative in 15% of studies. At this early stage, T2-weighted MR images, especially with fat saturation, are positive with high-signal intensity in areas of acute inflammation, edema, hemorrhage, or vascular engorgement in the affected bone. Recognition at this early stage is essential to prevent the complications of collapse and secondary osteoarthritis. As edema resolves, osteonecrosis may then show a "double line sign" consisting of a hypointense, fibrotic, peripheral, reactive rim around hyperintense marrow fat. In later stages, osteonecrosis becomes low signal intensity on T2-weighted MR images because hypointense fibrous tissue replaces marrow fat. These MRI features correlate with the radiographic findings of subchondral collapse. In addition to necroses in the epiphyses, MRI can also demonstrate well-demarcated medullary bone

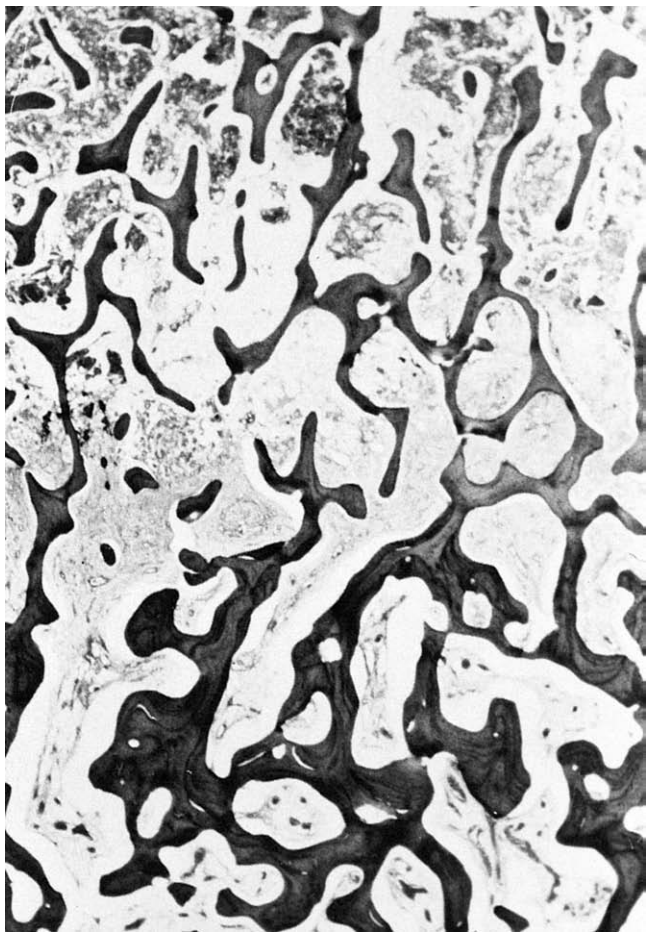


FIGURE 13 A major factor contributing to the increased density of the bone is the apposition of new bone upon necrotic trabeculae (creeping substitution) as seen on this photomicrograph.

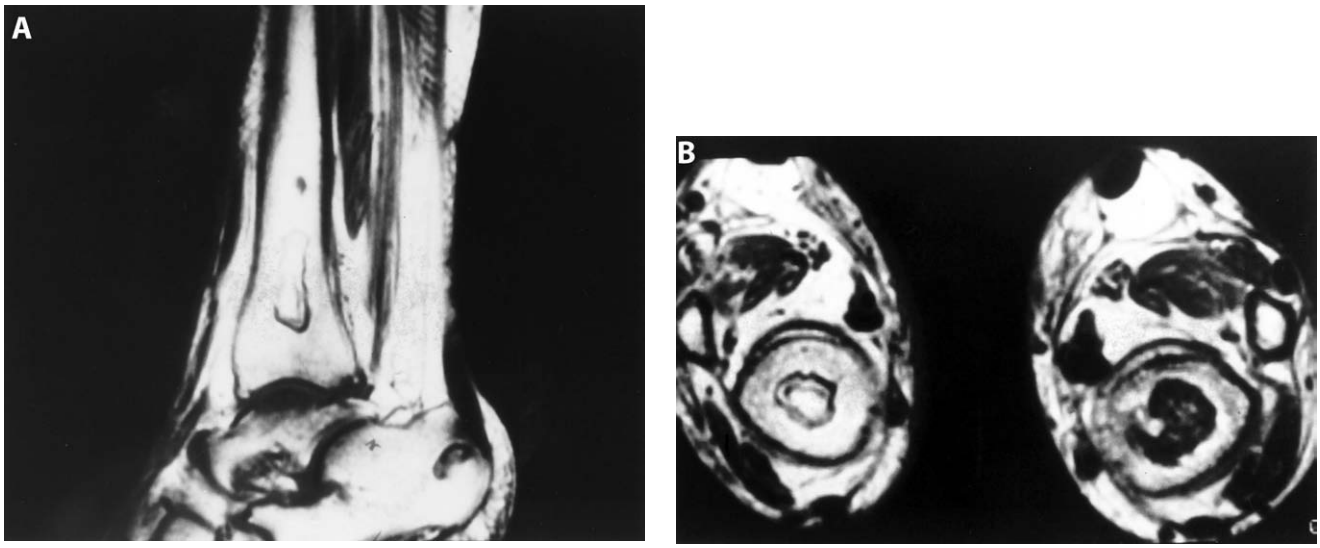


FIGURE 14 (A) Sagittal and (B) axial T1-weighted MR images (500/30) of the tibia show well-demarcated infarcts in the metadiaphysis in a 33-year-old woman with SLE who had received corticosteroid therapy for several years.

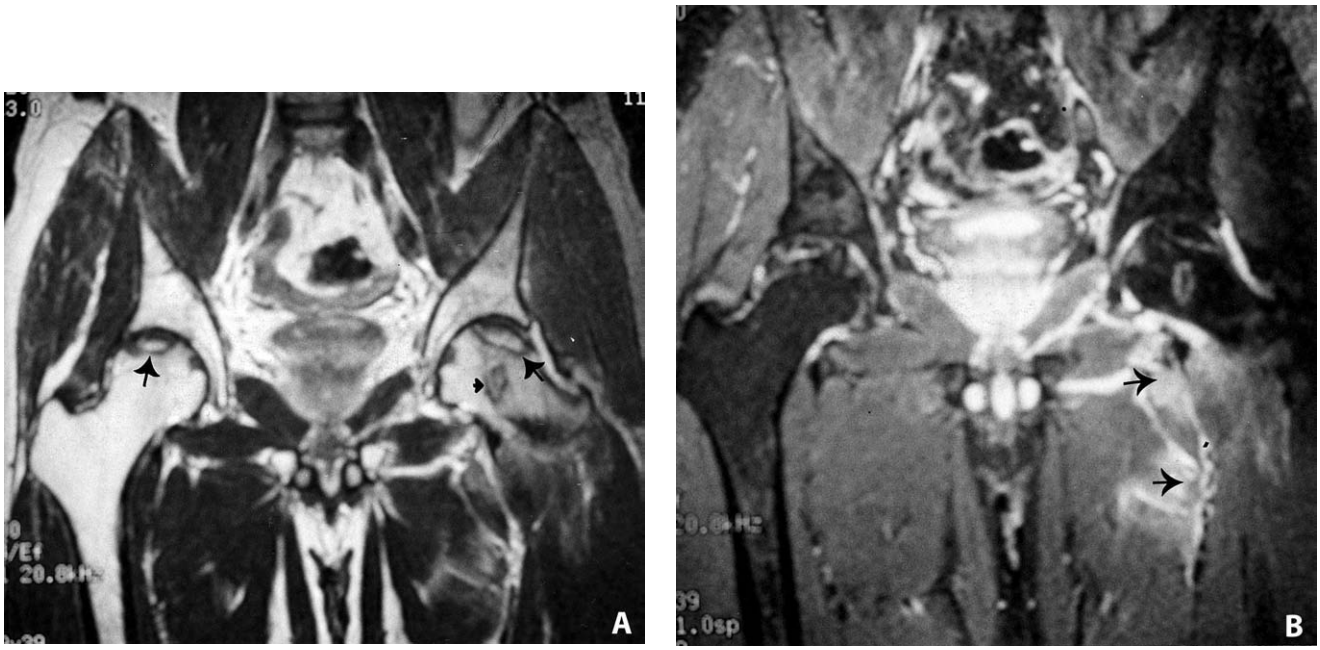


FIGURE 15 This 36-year-old man with SLE had bilateral hip and left thigh pain. (A) Coronal T1-weighted MR image (566/16) of the hips shows crescentic bilateral osteonecrosis of femoral heads (*large arrows*) and a well-defined medullary infarct (*arrowhead*) of the neck of the left femur. The left adductor magnus muscle displays heterogeneous signal intensity. (B) Coronal short tau inversion recovery (STIR) MR image (4900/89/150) shows the osteonecroses surrounded by high signal intensity (*arrowhead*). Normal marrow fat is low signal intensity. Fluid is present in the hip joints. The feathery pattern of high signal intensity in the left adductor magnus muscle (*arrows*) is consistent with myositis.

infarcts (Fig. 14). Although T1- and T2-weighted MR images in orthogonal planes are the standard sequences for evaluating osteonecrosis and infarcts, the addition of intravenous contrast medium can improve differentiation between acute medullary bone infarct and osteomyelitis (see section on osteomyelitis) [49a].

The complications of osteonecrosis are often more serious than the condition itself. In the later stages of ischemic necrosis, deformity of the articular surface may develop with risk of secondary osteoarthritis, which, when severe, necessitates arthroplasty. Norman [52] reported the conditions that predispose to secondary osteoarthritis as osteonecrosis (30%), "burnt-out" inflammatory arthritis (i.e., rheumatoid arthritis, ankylosing spondylitis) (28%), congenital dislocation of the hip (26%), and epiphysiolysis (9%). In osteonecrosis, the articular cartilage remains intact for a longer period of time than in idiopathic osteoarthritis. This explains why the joint is not affected initially in the degenerative process, whereas in primary osteoarthritis, the cartilage is the first tissue to undergo degeneration with cartilage loss and joint space narrowing. After the joint narrows, osteophytes form as part of the repair response. They ring the articular surface away from the zone of heavy weight-bearing. Within the pressure segment, the cartilage surface is denuded, and sclerosis, subarticular cysts, or pseudocysts form in the subchondral zone (Fig. 16A). The pseudocysts are metaplastic foci of cartilage and fibrous tissue. Characteristically, foci of articular cartilage remain intact in osteonecrosis, even in the weight-bearing segment. The pattern of joint cartilage destruction in secondary osteoarthritis is decidedly different from that of idiopathic osteoarthritis. When the hip, shoulder, or any other joint is put through a range of motion, some retention of joint space is likely to be present in the different positions. This radiographic sign may help differentiate osteoarthritis resulting from osteonecrosis from idiopathic osteoarthritis (Fig. 16B). When arthroplasty is performed for severe osteoarthritis, the stability of the arthroplasty or the presence of loosening, subsidence, and failure are evaluated on sequential radiographs. ^{99m}Tc MDP bone scans are also useful for evaluating infection, and arthrography can delineate areas of abscess formation.

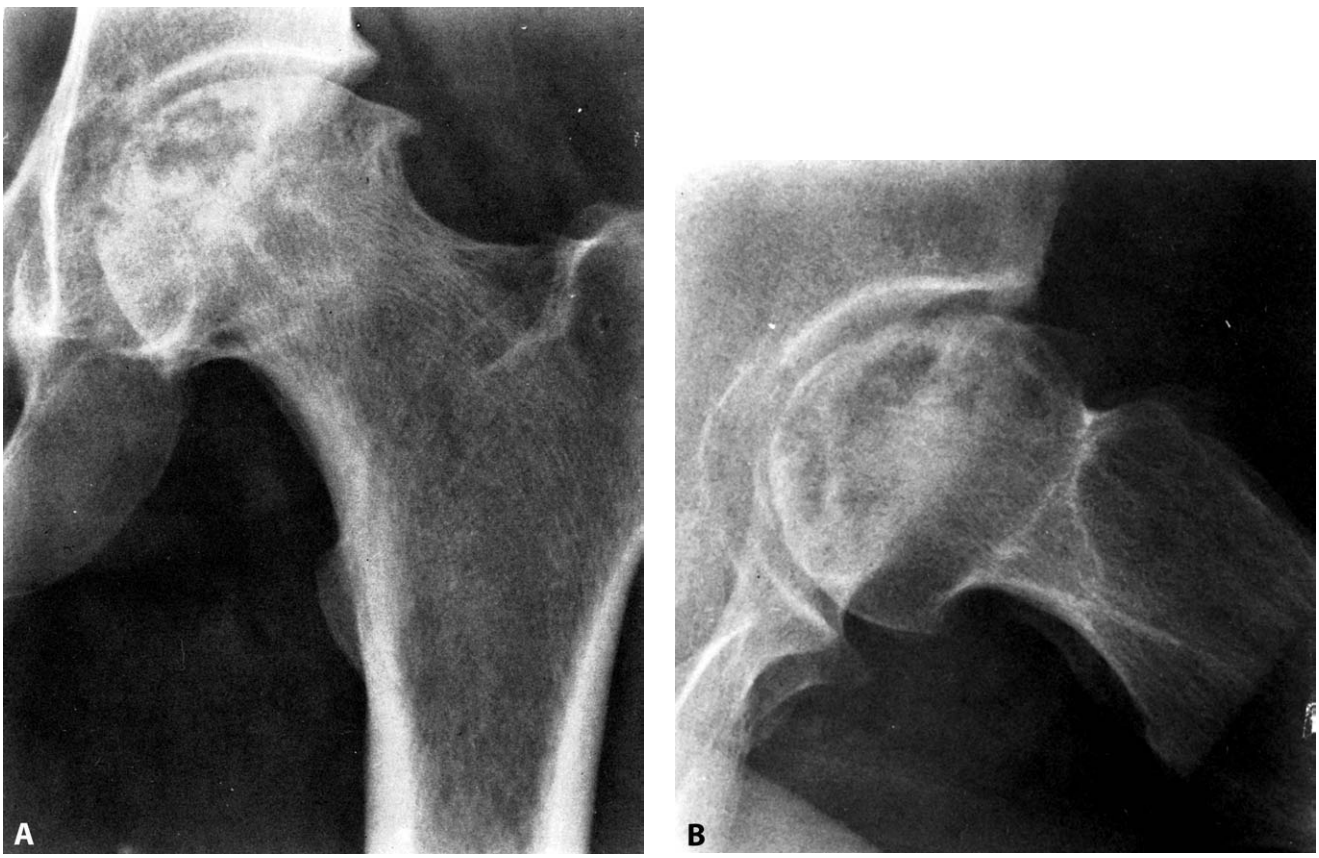


FIGURE 16 (A) Anterior–posterior radiograph shows secondary osteoarthritis complicating steroid-induced osteonecrosis in a 20-year-old woman with SLE. (B) The oblique radiograph of the hip shows retention of the joint space despite osteophytes. This would suggest persistence of articular cartilage.

Intravertebral Vacuum Cleft

The intravertebral vacuum cleft sign (Fig. 17), reported by Maldague *et al.* [56], represents a sign of osteonecrosis of the vertebral body. The radiographic gas shadow (cleft) within the vertebra associated with vertebral collapse is a rare but distinctive finding and aids in excluding a space-occupying lesion such as infection or tumor as the cause of the vertebral collapse. Often, these patients have been on corticosteroids. In one series, 7 of 10 patients with the vacuum cleft were receiving corticosteroid therapy.

Insufficiency Fractures

Patients with SLE who develop osteoporosis on long-term corticosteroid therapy are susceptible to insufficiency fractures that result from loads applied to weakened bones [57].

Radiographs may be negative for fracture in 50% of patients, but ^{99m}Tc MDP-delayed bone scans demonstrate increased uptake in areas of stress fracture. When bone scans are indeterminate, MRI can be useful for delineating the fracture as focal linear areas of high signal intensity on T2-weighted MR images, often associated with extensive high-signal-intensity edema in the adjacent bone marrow [57a].

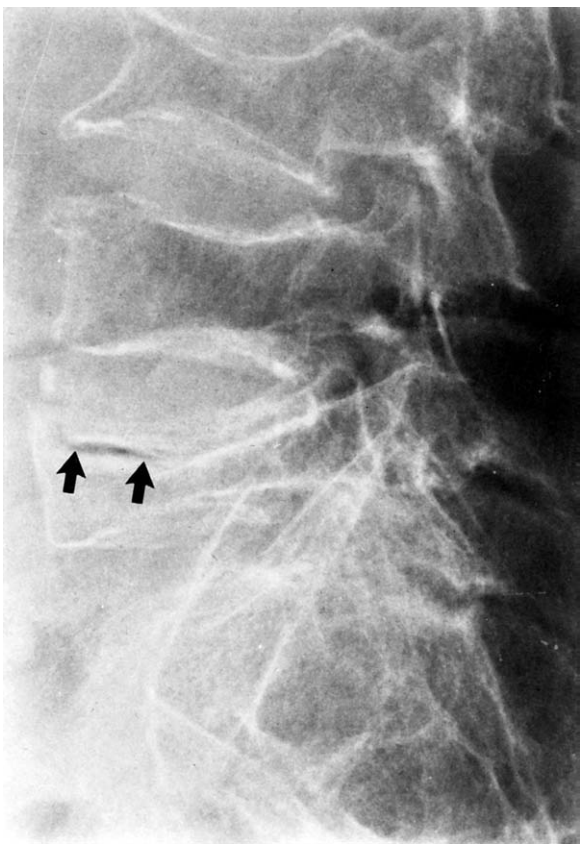


FIGURE 17 A rare finding of an intravertebral vacuum sign (*arrows*) in a lupus patient treated with corticosteroids.

Infections of Bones and Joints

Corticosteroid and immunosuppressive therapy predispose lupus patients to infection [58–60]. Investigators have shown that infection was the primary cause of death in 40% of patients with SLE [58]. When the daily dosage of prednisone is maintained above 40 mg/day, there is a five- to sevenfold increase in infection. The most common of sites of bacterial infection are the urinary tract, lungs, and skin. A small number of patients, however, develop infectious arthritis [58], osteomyelitis, or both. Septic arthritis can be monoarticular or polyarticular, and the onset is usually acute (within 24–72 hours). Suppurative arthritis associated with SLE can be a serious problem. One must be alert to the features of possible infection in order to establish the diagnosis, identify the organism, and institute appropriate treatment promptly. The knee is the most commonly affected joint, but other joints involved are the hip, ankle, and elbow, in descending order of frequency. *Neisseria* or gram-negative bacilli are often cultured from the infected joints of patients with SLE. In contrast, in rheumatoid arthritis, the characteristic organism is *Staphylococcus aureus* in 75% of patients [60]. Initially, radiographs may be normal or show periarticular osteoporosis without erosions. With progression, radiographs may demonstrate joint effusions and focal erosions at the periphery of the articular surface of the bone [58], or, with acute suppurative arthritis, joint destruction and pathologic subluxation [61, 62]. ^{99m}Tc MDP bone scans demonstrate increased radiopharmaceutical uptake of the involved joint. MRI shows the joint effusions that are sometimes loculated and thickened synovium (Fig. 18). High-signal-intensity edema may also develop in the adjacent bone. Power Doppler can detect the inflammatory tissue by showing increased blood flow [33c], and duplex sonography helps localize joint fluid for aspiration.

Suppurative osteomyelitis as an isolated complication of SLE is not as common as infectious arthritis. Hematogenous spread is the usual route of infection. In the past, ^{99m}Tc MDP scintigraphy was used extensively for detecting osteomyelitis. Currently, MRI, when available, is the preferred modality. MRI is as sensitive as bone scintigraphy in the early detection of osteomyelitis, but because of its superior spatial resolution, MRI is more specific for distinguishing osteomyelitis from arthritis, cellulitis, and soft tissue abscess [63, 63a]. On T2-weighted MR images, particularly on fast spin echo sequences, fatty marrow and osteomyelitis both appear hyperintense, and osteomyelitis may be obscured. With fat saturation (suppression), fatty marrow becomes low

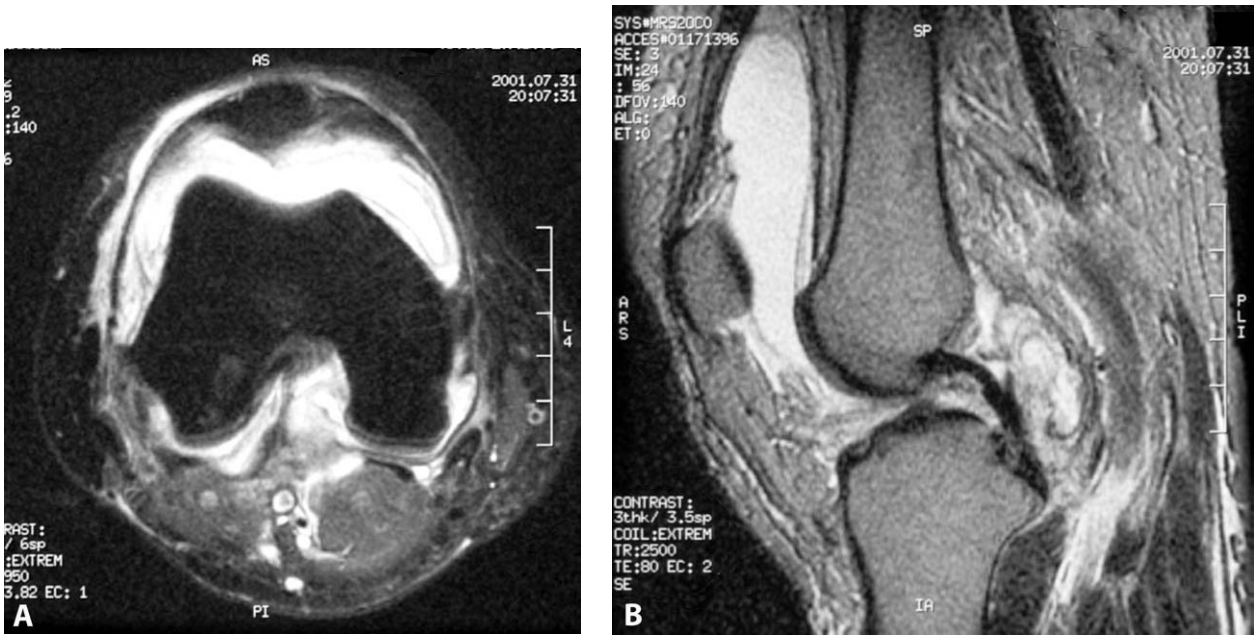


FIGURE 18 (A) Fast spin echo axial T2-weighted with fat saturation (3950/34) and (B) sagittal oblique T2-weighted MR images (2500/80) show a large effusion with thickened synovium and posterior loculation in this lupus patient with knee pain and infective arthritis. Culture from a biopsy grew out *Cocciodiomycosis immitis*.

signal intensity on the T2-weighted sequence. In contrast, osteomyelitis is hyperintense and conspicuous. The addition of contrast medium and fat saturation to the T1-weighted sequence also enhances the detection of osteomyelitis because intensely enhancing osteomyelitis contrasts with nonenhancing yellow marrow and mildly enhancing red marrow. Contrast-enhanced MRI also separates necrotic, gangrenous tissue that does not enhance from the cellulitis that does (Fig. 19). The identification of both necrosis and cellulitis assists the surgeon in selecting the areas for surgical debridement. Contrast-enhanced T1-weighted MR sequences may

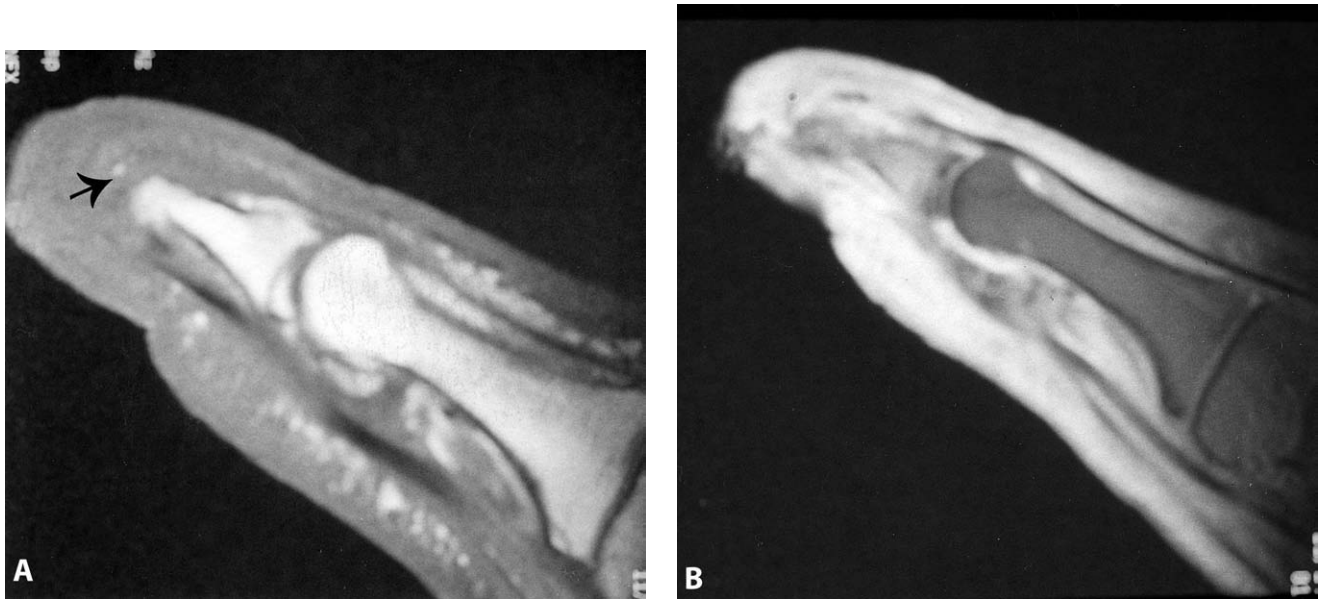


FIGURE 19 (A) Sagittal T1-weighted MR image (500/14) of the great toe shows extensive low signal intensity in the soft tissues and in the region of the poorly-defined distal phalanx (*arrow*) of a 53-year-old woman with SLE and a markedly swollen tender toe. (B) Sagittal contrast-enhanced T1-weighted with fat saturation MR image (566/14) shows the enhancing soft tissues, middle phalanx, and partially destroyed distal phalanx consistent with cellulitis and osteomyelitis.

also be helpful for differentiating the acute infarct with thin linear rim enhancement from osteomyelitis with more geographic and irregular marrow enhancement [49a]. Occasionally, radiographs can show findings of bone and soft tissue destruction in patients with severe vasculitis and gangrene of the fingers and toes and superimposed pyogenic infection (Fig. 20).

Osteomalacia

A rare complication of lupus treatment is the development of osteomalacia. One patient in our series had severe SLE with extensive vasculitis of the bowel and infarction of the intestines. A major resection of the diseased loops of bowel was performed to control her disease and symptoms. The sacrifice of a considerable length of intestine may result in malnutrition and severe osteomalacia.

Renal Osteodystrophy and Renal Failure

The lupus patient with renal osteodystrophy (with or without dialysis) may show multiple soft tissue and bone abnormalities, particularly soft tissue and vascular calcifications (Fig. 21), bone resorption, osteosclerosis, and osteoporosis at radiography [64]. Cortical thinning can be a manifestation of both secondary hyperparathyroidism and osteoporosis. In lupus patients with renal osteodystrophy and secondary hyperparathyroidism, ^{99m}Tc MDP bone scans may show the "super scan" or "metabolic bone scan" with diffuse increased radiopharmaceutical osseous uptake, although this is not as commonly seen as in primary hyperparathyroidism.

In lupus patients with renal failure, delayed ^{99m}Tc MDP whole body bone scans can demonstrate increased uptake in soft tissues as well as decreased renal parenchymal uptake because of decreased renal excretion of radiopharmaceutical (Fig. 22).

Myopathy

Eighteen of 228 lupus patients (8%) who were followed for at least a 6.5-year period manifested muscle disease. In another study, 50% of patients with SLE had muscle involvement [65]. The myopathy appeared to parallel the activity of the patient's disease. Most patients with lupus myositis experience myalgia, weakness, and tenderness in the muscle groups, especially around the shoulder and hip. In the pediatric age group, patients with SLE frequently have pain in the muscles of their extremities, which is almost as frequent as arthralgias. Local swelling in the muscle bellies is often circumscribed and transient.

The exact etiology of lupus myopathy is controversial. Other factors that may contribute to muscle symptomatology are referred pain from adjacent joints or a proximal myopathy developing as a complication of high-dose corticosteroid therapy. Isenberg and Snaith [65] studied the muscle biopsies specimens from 20 lupus patients with myopathy, 10 of whom had abnormal histology. They concluded that the findings were nonspecific.

MRI defines the extent of the myositis and the response to treatment. However, lupus patients who are on corticosteroids may also show atrophy with fatty infiltration of their muscles. On newer, fast spin echo T2-weighted MR images, both fatty atrophy of muscle and myositis produce linear areas of high signal intensity within the muscle. When fat suppression is used, fat in atrophic muscle will appear hypointense and the remaining hyperintense areas in muscle represent myositis (Fig. 23). Likewise, on contrast-enhanced, fat-suppressed T1-weighted MR images, areas of myositis will enhance intensely in contrast to normal or atrophic muscle, which shows only mild enhancement (Fig. 23). Pyomyositis [66] must always be excluded in SLE and cannot be differentiated from noninfected, inflammatory myositis unless associated abscesses are identified. Sonography or CT provides guidance for abscess localization and drainage.

IMAGING OF DERMATOLOGIC SLE

Soft tissue calcification, although more common in dermatomyositis and scleroderma, is occasionally seen in SLE [20, 21, 67–76]. Budin and Feldman [75] described the four different patterns of calcifications in SLE at radiography as diffuse, linear, streaky, and nodular. They emphasized that soft tissue calcifications may be present in the skin and subcutaneous tissues and in the peripheral vasculature in lupus patients without renal osteodystrophy (Fig. 24). Calcifications are usually localized (the circumscripta type) but may be generalized (universalis type). Shapeero and Miller [20] found only 1 of 141 patients with the generalized form, with subcutaneous calcifications of the chest, abdomen, and extremities (Fig. 25). Different factors have been proposed to explain soft tissue calcification in SLE, including infection, vasculitis, and autoimmune response at the cell membrane with resultant cell necrosis and dystrophic calcification [75]. Soft tissue calcification in the lupus patient may also be a manifestation of renal osteodystrophy as discussed in that section (Fig. 21).

Increased uptake of ^{99m}Tc hydroxydiphosphonate in extraosseous sites has also been reported in SLE

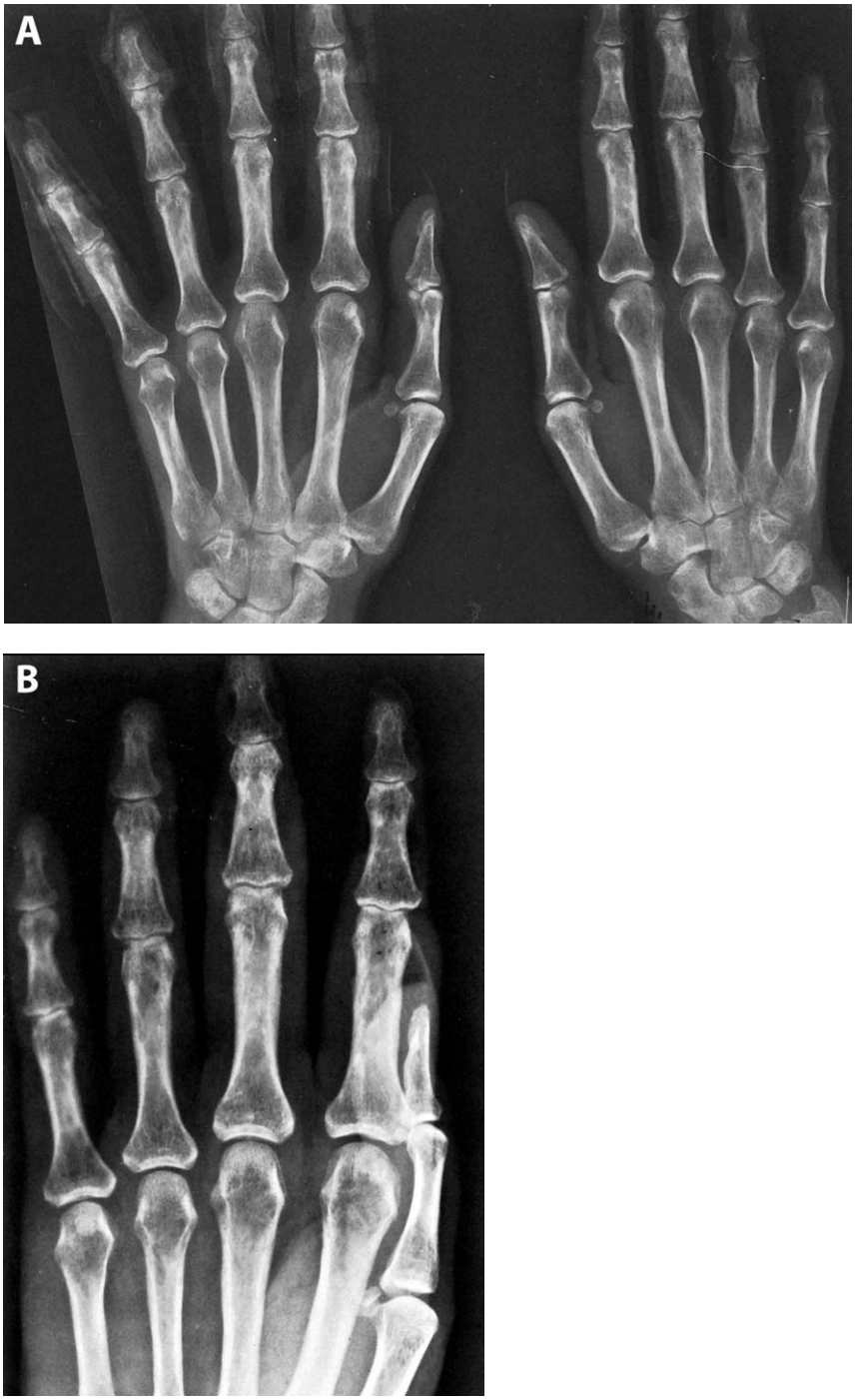


FIGURE 20 This 59-year-old woman had a 30-year history of SLE. She developed severe vasculitis complicated by gangrene of the digits of both hands. (A) The status at onset. (B) Loss of soft tissue at the peripheral parts of the digits.

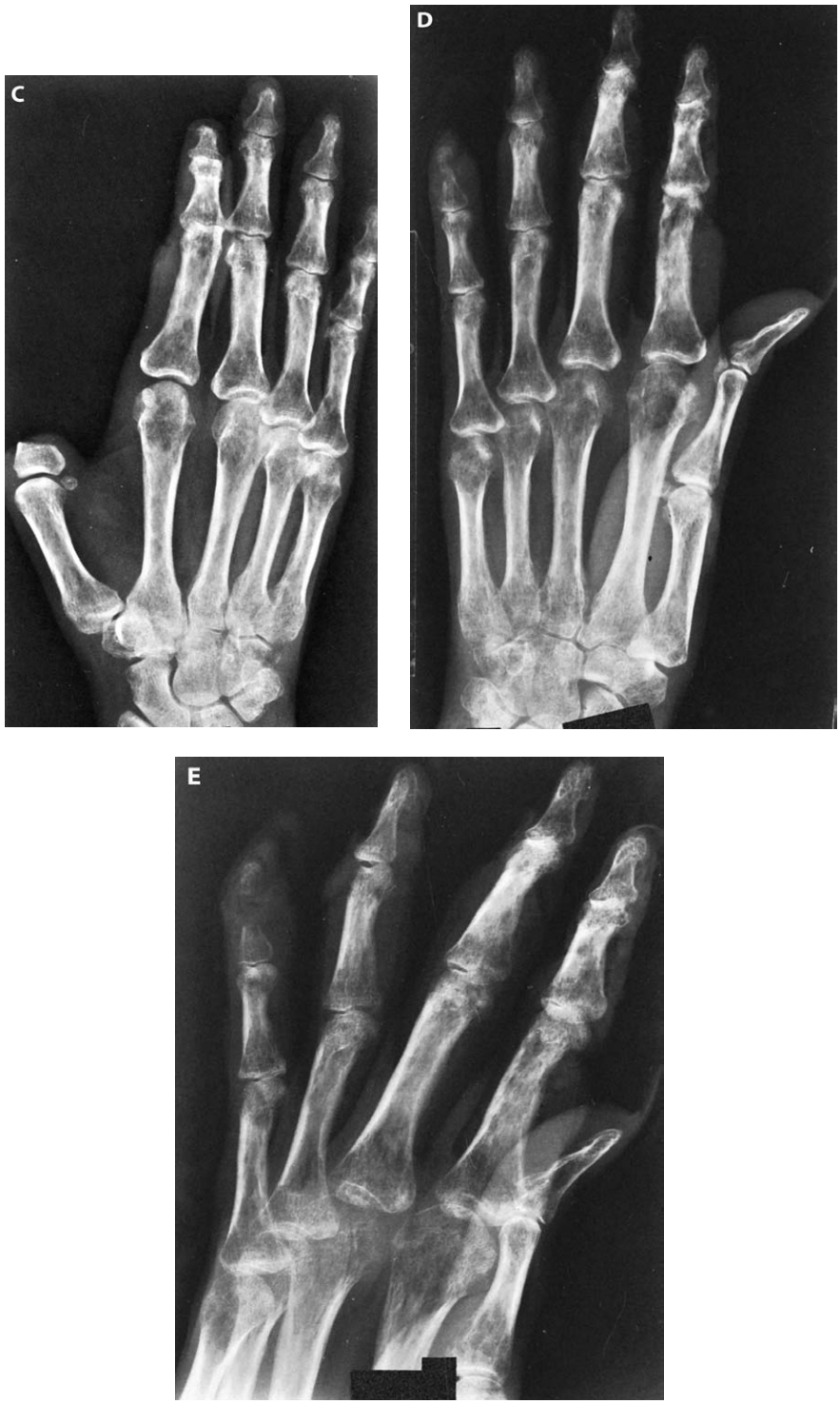


FIGURE 20 (*Continued*) (C) Severe gangrene and slough of the soft tissue of the index and middle fingers. The right thumb was amputated because of gangrene. (D) One year later, an osteomyelitis destroyed the terminal phalanx of the little finger. (E) Two years, later the osteomyelitis has progressed to involve the proximal phalanx of the index finger, with more extensive involvement of the infection into the little digit and more extensive gangrene.

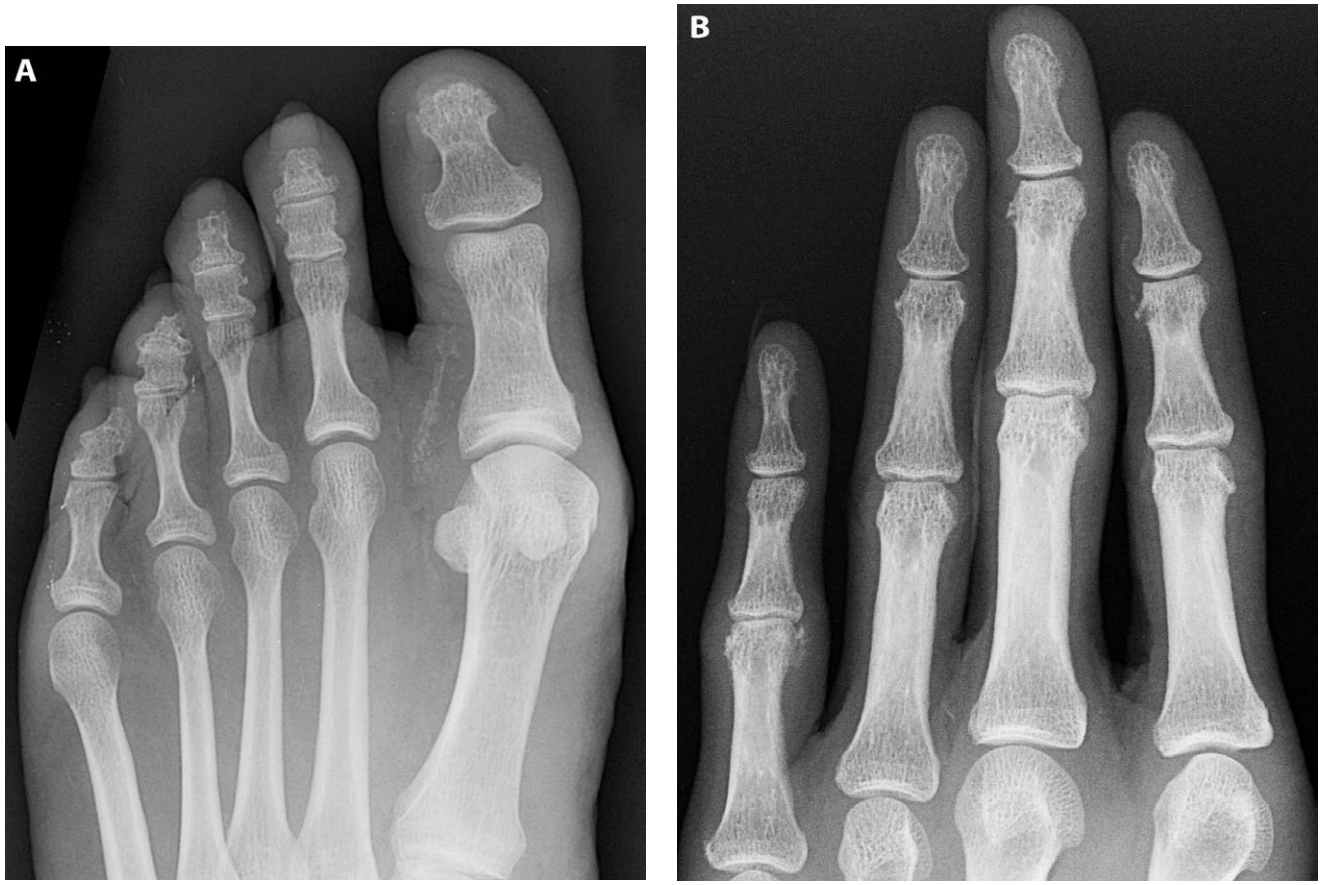


FIGURE 21 Anterposterior radiographs of the (A) foot and (B) hand show linear vascular and focal juxta-articular calcifications in the soft tissues and subperiosteal absorption of the radial aspect of the proximal phalanges in a 37-year-old dialysis patient with SLE and with renal osteodystrophy.

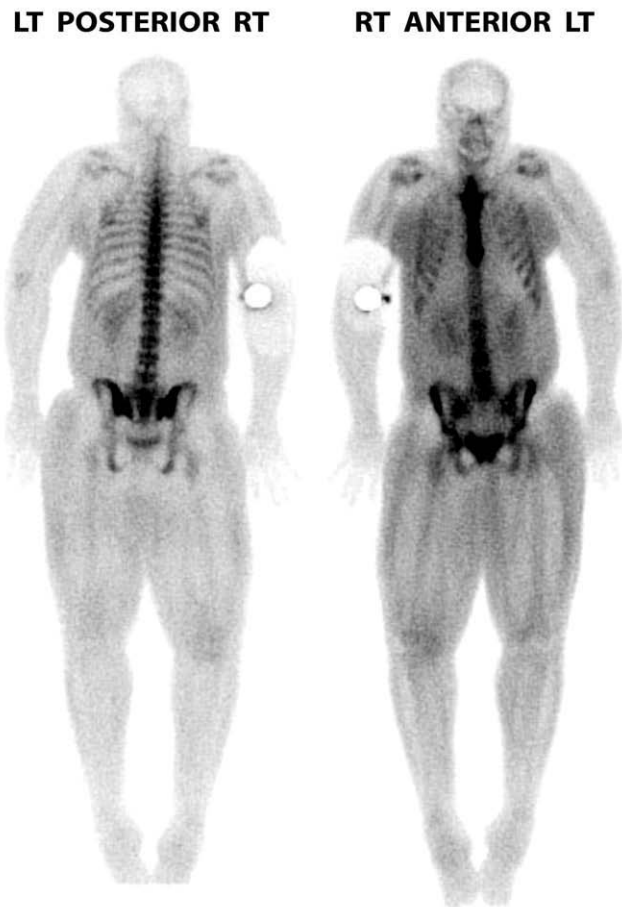


FIGURE 22 Whole body delayed anterior and posterior planar scintigraphic images 3 h after injection of 99m-Tc HDP show diffuse soft tissue uptake with bilateral decreased renal parenchymal uptake consistent with renal failure in this patient with lupus nephritis.

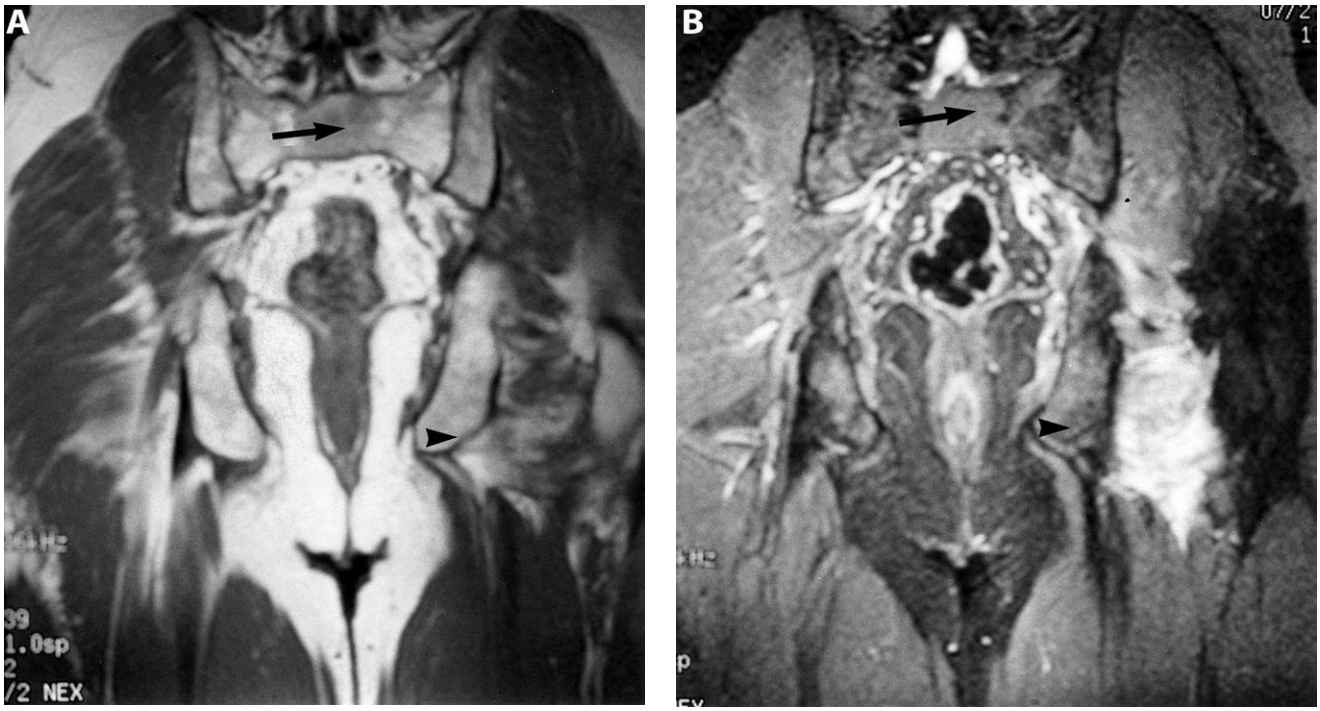


FIGURE 23 (A) Coronal T1-weighted MR image of the pelvis shows heterogeneous signal intensity of the left thigh muscles (obturator internus, obturator externus, gemelli, and adductor magnus) that could result from fatty atrophy or inflammation/hemorrhage in a patient with SLE. (B) Coronal short tau inversion recovery MR image (fat is low signal intensity) shows diffuse high signal intensity in this region consistent with myositis (*arrow*, sacrum; *arrowhead*, ischium).

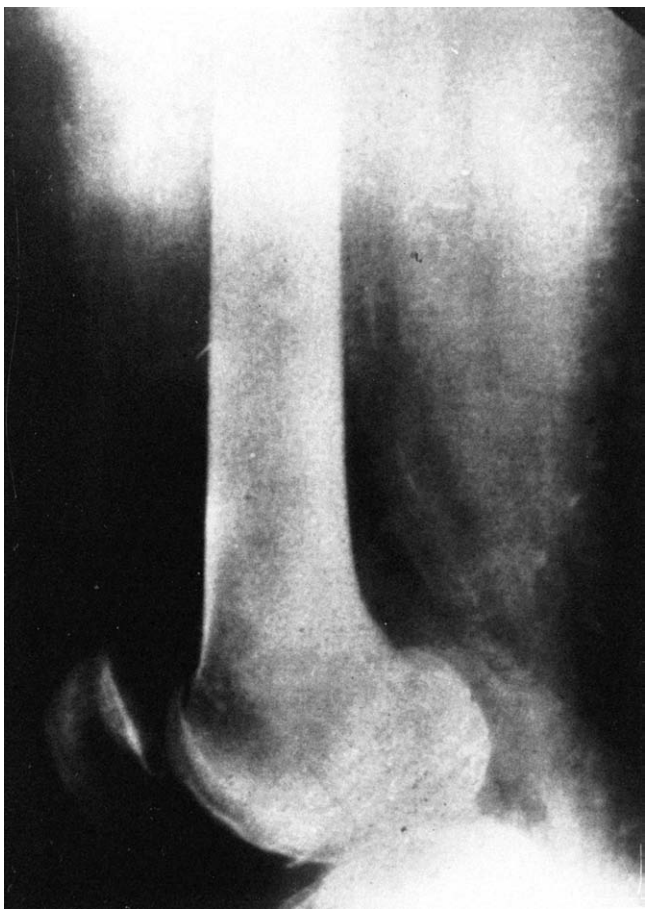


FIGURE 24 Lateral radiograph of left knee shows calcific plaques and heavily calcified femoral and popliteal arteries in a 19-year-old woman with SLE without renal osteodystrophy From Budin and Feldman [75], with permission.

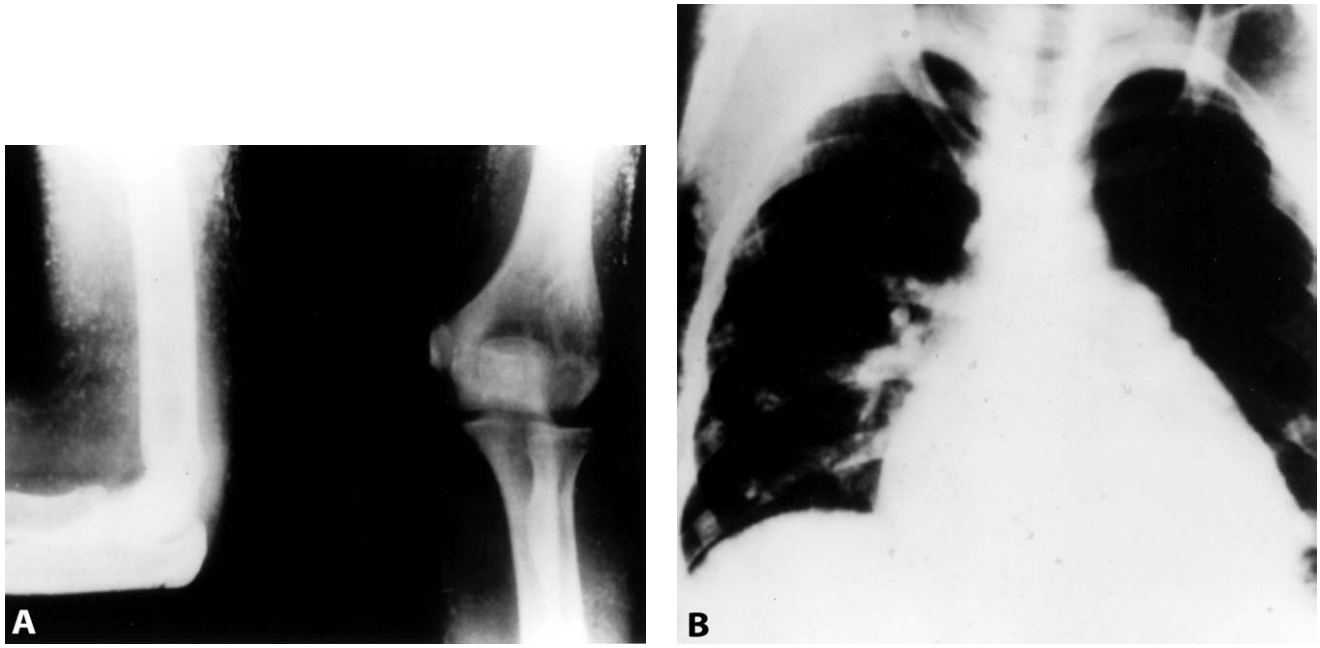


FIGURE 25 (A) Anteroposterior and lateral radiographs of a right elbow show multiple linear and nodular calcifications in the superficial and deep soft tissues. (B) Posteroanterior chest radiograph shows diffuse calcification of the soft tissues of the chest in an 18-year-old woman with SLE with calcinosis universalis and cutis. From Shapeero and Miller [20], with permission.

even though no abnormal soft tissue calcifications were seen on the radiographs. The basic mechanism for nonosseous uptake of radiopharmaceuticals is not known. Various mechanisms have been suggested: absorption to soft tissue calcium and immobile collagen, binding of the hormone or enzyme receptors, and tagging to denatured protein [76].

As discussed in the section on renal failure and musculoskeletal abnormalitites, diffuse increased radiopharmaceutical uptake in the soft tissues on delayed ^{99m}Tc MDP bone scans can occur in lupus patients with a decreased renal excretion of radiopharmaceutical (Fig. 22).

IMAGING OF PLEUROPULMONARY SLE

Pleural Effusions

Pleural involvement is the most common radiographic manifestation of SLE. Pleural effusions (Fig. 26) are often small, transient, recurrent, and bilateral [77–80] but may be massive and refractory to treatment [81]. Pleural effusions may also develop in the lupus patient with pulmonary infarcts, pulmonary infection, or congestive heart failure secondary to myocarditis, hypertension, or uremia. Lupus pleural effusions have normal glucose concentration in contrast to the low glucose level in infectious or rheumatoid pleural effusions. The majority of effusions reported in the literature are exudates; LE cells may be present [82, 83]. The posterior–anterior, lateral, and lateral decubitus radiographs identify the pleural effusion, and sonography may be useful for guiding thoracentesis.

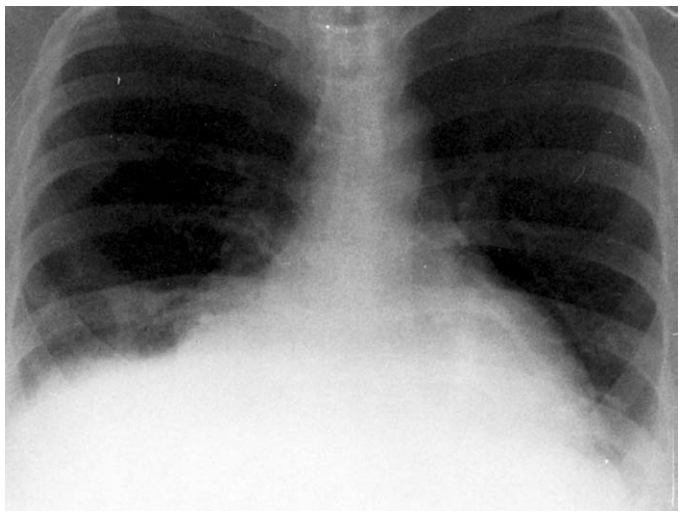


FIGURE 26 Posteroanterior chest radiograph shows bilateral pleural effusions in a 19-year-old woman with lupus pleuritis.

Lupus Pneumonitis

Radiographically, acute lupus pneumonitis usually appears as unilateral or bilateral basilar opacities (Fig. 27) that do not change with antibiotics but respond to corticosteroid or immunosuppressive therapy. Patients usually have high fevers, tachypnea, tachycardia, and cyanosis, but few physical findings [84–86]. Sputum cultures show no pathologic growth. The combined clinical and radiographic findings suggest the diagnosis of acute lupus pneumonitis. Histologic examination shows hyaline membranes, alveolitis with focal necrosis and ateletectasis, interstitial edema, interstitial lymphocytic infiltrate, bronchiolitis, and capillary and arteriolar thrombi [87–91]. Electron microscopy occasionally identifies microtubular structures within the cytoplasm of the pulmonary capillary endothelium similar to those seen in the kidney [92, 93]. This acute process, which occurs in 0.9–11.7% of lupus patients [86, 87], may resolve completely or regress to a more chronic interstitial pneumonitis and fibrosis.

Sudden development of bilateral infiltrates is not uncommon in SLE and may also result from hemorrhage, congestive heart failure, and uremia [94]. In addition, cytotoxic drug therapy, such as cytosine arabinoside, may produce a diffuse alveolar, interstitial, or a mixed pattern without signs of congestive heart failure (Fig. 28) [95]. After cessation of the drug, the alveolar consolidation regresses rapidly within 3–7 days whereas the interstitial pattern disappears more gradually [66].

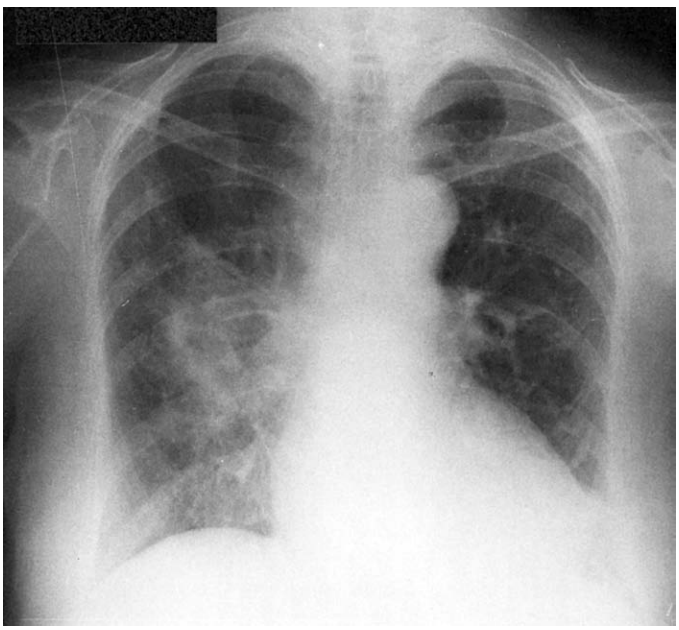


FIGURE 27 Posteroanterior chest radiograph demonstrates bilateral lower lobe lupus pneumonitis in a 43-year-old woman with tachycardia, high fevers, and negative sputum.

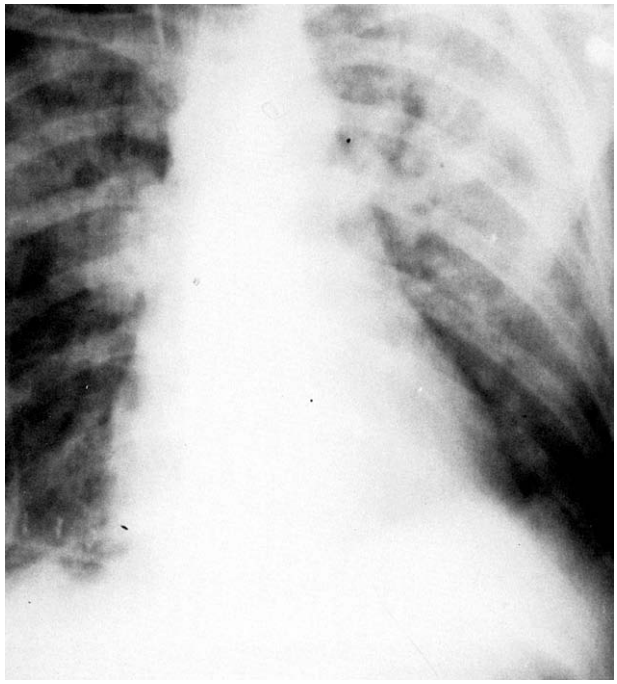


FIGURE 28 Anteroposterior portable chest radiograph shows a bilateral interstitial-alveolar pattern in a 27-year-old lupus patient with cyclophosphamide toxicity. The chest radiograph returned to normal with cessation of cyclophosphamide therapy.

However, in lupus patients, especially those on corticosteroid therapy, alveolar or interstitial patterns and cavitary nodules on radiographs most frequently represent infection, particularly secondary to opportunistic organisms, such as gram-negative organisms, fungi, cytomegalic inclusion disease, *Pneumocystis carinii*, and *Mycobacterium tuberculosis* [58, 77, 96–99]. Although the radiograph can demonstrate alveolar or interstitial pneumonia (Fig. 29A), CT may be needed to define cavitation (Fig. 29B). Contrast-enhanced CT and MRI, particularly with its triplanar capabilites, can delineate the extension of the abscesses into the soft tissues outside the thoracic cavity (musculature, neck including brachial plexus).

In SLE, other nonspecific pulmonary findings (fleeting, migratory, recurrent densities, honeycombing reticulation, parenchymal or subpleural cysts, and basilar linear shadows) develop in one-third to two-thirds of patients [58, 79, 80, 85, 86, 100, 100a]. Various authors have called these findings *diffuse interstitial lung fibrosis*, *fibrosing alveolitis*, and *interstitial pneumonitis* [58, 77, 101–104]. Some investigators have found 67-gallium scintigraphy useful for diagnosing active interstitial inflammation and for monitoring its response to treat-

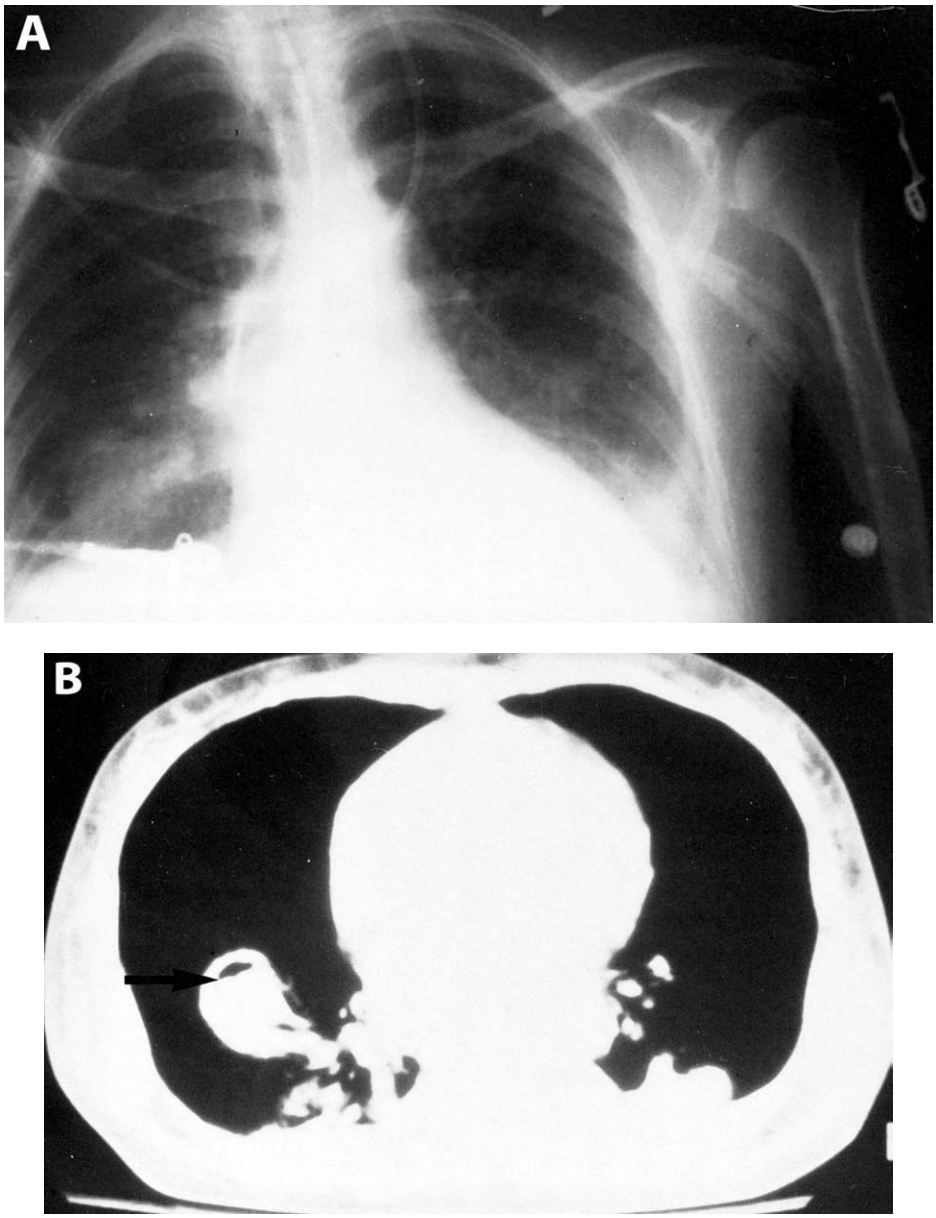


FIGURE 29 (A) Posteroanterior chest radiograph shows consolidation in both lower lobes. (B) Axial CT scan done on the same day as (A) shows a cavity in the right lower lobe. Brush biopsy showed *Aspergillus fumigatus*.

ment [105, 105a]. In fibrosing alveolitis, rupture of a bleb or cyst may lead to pneumothoraces and mediastinal emphysema [79, 106–109]. Radiographs sometimes may not identify the blebs, whereas they are easily defined at CT [109]. Hemopneumothorax has also been reported in SLE [110]. Both primary factors (alveolar hemorrhagic lesions, focal subpleural petechiae, pleuritis, mucopurulent mucous plugs with atelectasis, and ball valve effect) and secondary factors (pulmonary emboli, bacterial infection, and bleeding diathesis) may predispose to hemopneumothorax [110].

Mixed connective tissue disease may present with similar findings as SLE with basilar interstitial changes, pneumonitis, pleural effusions, and pleural thickening [111].

In addition to other pleuropulmonary findings in SLE, sequential chest radiographs may show progressive volume loss, the "shrinking lung," associated with high diaphragms and restrictive pulmonary function (Fig. 30) [112–117]. Some lupus patients with elevated diaphragms have normal diaphragmatic strength [117]; others have found weakened diaphragmatic muscle

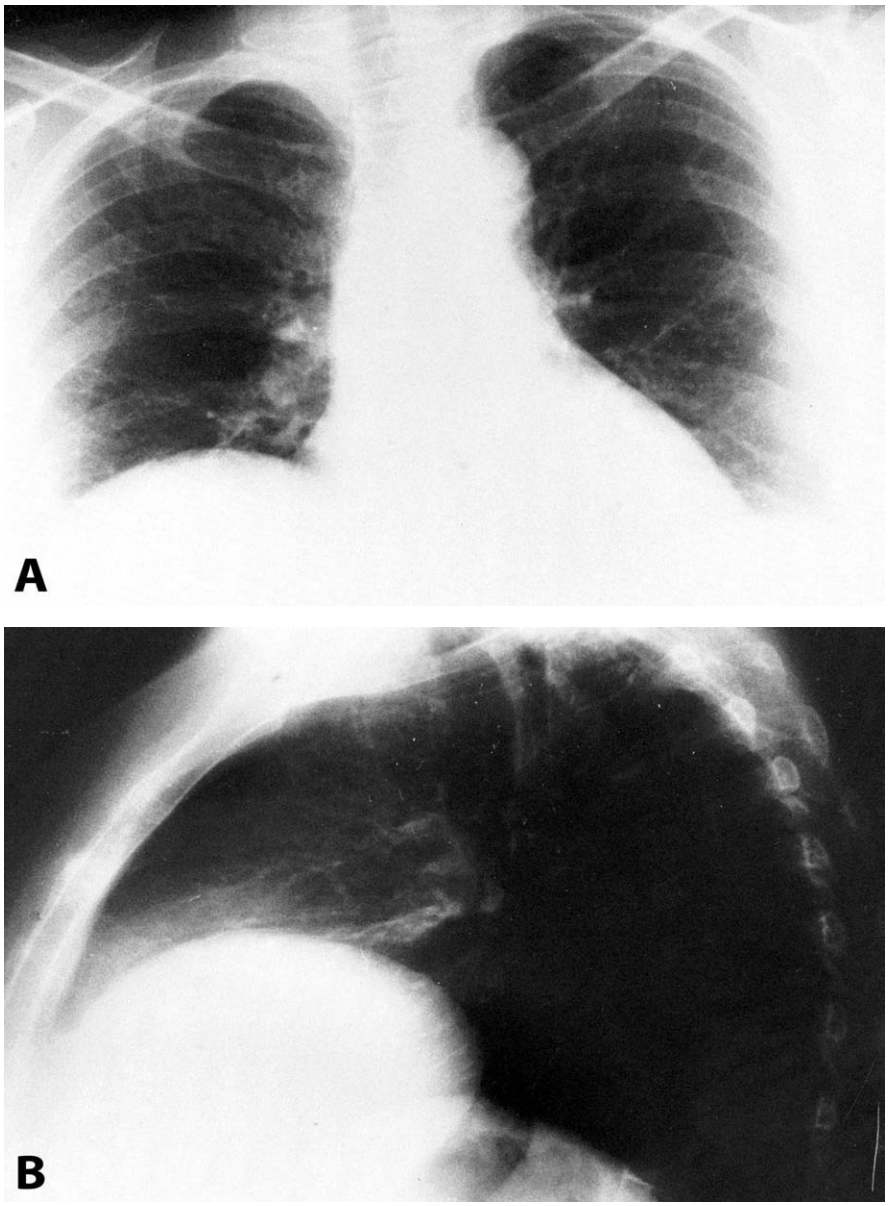


FIGURE 30 (A) Posteroanterior and (B) lateral chest radiographs show an elevated right diaphragm in a lupus patient with unilateral shrinking lung. Courtesy of Dr. M.-F. Kahn, University of Paris.

along with a restrictive pulmonary function pattern [92, 113]. In two patients, beta-agonists improved diaphragmatic performance [87, 114a]. Even when chest radiographs are normal, lupus patients may experience considerable impairment of pulmonary function, such as a restrictive respiratory pattern and low diffusing capacity [77, 118].

Thin-section, high-resolution CT is an important adjunct for the early assessment of pleuropulmonary disease in SLE. Several studies have shown that this technique identified lung disease in patients with SLE who had no clinical or radiographic evidence of lung involvement [119, 119a, 120]. Pulmonary abnormalities were identified in 38 to 72% of patients at high-resolution CT as compared with 6 to 34% at radiography, respectively. In one study [119], the interstitial changes correlated with decreased pulmonary function: decreased single-breath diffusing capacity for carbon monoxide (DLCO) ($r = 0.77$) and ratio of forced expiratory volume in 1 second to forced vital capacity (FEV^1/FVC) ($r = 0.8$) but did not correlate with the residual volume/total lung capacity (RV/TLC) ratio. A second study [120] found abnormal pulmonary function tests in only 41% of patients with abnormal high-resolution-CT findings. In a third series [120], a trend toward significance was found between disease activity

and interstitial lung disease on high-resolution CT, although no statistical significance was found between abnormal pulmonary function tests and interstitial lung disease. In these three studies, high-resolution CT demonstrated various findings of interstitial lung disease with "ground glass" appearance and interlobular septal and intralobular interstitial thickening associated with architectural distortion and bronchial wall thickening and dilatation. The "ground glass" appearance reflected two processes: (1) acute alveolitis and (2) fine reticular fibrosis, which appeared as an amorphous increase in lung attenuation because it was below the limits of CT resolution. In the nonlupus patient, certain interstitial findings at high-resolution CT appear to correlate with histology at open lung biopsy and may help predict response to therapy and patient outcome [120, 121]. The use of high-resolution CT as a prognostic indicator has yet to be proved in SLE.

In addition to its role for diagnosing and evaluating extent of disease, high-resolution CT is effective in guiding procedures such as lung biopsy and bronchoalveolar lavage to areas of specific SLE involvement.

Pulmonary Infarcts

Pulmonary infarcts in SLE result from thromboembolism [122, 122a], *in situ* vasculitis, or circulating anticoagulants and antiphospholipid antibodies [123, 123a, 124] and rarely are the presenting manifestation of SLE [124a]. Scintigraphy shows abnormal ventilation–perfusion defects discordant with ventilation defects, when present (Fig. 31). Radiographs may be normal or may show pleural effusions, atelectases (Fig. 32), or, infrequently, enlarged central pulmonary arteries in chronic embolism. In one case report [99], cavitary nodules proved to be necrotic pulmonary infarction at autopsy. It should be emphasized that because infection is common, cavitary pulmonary vasculitis should be a diagnosis of exclusion and a thorough investigation, including biopsy, should be performed before a final diagnosis of vasculitis is made [99]. Helical CT allows rapid evaluation of the pulmonary circulation with breath holding and gives improved demonstration of thrombi in pulmonary arteries [122a, 123a].

The imaging features of pulmonary "vasculitis" are best defined at high-resolution, helical CT but are not specific for SLE and may be also seen in other vasculitidies, such as Wegener's granulomatosis and scleroderma–polymyositis overlap syndrome. The inflamed pulmonary vessels produce a perivascular inflammatory infiltrate with hemorrhage and necrosis that appears as centrilobular, hazy densities at CT.

Pulmonary Hemorrhage

Pulmonary hemorrhage is an unusual and often fatal complication of SLE. Patients present with acute symptoms of hemoptysis, respiratory failure, tachycardia,

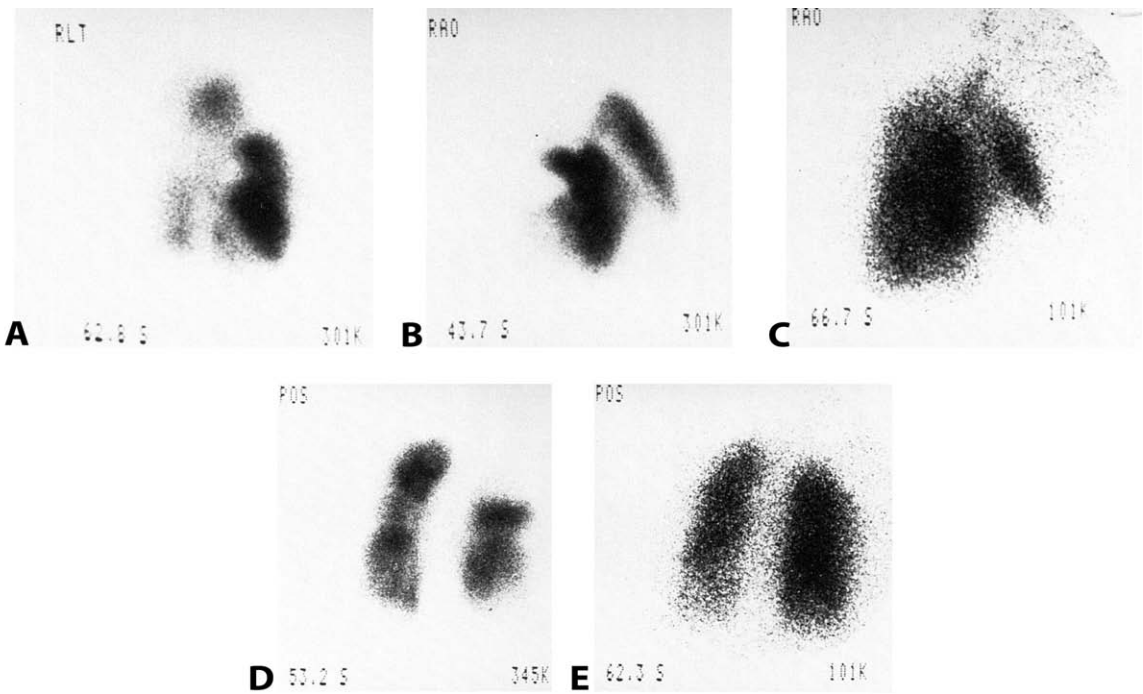


FIGURE 31 Lung scintigrams in a lupus patient with thromboembolism shows mismatch in perfusion (A, B, D) and ventilation (C, E) defects.

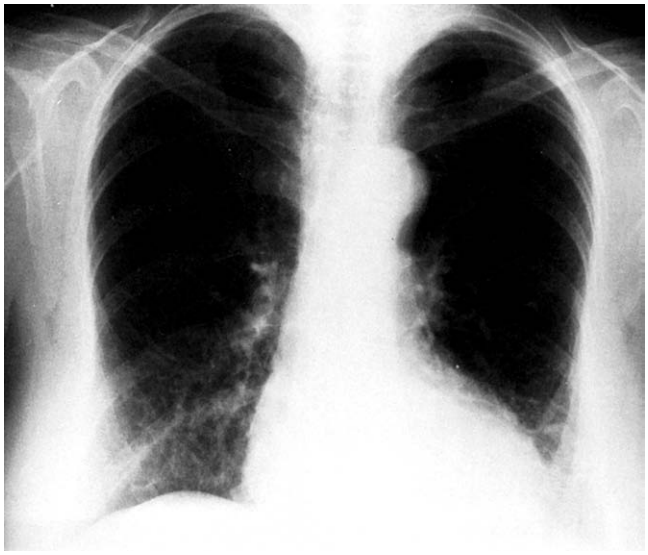


FIGURE 32 Posteroanterior chest radiograph shows subsegmental atelectases in the lower lobes in a patient with SLE and pulmonary emboli.

and high fevers and may survive for 2 hours to 3 years after the first hemorrhage [124–134]. In children, pulmonary hemorrhage may precede SLE and be indistinguishable from pulmonary hemosiderosis. Diffuse parenchymal abnormalities, especially with unexplained blood loss or signs and symptoms of connective disease in a child, should alert the clinician to consider this diagnosis [135].

When hemoptysis is absent, iron-laden macrophages on transbronchial biopsy may suggest the appropriate diagnosis [129]. Occult pulmonary hemorrhage can also be measured by inhaled carbon monoxide (CO) uptake $C^{15}O$ clearance studies, although this is performed infrequently. By this technique, extravascular blood takes up additional inhaled $C^{15}O$ so that the $C^{15}O$ uptake is elevated. In addition, when $C^{15}O$ is inhaled, extravascular blood labeled with the $C^{15}O$ is stagnant and slows radiopharmaceutical clearance. Thus pulmonary hemorrhage is characterized by increased $C^{15}O$ uptake and delayed $C^{15}O$ clearance [136].

The radiographic features of pulmonary hemorrhage are predominantly extensive, bilateral, homogeneous densities (the "ground glass" appearance) (Fig. 33) [127, 129] and nodular, acinar infiltrates [127, 132]. Ventilation-perfusion radionuclide scans are normal [132]. CT defines the character and distribution of pulmonary hemorrhage and efficacy of therapy more clearly than conventional radiography [137]. With effective corticosteroid, immunosuppressive, and sometimes plasmapharetic therapy, patients may improve with resolution of imaging findings. Despite therapy, patients with pulmonary hemorrhage may show rapid deterioration with demise.

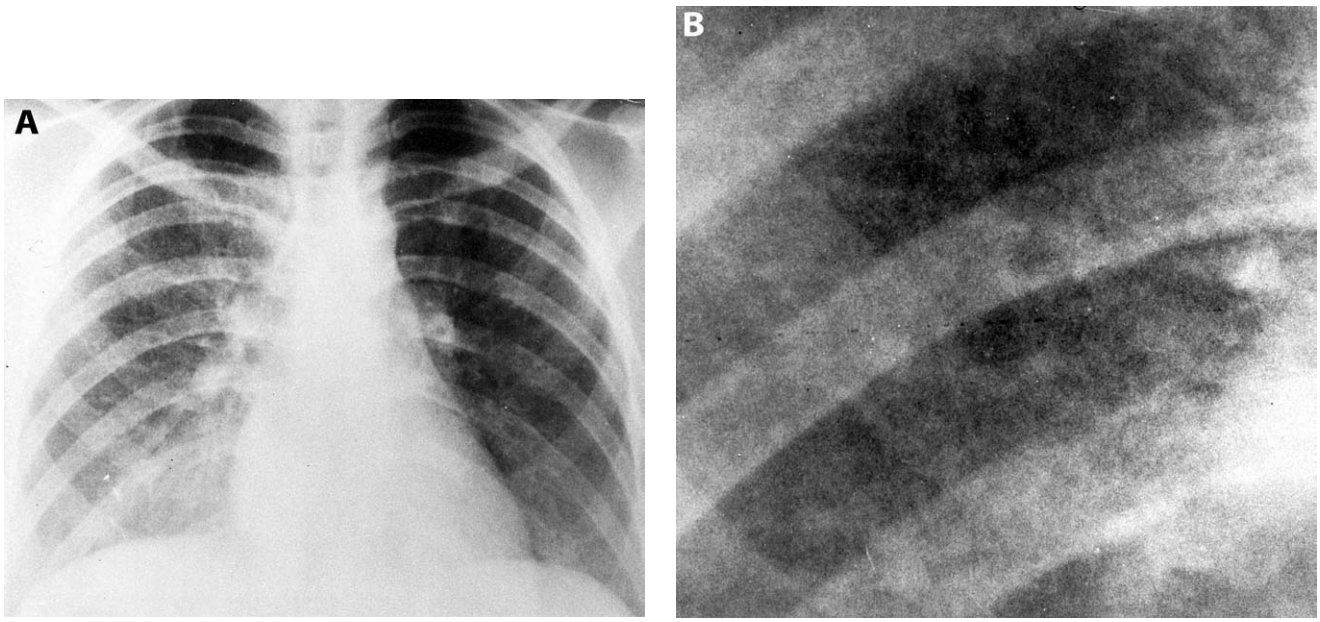


FIGURE 33 (A) Posteroanterior chest radiograph shows diffuse, bilateral increased lung density partially obscuring vessels with absence of air bronchograms. (B) Magnification of the right midlung: the typical ground-glass pattern sometimes seen in pulmonary hemorrhage partially obscures the pulmonary vessels in a 22-year-old patient with SLE. From Gamsu and Webb [129], with permission.

Pulmonary hemorrhage probably results from several mechanisms. An immune complex-mediated etiology has been suggested because electron microscopy identifies granular electron deposits (characteristic of immune complex aggregates) in the alveolar and vascular walls [77, 92, 127, 128, 131, 132]. Brentjzens *et al*. [138] also demonstrated similar deposits in experimental animal models of serum sickness-induced pulmonary disease. Other factors contributing to pulmonary hemorrhage are bleeding diathesis, intercurrent infection, hemorrhagic shock, and pulmonary embolism and infarction that are sometimes associated with lupus anticoagulants [92, 124, 127, 139, 140].

Pulmonary hemosiderosis with hemosiderin-laden macrophages has also been described in patients with SLE [141], but chest radiographs show no abnormalities.

Pulmonary Hypertension

Pulmonary hypertension may have an insidious onset, masquerade as pleuritis, pneumonitis, or recurrent emboli, and may be the terminal feature of lupus. Previously, pulmonary hypertension was thought to be an unusual manifestation of lupus, perhaps because prior techniques did not allow adequate diagnosis [142–153]. However, with current Doppler echocardiographic equipment, Simonson *et al.* [154] showed that pulmonary hypertension is common in SLE, progresses slowly over time, and is related to an increase in pulmonary resistance as evidenced by the change in prevalence from initial and final evaluations during a 5-year follow-up. On the initial echocardiographic studies, the prevalence of pulmonary hypertension was 14% and was clinically unsuspected in 80% of their patients. In 5-year follow-up studies, the prevalence had increased to 43% [155]. Doppler echocardiographic measurements of tricuspid insufficiency with saline contrast enhancement, when needed, were used to calculate pulmonary artery systolic pressure, Doppler echocardiographic calculations of cardiac output were done at rest for each patient, and pulmonary resistance was determined by dividing the pulmonary artery systolic pressure by the cardiac output. A significant increase in mean systolic pulmonary artery pressure was found in lupus patients at 5-year follow-up. Because pulmonary resistance increased between the initial and follow-up echocardiographic studies, it was suggested that the causal mechanism for pulmonary hypertension was increased pulmonary vascular resistance. Another Doppler echocardiographic study [156] evaluated the pulmonary artery pressure response to exercise in patients with SLE. Exercise duration was significantly reduced in lupus patients to 8.1 minutes in lupus patients in contrast to 14.4 minutes in control patients. Pulmonary artery pressure was significantly greater in lupus patients at rest and for each stage of exercise as compared with controls and may be a contributory factor to decreased exercise tolerance. Similar cardiac indexes at rest in normal and lupus patients further support the hypothesis that the mechanism for pulmonary hypertension is an increase in pulmonary vascular resistance.

Raynaud's phenomenon is more frequent in lupus patients with elevated pulmonary pressure (75%) than those with normal pressures (40%), although this is not of statistical significance. Pulmonary hypertension is also associated with Raynaud's phenomenon in patients with scleroderma [157] and MCTD [158].

In more severe pulmonary hypertension, echocardiography may show enlargement of the right atrium and right ventricle, midsystolic closure of the pulmonic valve, mild mitral and tricuspid valve prolapse, and paradoxical septal motion as a manifestation [143, 144] (personal communication, Nelson Schiller, University of California at San Francisco) (Fig. 34). Chest radiographs are frequently normal but may show cardiomegaly with right atrial and right ventricular hypertrophy and central pulmonary arterial enlargement (Fig. 35). An unusual cause of severe pulmonary hypertension is thrombosis of the main pulmonary arteries that can be detected at high-resolution helical CT [123a].

Although the pathogenesis of increased pulmonary vascular resistance is not clear, different mechanisms have been proposed: (1) loss of pulmonary vascular reserve secondary to interstitial lung disease, (2) narrowing of pulmonary vasculature resulting from pulmonary vasculitis, *in situ* thrombosis of pulmonary arterioles, or pulmonary emboli, (3) generalized vasoconstriction of pulmonary arterioles, (4) thrombosis of pulmonary arterioles because of a hypercoaguable state associated with antiphospholipid antibodies, (5) vasoreactivity of pulmonary arterioles due to antiphospholipid antibodies that cause decreased endothelial protacyclin formation, and (6) vasospasm of pulmonary arteries associated with the peripheral arterial vasospasm of Raynaud's phenomenon [123a, 144–154].

Hilar Adenopathy

Hilar lymphadenopathy [58, 159, 159a] is a rare manifestation of SLE and has been associated with mediastinal adenopathy on chest radiographs but is best detected at CT and MRI. Mediastinoscopy confirms the enlarged lymph nodes, and histologic examination shows areas of necrosis and intracellular and extracellular hematoxylin bodies compatible with the diagnosis of SLE [58]. Hilar lymphadenopathy with or without pulmonary nodules has also been

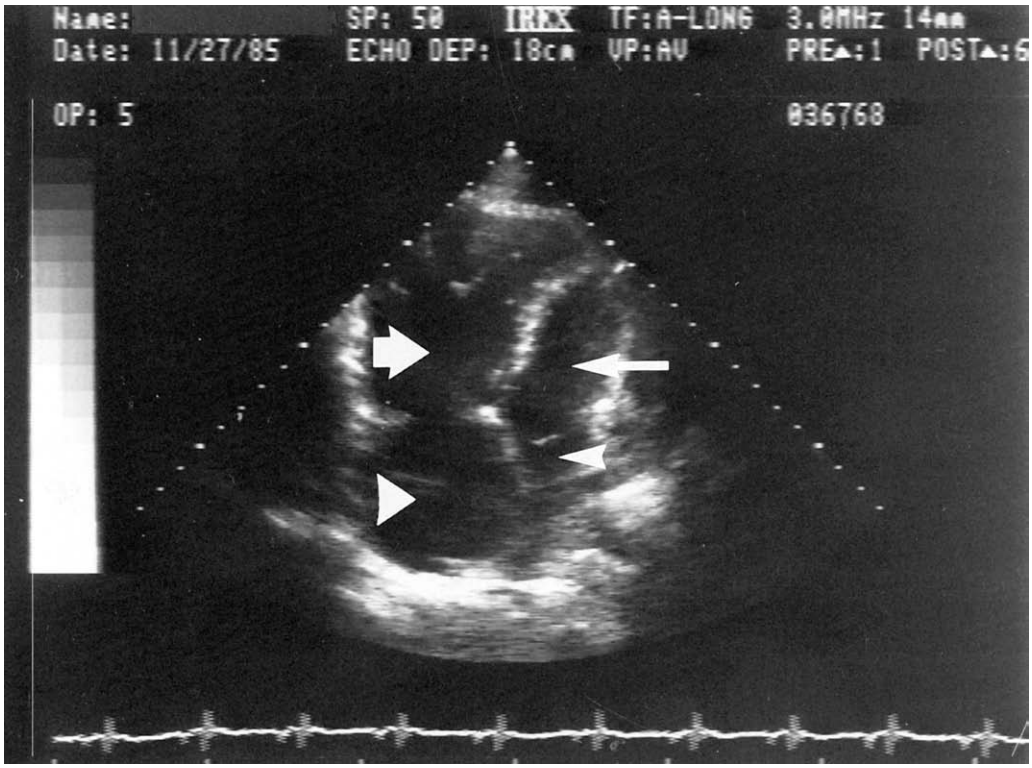


FIGURE 34 Apical four-chamber echocardiogram of a patient with long-standing SLE and pulmonary hypertension. The right ventricle (*large arrow*) and right atrium (*large arrowhead*) are considerably larger than the left ventricle (*thin arrow*) and left atrium (*thin arrowhead*) with bulging of the interatrial septum from right to left. Courtesy of Dr. N. B. Schiller, University of California, San Francisco.

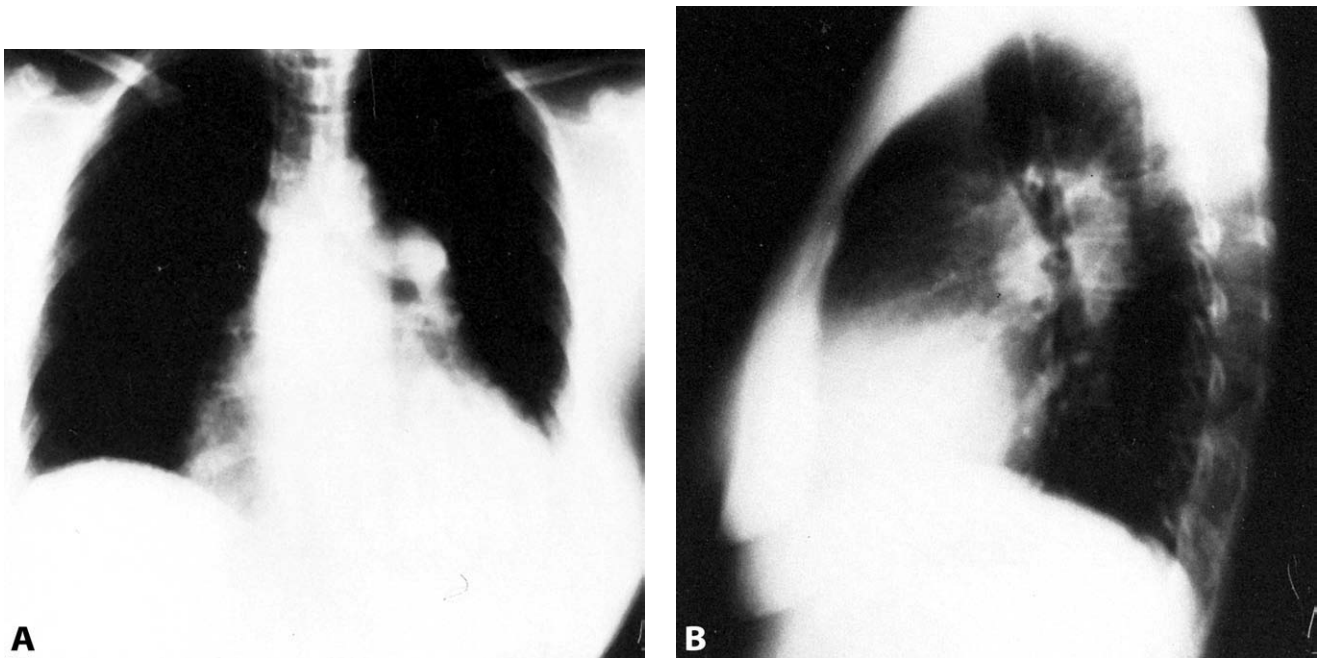


FIGURE 35 (A) Posteroanterior and (B) lateral chest radiographs show enlarged central pulmonary arteries in a 55-year-old patient with SLE and pulmonary arterial hypertension.

described in SLE associated with sarcoidosis [160, 161]. Furthermore, hilar lymphadenopathy may be the presenting manifestation of lymphoma in the lupus patient, and CT or MRI and, most recently, F-18-fluorodeoxyglucose positron emission tomography (FDG-PET) should be used for staging this disease and following its response to therapy (see section on reticulodendothelial SLE).

IMAGING OF CARDIOVASCULAR SLE

Pericardial Disease

Cardiovascular abnormalities of SLE develop in the majority of patients at some time during the course of their illness [162–177]. The most frequent clinical cardiovascular manifestation in SLE is acute pericarditis in 17–50% of patients [20, 77, 80, 166], although autopsy series reveal a higher prevalence of pericardial involvement ranging from 61 to 83% [176]. Pericardial effusions are associated significantly with active disease and pericardial pain. In only 14% of patients, the chest radiograph suggests the diagnosis when patients are symptomatic and pericardial effusions are large enough to displace the anterior fat pad. Without a positive anterior fat pad sign, a globular cardiac configuration may represent either a pericardial effusion or an acute myocarditis (Fig. 36) [20]. With an unusual sequela of pericardial effusion, constrictive pericarditis, radiographs show a marked decrease in the cardiac configuration (Fig. 37) [178]. MRI and echocardiography show pericardial thickening combined with a dilated inferior vena cava, hepatic veins, right atrium, and a normal or reduced volume right ventricle. In addition, an elongated narrow-shaped right ventricle with a S-shaped ventricular septum may be seen [178a].

Echocardiography detects pericardial effusions in 21–54% of lupus patients and is the most sensitive and inexpensive method for diagnosing and following most pericardial effusions (Fig. 38A) and thickening [162, 163, 166, 167, 171, 179–186]. Echocardiography depicts most effusions as anechoic fluid and pericardial fibrosis as an echogenic, thickened pericardium. Acute hemorrhage into the pericardium may cause an echogenic effusion. MRI (Fig. 38B) and CT occasionally may be used to evaluate pericardial effusions, particularly for delineating an anterior effusion or for defining a hemorrhagic effusion. Acute hemorrhage shows high attenuation at CT [187–189]. After the first 24–48 h, MRI is the most specific test for defining subacute hemorrhage, which characteristically is high signal intensity on electrocardiographic-gated, T1-weighted MR images. A rare manifestation of lupus, cardiac tamponade [190–193], can be recognized at echocardiography as right atrial collapse, right ventricular expiratory collapse, and paradoxical wall motion [165]. Cardiac tamponade may resolve with indomethacin [194] or may require sonographically-guided pericardiocentesis with its attendant risks of laceration of the myocardium and coronary arteries [194, 195]. Purulent pericarditis with or without tamponade is another unusual complication of SLE [165, 196].

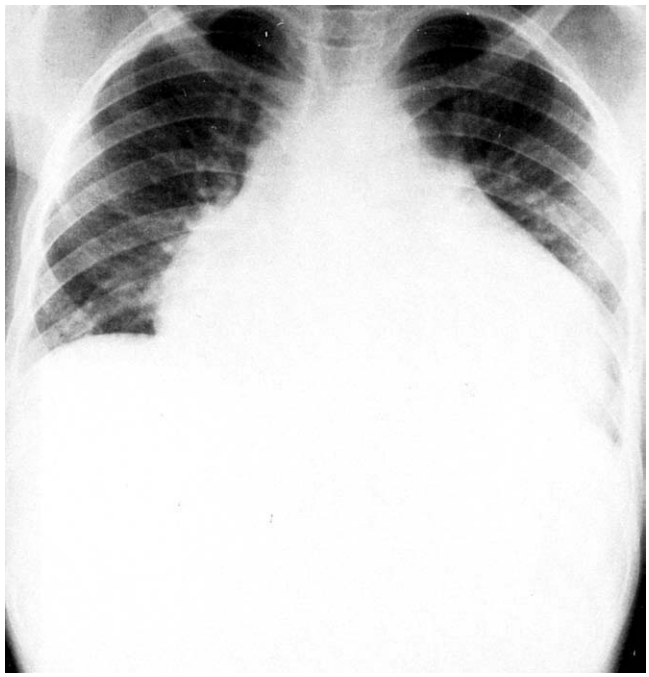


FIGURE 36 Posteroanterior radiograph shows a globular cardiac silhouette in a 10-year-old girl with SLE and a pericardial effusion.

As with pleural effusions, pericardial fluid is usually an exudate, with numerous leukocytes, LE cells, antinuclear antibodies (ANAs), immune complexes, and antirheumatoid factor. Glucose levels are normal [165, 174, 197].

Cardiac Abnormalities

Conventional radiography shows both generalized and localized cardiomegaly that may be associated with various conditions: (1) myocarditis (Fig. 39) and Libman–Sacks endocarditis; (2) low or high output congestive heart failure; (3) pulmonary hypertension with right-sided enlargement; and (4) hypertensive cardiovascular disease secondary to corticosteroid therapy, accelerated arteriosclerosis, and lupus nephritis.

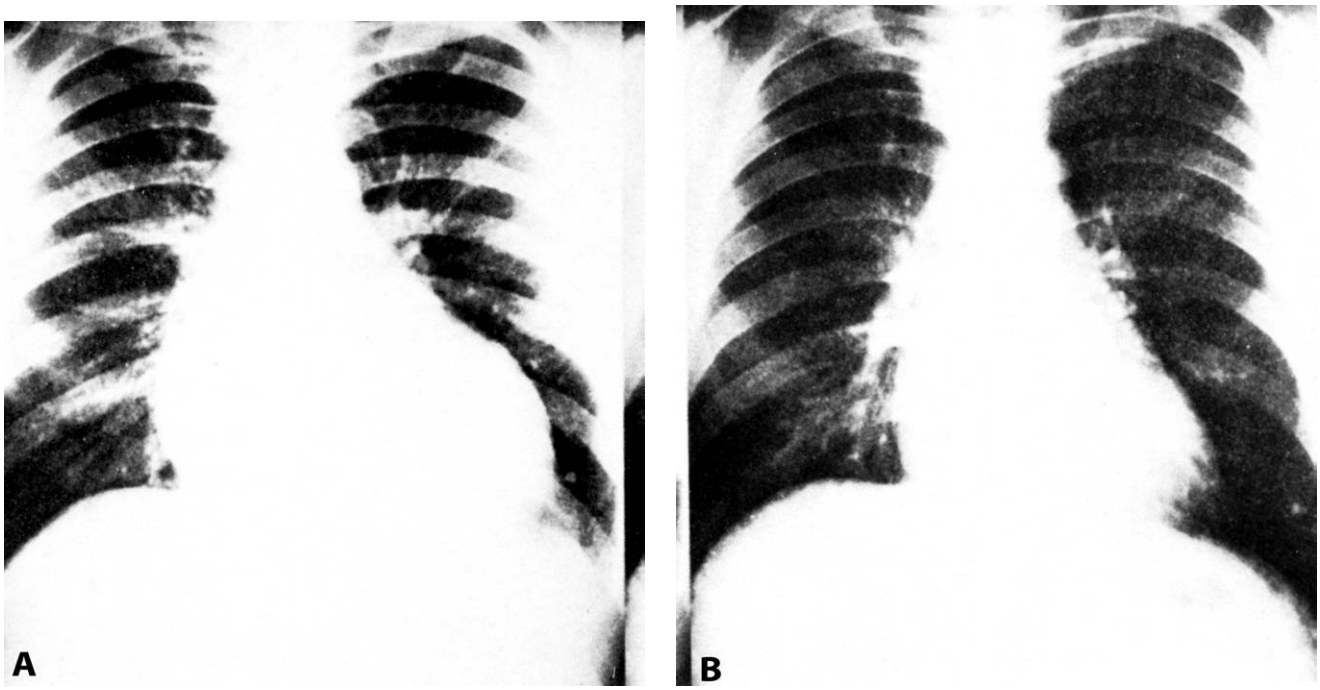
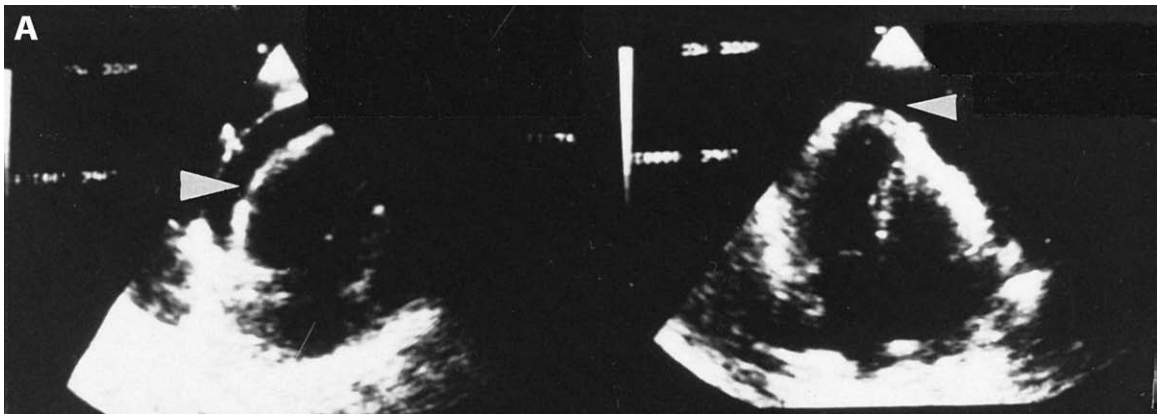


FIGURE 37 (A) Initial posteroanterior radiograph shows enlarged cardiac configuration in a 28-year-old man with SLE and pericardial effusion. (B) Posteroanterior chest radiograph taken 6 weeks later, when symptoms of pericardial constriction had developed, shows a decrease in the size of the cardiac silhouette. From Starkey and Hahn [178], with permission.



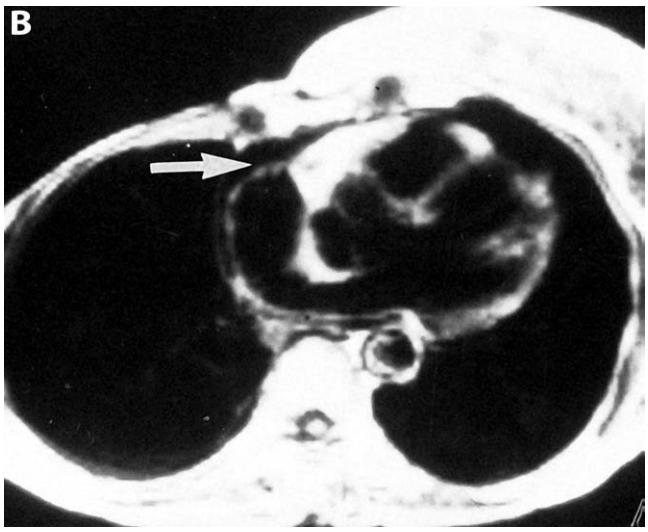


FIGURE 38 (A) Two-dimensional echocardiogram shows an anechoic anterior pericardial effusion (*arrow*) in a patient with SLE. (B) Axial ECG-gated T1-weighted MR image shows an anterior low-signal-intensity pericardial effusion (*arrow*).

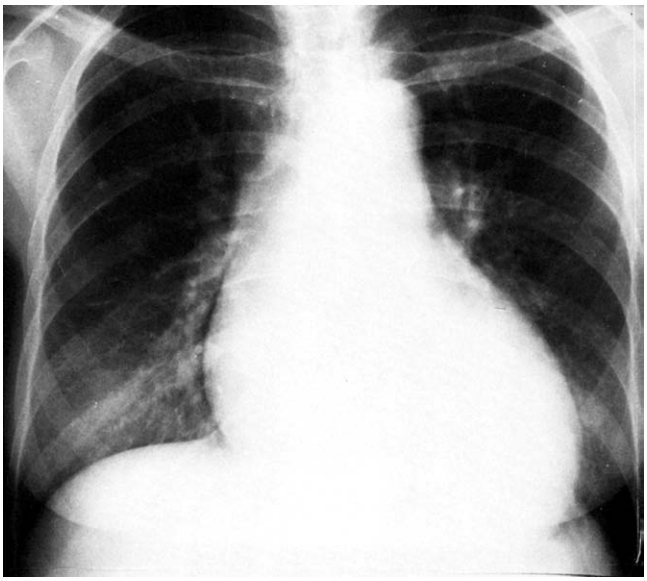


FIGURE 39 Posteroanterior chest radiograph demonstrates generalized cardiomegaly in a 28-year-old woman with SLE and myocarditis.

ECG, whether transthoracic or transesophageal, two dimensional (2D), three dimensional (3D), pulsed, or color flow Doppler, has become the primary method for defining anatomic and functional cardiac disease in SLE [162–171, 185, 186, 198–209]. Myocardial perfusion scintigraphy with ^{201}thallium alone or the ^{201}thallium/^{99m}Tc sestamibi (tetrofosmin) dual isotope technique is useful for detecting myocardial ischemia [210–211a]. The newer modalities of ultrafast, electron beam CT (EBCT), FDG-PET, and fast echoplanar MRI and magnetic resonance angiography (MRA) may add a new dimension to our understanding of the cardiac anatomy, dynamics, and ischemia in the lupus patient [212–219]. Prior to the development of these methodologies and ECG, cardiac catheterization was required to identify specific myocardial and valvular lesions [220–228] and is still used for the preoperative evaluation of coronary and endocardial abnormalities.

Endocardial Lesions

ECG readily detects anatomic and functional abnormalities of cardiac valves of patients with SLE [167, 201, 207, 229–243]. On radiographs, however, classic lupus endocarditis is difficult to diagnose because calcified vegetations are rarely seen. Senningen *et al*. [233] reported the radiographic findings of a Libman–Sacks endocardoma as an area of amorphous calcification just below the mitral valve (Fig. 40A) and the angiocardiographic findings as a multiobular intraventricular mass below the posterior mitral valve leaflet (Figs. 40B and 40C).

Transthoracic ECG (2D, 3D, and color flow Doppler) shows valvular abnormalities in 23–44% of lupus patients [165–167, 209, 243]. More recently, transesophageal (ECG) [207, 244] detected valvular lesions in 74% of lupus patients. The classic verrucous Libman–Sacks lesion (Figs. 41 and 42), when greater than 2 mm, appears as an irregular mass of heterogeneous echogenicity with diffuse valvular thickening. This thickening most commonly involves the mitral valve and sometimes the aortic valve with minimal involvement of the subvalvular apparatus. Any valve, however, may be affected [164, 179, 198, 201, 207]. When localized thickening is present, the midportion or base of the leaflet is involved more frequently. The overall prevalence of valvular regurgitation in SLE patients at transesophageal echocardiography is 61%, and moderate or severe regurgitation develops in 42% of patients. Libman–Sacks verrucae and focal myocardial scars affecting the papillary muscle may also lead to mitral prolapse, which was reported to have a significantly higher prevalence in SLE than in the general population [200].

The effect of corticosteroids on valvular disease in SLE remains a controversial tissue. Galve *et al*. [201] suggested that two types of endocardial valvular lesions, the verrucous mass and the fibrosed valve, may relate to corticosteroid usage differently. In their lupus patients, the verrucous lesion was usually seen in the younger patient, with active disease of shorter duration, who had received a smaller dose of corticosteroids. The rigid, fibrosed, thickened, and sometimes calcified valve was found more frequently in patients with disease of long duration who had received long-term corticosteroid therapy. Bulkey *et al*. [232] suggested that corticosteroids promoted healing of valvular vegetations, and scarring and cusp retraction subsequently may result. Another report, however, stated that valvular disease did not correlate with duration, activity, severity, or treatment of SLE [207], and that the extent of valvular disease at presentation did not predict permanent or progressive valvular dysfunction [207]. The incidences of valvular abnormalities at initial and follow-up evaluation (29 and 13 months later) were similar for valvular thickening for initial and follow-up studies (51 and 52%, respectively), but the incidences of initial and follow-up studies differed for vegetations (30 and 20%), for regurgitation (25 and 28%), and for stenoses (3 and 4%). Valvular lesions persisted or changed in size or appearance between the two studies but could also resolve. In addition, some new lesions appeared during the study.

Echocardiography should also be performed following biosynthetic valves to evaluate for postoperative

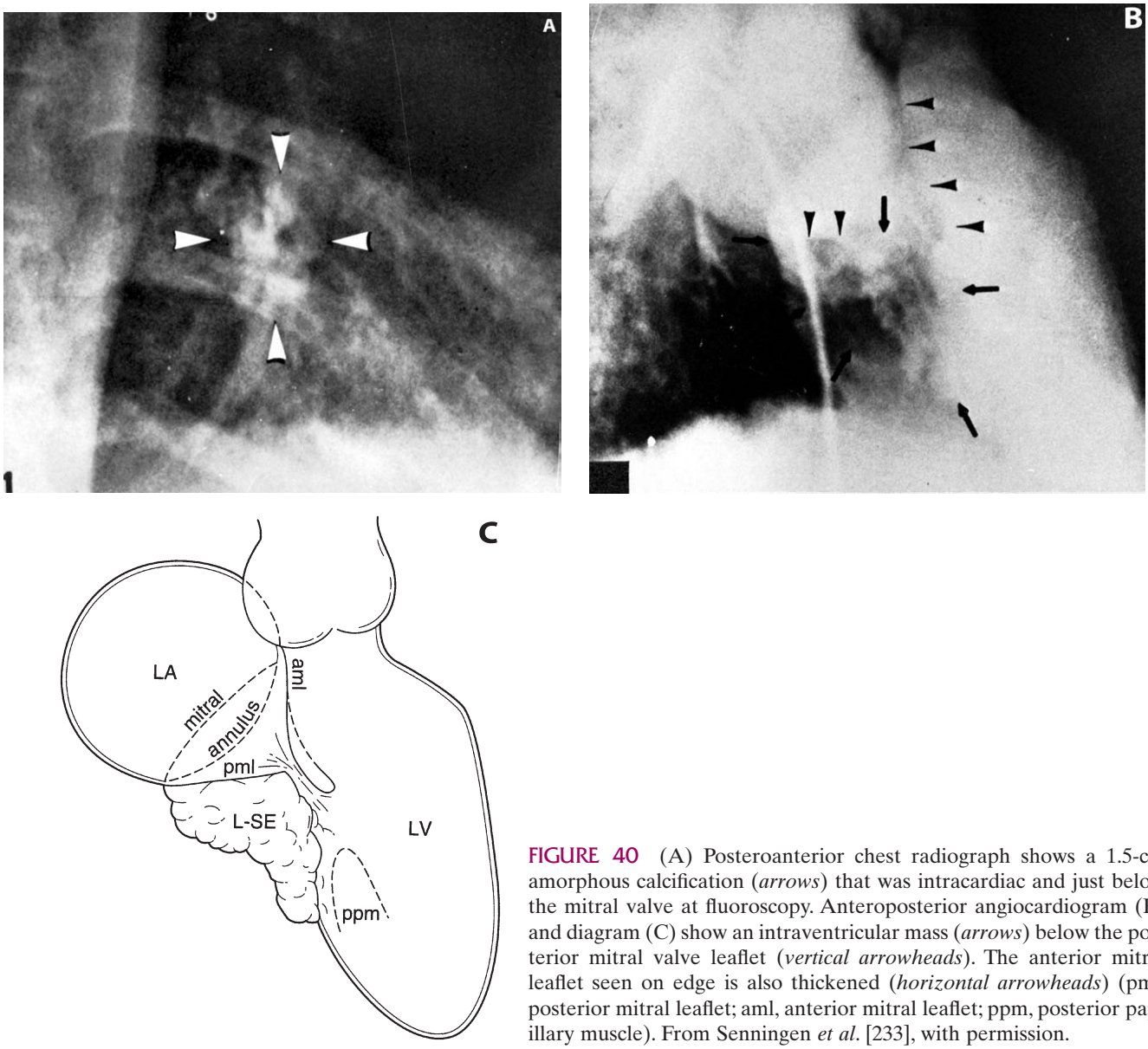


FIGURE 40 (A) Posteroanterior chest radiograph shows a 1.5-cm amorphous calcification (*arrows*) that was intracardiac and just below the mitral valve at fluoroscopy. Anteroposterior angiocardiogram (B) and diagram (C) show an intraventricular mass (*arrows*) below the posterior mitral valve leaflet (*vertical arrowheads*). The anterior mitral leaflet seen on edge is also thickened (*horizontal arrowheads*) (pml, posterior mitral leaflet; aml, anterior mitral leaflet; ppm, posterior papillary muscle). From Senningen *et al*. [233], with permission.

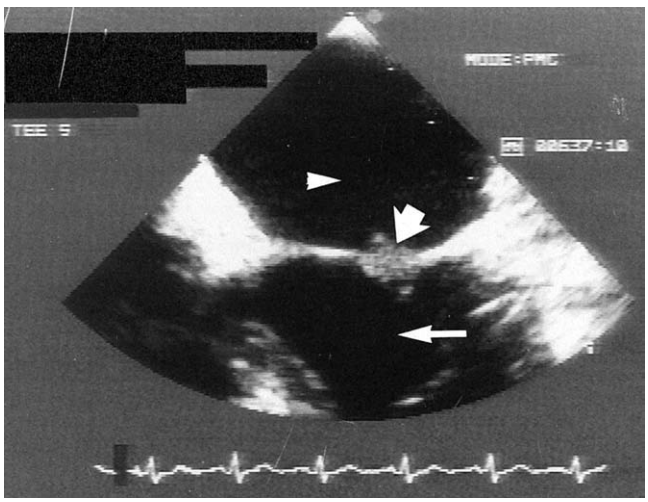


FIGURE 41 Transesophageal echocardiogram in an acutely ill patient with Libman–Sacks endocarditis shows thickening (*thick arrow*) of the anterior and posterior mitral valve leaflets (LA, arrowhead; LV, thin arrow). Courtesy of Dr. N. H. Silverman, University of California, San Francisco.

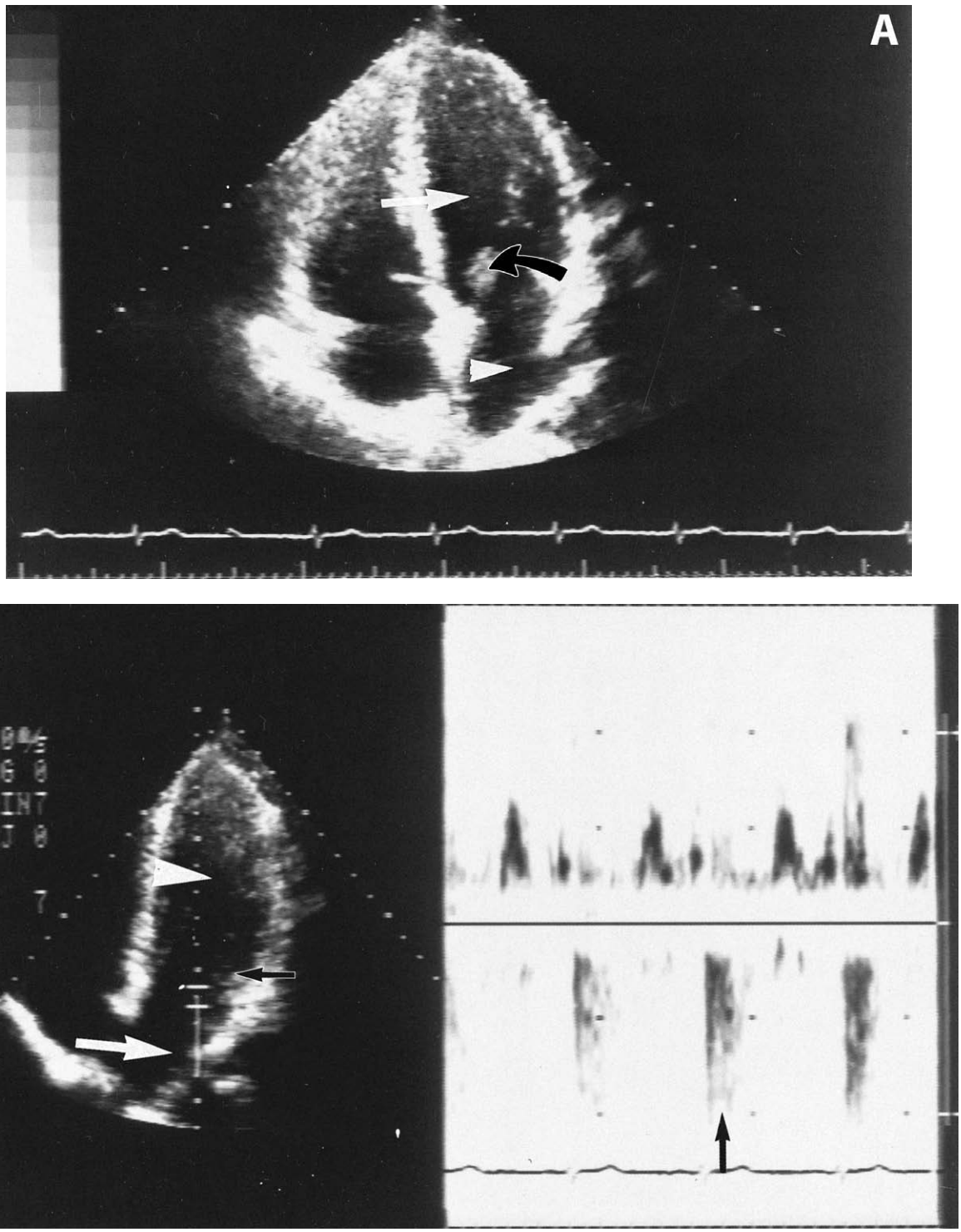


FIGURE 42 (A) Apical four-chamber echocardiographic image shows a thickened mitral valve leaflet (*black curved arrow*) (LV, *white arrow,* LA, white arrowhead). (B, left) An apical four-chamber image with Doppler sample volume on the left atrium side (*arrow*) of the mitral valve (LV, *arrowhead*); (right) Doppler signal (*black arrow*) identifying the presence of mitral regurgitation arising from the valve abnormality in A. Courtesy of Dr. N. H. Silverman, University of California, San Francisco.

complications of dehiscence and for recurrent lupus valvulitis of the bioprosthetic valve [235].

Several investigators [166, 236–240, 243, 245, 246] have described a relationship among antiphospholipid antibodies, lupus anticoagulant, and valvular disease. In these studies, valvular vegetations, left ventricular hypertrophy, and cerebral arterial occlusions were more common among lupus patients with antiphospholipid antibodies than among those without elevated levels. Others have found that patients with SLE had a similar frequency and severity of valvular disease regardless of the presence or absence of antiphospholipid antibodies [242, 244].

Libman–Sacks endocarditis should be distinguished from rheumatic valvular disease. Lupus endocarditis usually presents as diffuse valvular leaflet thickening that infrequently calcifies. When thickening is present, the mid and base of the leaflet, rather than the tips, are more commonly involved. In contrast, in rheumatic fever, valvular masses predominate in the valve tips and are frequently calcified [201].

In SLE, infective endocarditis may simulate or complicate Libman–Sacks endocarditis and may cause significant morbidity and mortality and must be excluded in the febrile lupus patient with valvular disease [165, 201, 207]. Sometimes infected masses may differ from Libman–Sacks endocarditis in their location, appearance, and mobility [244]. Infective lesions usually are homogeneously echogenic, tend to be smaller, and attach to the valves at their line of closure without prolapsing into the cardiac cavity. In contrast, Libman–Sacks lesions display heterogeneous echogenicity, are more diffuse, and are located near the mid and base of the leaflet. Furthermore, lupus masses move parallel to the motion of the associated leaflet, whereas infected masses demonstrate vibratory or rotary motion partly independent from valvular motion [244]. Another complication of cardiac infection in lupus is the mural ventricular vegetation or thrombus [199–199b]. Intracavitary thrombi are usually associated with abnormal myocardial function [199a, 199b]. Rarely mural thrombi can develop as an isolated lesion without underlying myocardial abnormality [199c]. Intracavitary mural thrombi, whether infected or not, and Libman–Sacks endocarditis, when arising from the chordae tendinae and projecting into the outflow tract, may all present as echogenic intracardiac masses at echocardiography [199, 199a–c, 241].

Echocardiography also has an instrumental role in defining endocardial disease when clinically occult. For example, clinically silent valvular disease may be the source for thromboembolism in patients who present with cerebrovascular accidents or transient ischemic attacks.

Myocardial Functional and Anatomic Abnormalities

Echocardiography demonstrates multiple functional abnormalities in SLE: global left ventricular hyperkinesis, discrete segmental abnormalities of left ventricular wall motion, decreased end systolic volume index, prolonged isovolumic relaxation time, decreased left ventricular ejection fraction percentage and abnormal left ventricular diastolic filling dynamics, prolonged isovolumic relaxation time, reduced peak early diastolic flow velocity (peak E), increased peak late diastolic flow velocity (peak A), reduced E/A ratio, and lower deceleration rate of early diastolic flow velocity [168, 170, 171, 180, 184, 202, 204–206, 247, 248, 248a].

Anatomic abnormalities at echocardiography include left ventricular hypertrophy, left atrial dilatation and right ventricular enlargement, increased mass of the posterior left ventricular wall and interventricular septum, aneurysmal dilatation and intracavitary thrombus [171, 180, 184, 202, 206, 230, 241].

Winslow *et al*. [168] followed the natural history of left ventricular size and function in SLE in a 5-year echocardiographic study. Abnormalities of systolic and diastolic left ventricular function (increases in left ventricular mass index, mean wall thickness, end systolic volume, and decreases in ejection fraction) were increased significantly in patients with SLE as compared with a control population ($P \leq 0.05$). These findings progressed over the 5-year time period and were related to the coexistence of hypertension and coronary artery disease. In normotensive lupus patients, no significant differences in left ventricular mass index, volumes, or ejection fraction were found when compared with the control group. Primary lupus cardiac involvement did not lead to clinically significant changes in left ventricular size or function [168].

Myocardial infarction, once considered to be rare in lupus, is now recognized as a cause of late death [249–252]. Myocardial function is frequently depressed or abnormal [179, 184, 250] in some lupus patients who do not have clinical cardiac disease. In a prospective study, Hosenpud *et al*. [210] demonstrated a 38.5% prevalence of segmental myocardial perfusion abnormalities on exercise ^{201}thallium cardiac scintigraphy in SLE. Patients had reversible defects suggestive of ischemia, persistent defects consistent with scar, and both types of defects (reversible and irreversible). They found no correlation among several factors: a positive thallium study, the duration of disease, the quantity of corticosteroid therapy, major organ system involvement, or age. Only a history of pericardial disease was associated with positive scintigraphic findings.

For evaluation of cardiac dyskinesia, EBCT with cine-loop produces multilevel–multisecond continuous images of the beating heart. Intravenous injection of contrast media is required to allow differentiation between the blood pool and myocardium but would be contraindicated in the lupus patient with renal failure. EBCT can be performed during exercise stress and can characterize right and left ventricular performance because of a real-time technique [253].

Prior to the late 1990s, MRI, MR spectroscopy (MRS), and FDG-PET [214–218a] have been used in experimental protocols for evaluating the myocardium but not as part of standard clinical care. However, since the late 1990s, these modalities increasingly have become available and have been applied to the study of myocardial disease in populations at risk. In the only MRI myocardial study on lupus patients, the T1 relaxation times of myocardium were reported as longer in patients with active myocardial disease and decreased complement [214]. In patients without SLE and with unstable angina and infarction, T2-weighted MR images of the myocardium showed a hyperintense signal in the infarcted myocardium, which enhances on contrast-enhanced MRI [217]. Newer, faster contrast-enhanced MRI sequences (turbofast low-angle shot and echoplanar imaging) were developed to evaluate tissue perfusion [218, 218a] and the difference in viable and nonviable tissue in myocardium and other muscle and soft tissue [218, 218a]. These new MRI techniques can determine myocardial status, including myocardial perfusion reserve and region blood flow and volume. For example, during the first pass of contrast medium through the heart with fast contrast-enhanced MR sequences, the central region of infarcts may show hypoperfusion or no perfusion immediately consistent with irreversible injury as confirmed on electron microscopic examination. In contrast, the infarct periphery shows enhancement compatible with reversible injury and viable tissue [218a].

Metabolic abnormalities of the myocardium have been studied at FDG-PET and MRS. With its transverse tomographic images, FDG-PET allows spatial separation of different segments of the left ventricle so that differential radiopharmaceutical uptake reflects myocardial regional blood flow and metabolism. MRS, with ^{31}P and ^{13}C, produces spectra that correlate with changes of ischemia (e.g., lactate accumulation or depletion of phosphate high-energy stores) [215].

Coronary Artery Disease

Although accelerated arteriosclerosis is the predominant cause of myocardial infarction in SLE, vasculitis and thrombotic phenomena are also contributory factors [254–263a, 263b]. Cardiac catheterization has been the standard imaging modality for defining vascular abnormalities. Smooth, focal stenoses, aneurysmal dilatation, and lesions that change over a relatively short period of time characterize the vasculitis [260–262] and can be distinguished from the fixed, stenotic lesion of coronary arteriosclerosis. Heibel *et al*. [262] and Homcy *et al*. [260] emphasized that serial examinations should be obtained in order to differentiate the more rapidly evolving coronary vasculitis (Fig. 43) from the slowly progressing arteriosclerosis. A reversible arterial spasm has been documented at coronary angiography and may also be a factor in ischemic cardiac disease in SLE [264]. Although not reported in a cohort of lupus patients, three-dimensional contrast-enhanced ultrafast MRA, with a submillimeter resolution of structures, can be useful for evaluating coronary blood flow and flow reserve [264a].

EBCT has been applied to the detection of calcified coronary arteriosclerosis in the general population and in patients at risk including the patients with SLE [263a–263c]. In a pilot study on 13 patients (ages 33–48) with SLE with two or more risk factors, Von Feldt [263b] found that 2 patients had coronary calcification scores in the 70th percentile matched with women without known coronary disease and three had scores in the 90th percentile. In unpublished data in a review, Manzi and coworkers described the EBCT coronary artery findings in 40 patients with SLE, 20% of whom had a previous event (4 transient ischemic episodes or stroke, 3 myocardial infarction or angina, and 1 with both cerebrovascular and coronary events). The coronary calcification scores were significantly higher in women with a previous event than in those without a previous event [263c].

Coronary artery aneurysms (Fig. 44), a rare complication of SLE, are thought to result from accelerated arteriosclerosis or lupus vasculitis and have been described in the left main, left anterior descending, and right coronary arteries [265, 266]. Mycotic aneurysm of the coronary artery in patients with SLE is rare and was associated with salmonella infection in one report [266a]. With a coronary artery aneurysm, whether infected or not, new generation fast helical CT or electron beam CT and MRA or standard coronary angiography [262, 266] show the aneurysmal dilatation and its relationship to the remainder of the coronary vasculature and myocardium.

Aortic, Great Vessel, and Peripheral Arterial Occlusive Disease

Aortic root thrombus [267], aortic arch syndrome (thrombosis of a major arch vessels) [268, 269], aortic occlusion [270], aortoarteritis [271, 272], and superior

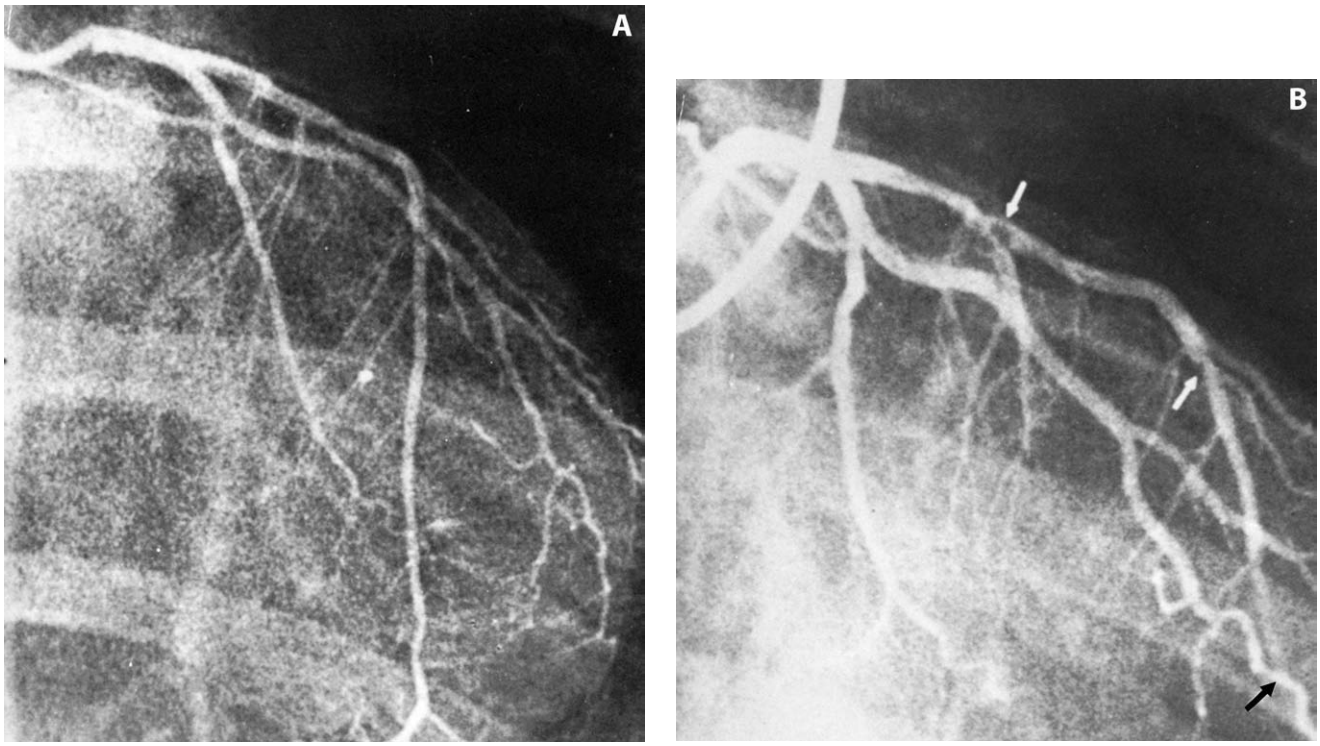


FIGURE 43 Selective arteriogram of the left coronary artery in right anterior oblique projection. (A) First study. No significant lesion of the left anterior descending coronary artery. (B) Second study, 18 days after (A) significant narrowing in the left anterior descending coronary artery at the origin of the large septal perforation (*uppermost arrow*), at the origin of diagonal branch (*middle arrow*), and in the distal portion of the vessel, consistent with coronary artery arteritis (*lowest arrow*). From Heibel *et al*. [262], with permission.

vena cava thrombosis [273] occasionally develop in lupus patients and may or may not be associated with antiphospholipid antibodies [268–270, 273]. Two-dimensional ECG, particularly with the transesophageal approach, can identify the aortic root thrombus, whereas conventional angiography and MRA define the aortic thrombosis in the thrombotic syndromes and the arteritis of the large vessels with alternating areas of narrowing and dilatation. Patients with SLE rarely develop dissecting aneurysms [274, 274a] (Fig. 45), which may be associated with atherosclerosis or Takayasu's aortitis [274b] or may be mycotic in etiology [275]. For rapid evaluation, helical CT or EBCT is carried out during the acute dissection or for the infected aneurysm that needs immediate treatment, and MRA is done for following the chronic dissection.

In SLE, peripheral arterial occlusion results from vasculitis or from thrombosis secondary to a hypercoaguable state associated with elevated lupus anticoagulant or antiphospholipid antibodies [276–281]. Ulceration and gangrene can follow. Vasospasm that may accompany the vasculitis is particularly associated with Raynaud's phenomenon (see sections on musculoskeletal and pleuropulmonary SLE). Conventional angiography and MRA (Fig. 46) show the peripheral vasculitis, thrombus, arteritis, and arteriosclerotic plaques [282–285].

Peripheral Venous Occlusive Disease

Thrombophlebitis and deep venous thrombosis occur in 4.6–12.2% of patients with SLE [77, 286]. Several factors predispose to deep venous thrombosis: chronic disseminated intravascular coagulation, small vessel angiitis with propagation of thrombus into larger vessels, venulitis, compensatory lupus anticoagulants, antiphospholipid antibodies, and prolonged patient immobility [276, 277, 286–290]. Conventional venography (Fig. 47) and, more recently, MRA and power Doppler/duplex sonography (Fig. 48) are usually used for studying the central veins. Previously, radiographic and radionuclide venography were the primary imaging modalities for evaluating extremity thrombosis [69, 288–291]. However, color flow Doppler/duplex

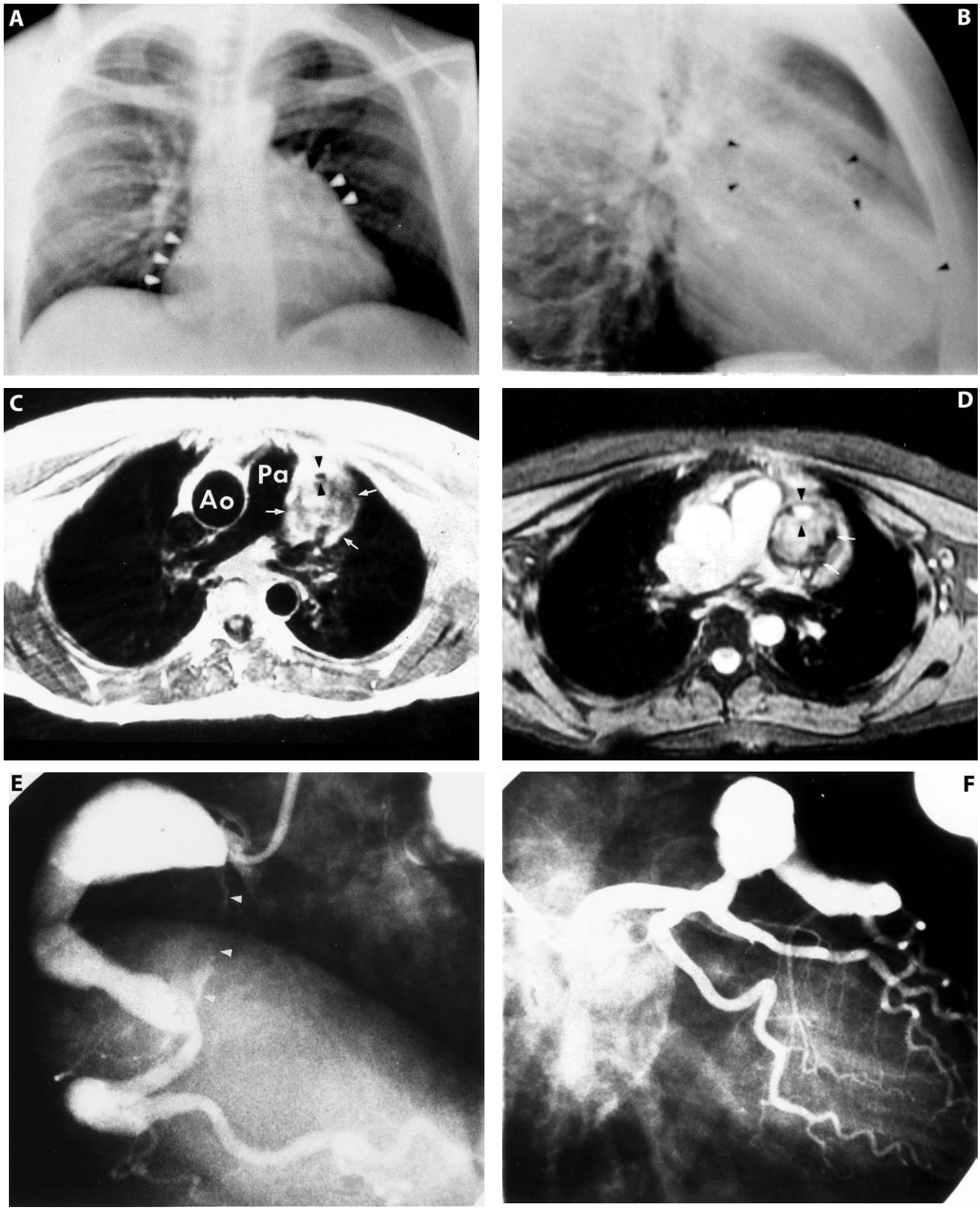


FIGURE 44 (A) Posteroanterior radiograph of a 26-year-old woman with SLE without cardiac symptoms shows a round calcified structure (*white arrowheads*) at the upper left margin of the heart and at the right atrial region. (B) Lateral radiograph shows that the upper calcifications are near the aortic root and the inferior calcifications overlie the right ventricle (*black arrowheads* outline the margin of the calcifications). Axial MR images (C and D) show an aneurysm of the left anterior descending coronary artery (*white arrows*). The aneurysm has an extensive thrombus that compresses the residual lumen (*black arrowheads*). The aneurysm is larger in diameter than both the aorta (Ao) and the main pulmonary artery (Pa). (E) Coronary angiogram in the left anterior oblique projection shows aneurysmal dilatation of the right coronary artery with peripherally calcified thrombus (*white arrowheads*). (F) Right anterior oblique projection shows a left anterior descending aneurysm. From Nobrega *et al.* [266], with permission.

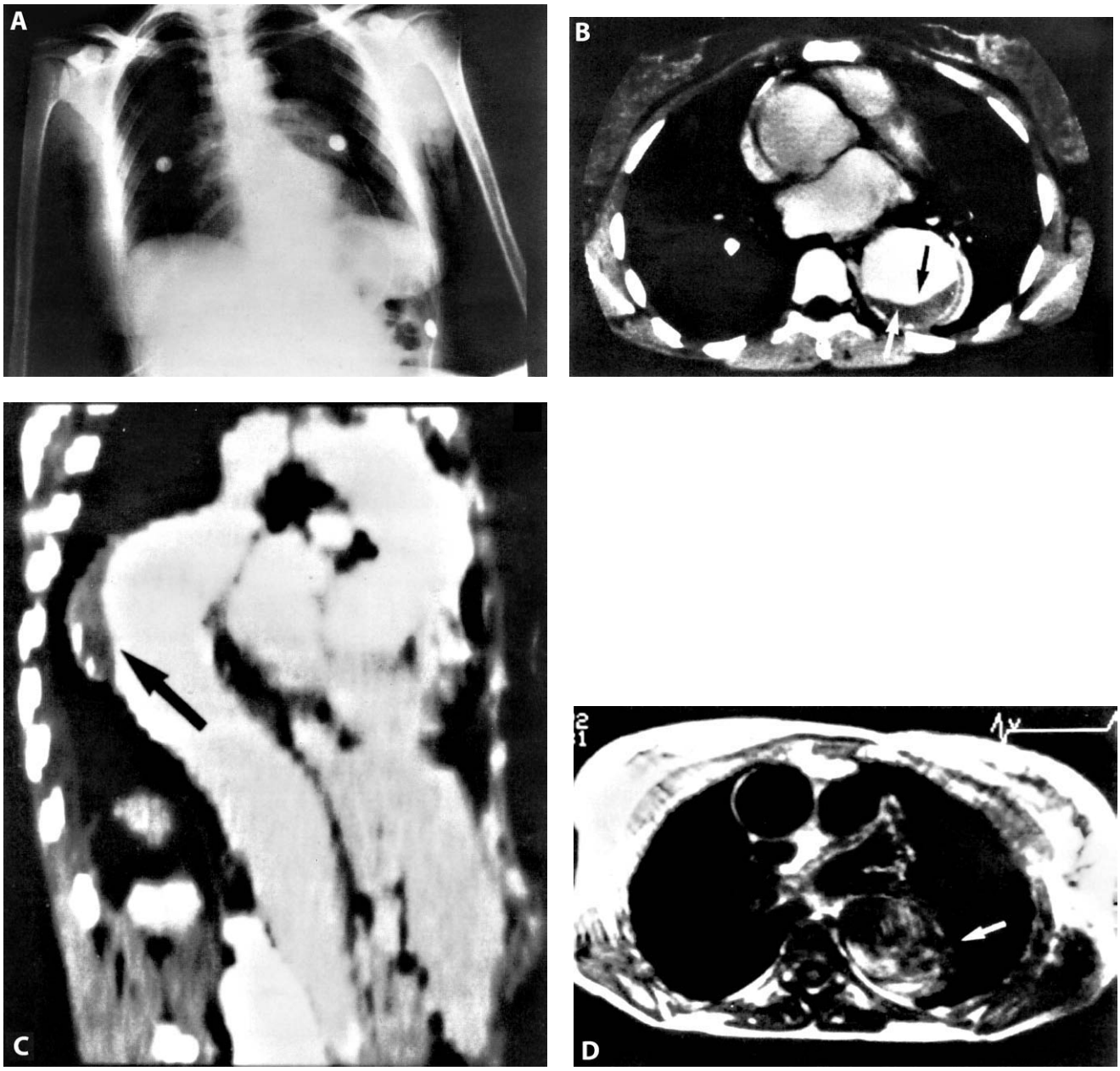


FIGURE 45 (A) Posteroanterior radiograph shows a widened mediastinum secondary to an enlarged descending aorta in a 30-year-old woman with SLE and a 10-day history of chest pain. (B) Axial contrast-enhanced CT shows a wide ascending and descending aorta with enhancing true lumen (*black arrow*) and nonenhancing false lumen (*white arrow*) consistent with aortic dissection. (C) CT coronal reconstruction defines the region of the tear of the descending aorta (*arrow*). (D) Axial T1-weighted MR image shows the wide descending aorta (white arrow) and the irregular intima. From Sclair *et al*. [274], with permission.

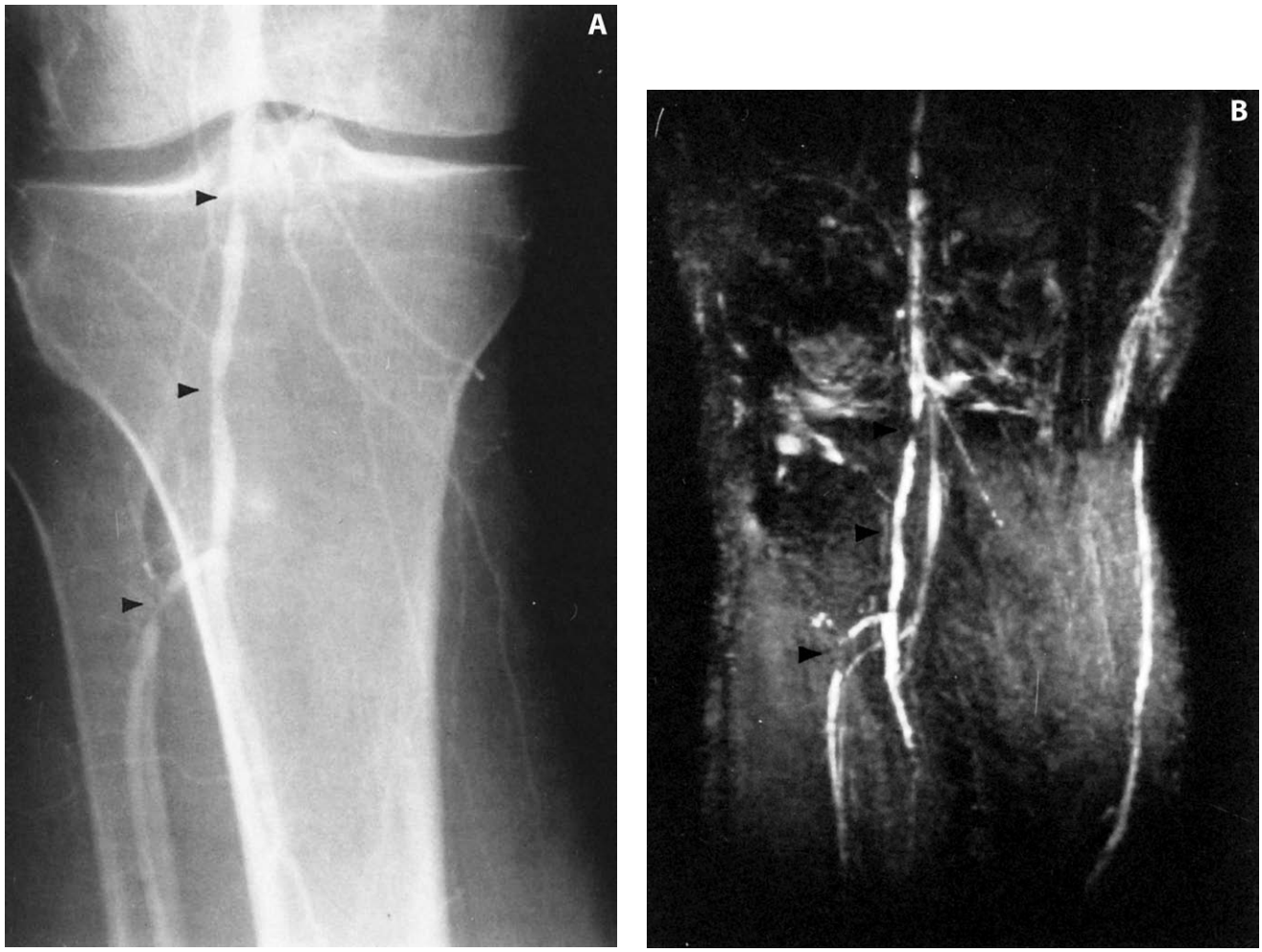


FIGURE 46 (A) Conventional anteroposterior angiogram of the calf shows arteriosclerotic changes of the popliteal and anterior tibial arteries. (B) Long TE and TR ECG-gated MR angiogram with a 90° flip angle shows the same lesions as on the conventional angiogram.

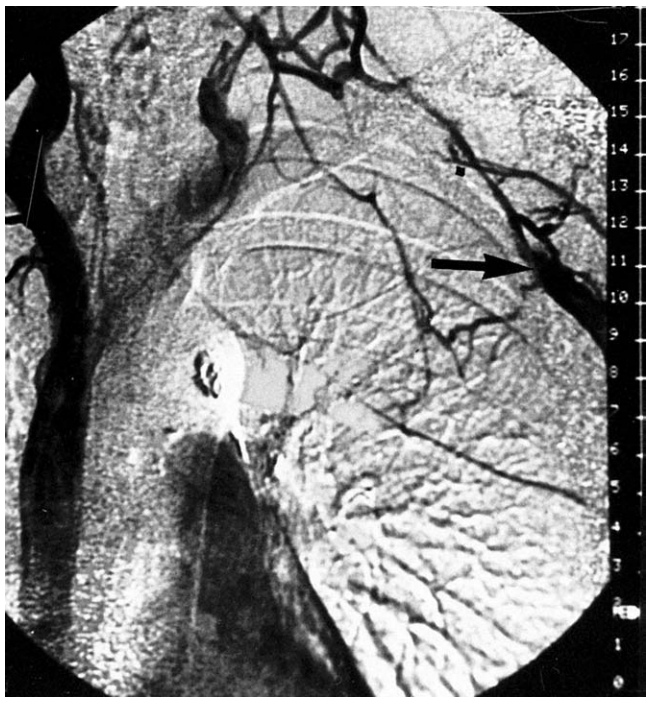


FIGURE 47 Venogram shows occlusion of the left axillary vein (arrow) with collateral filling of the innominate vein via the jugular collaterals in a patient with SLE. Courtesy of Dr. M.-F. Kahn, University of Paris.

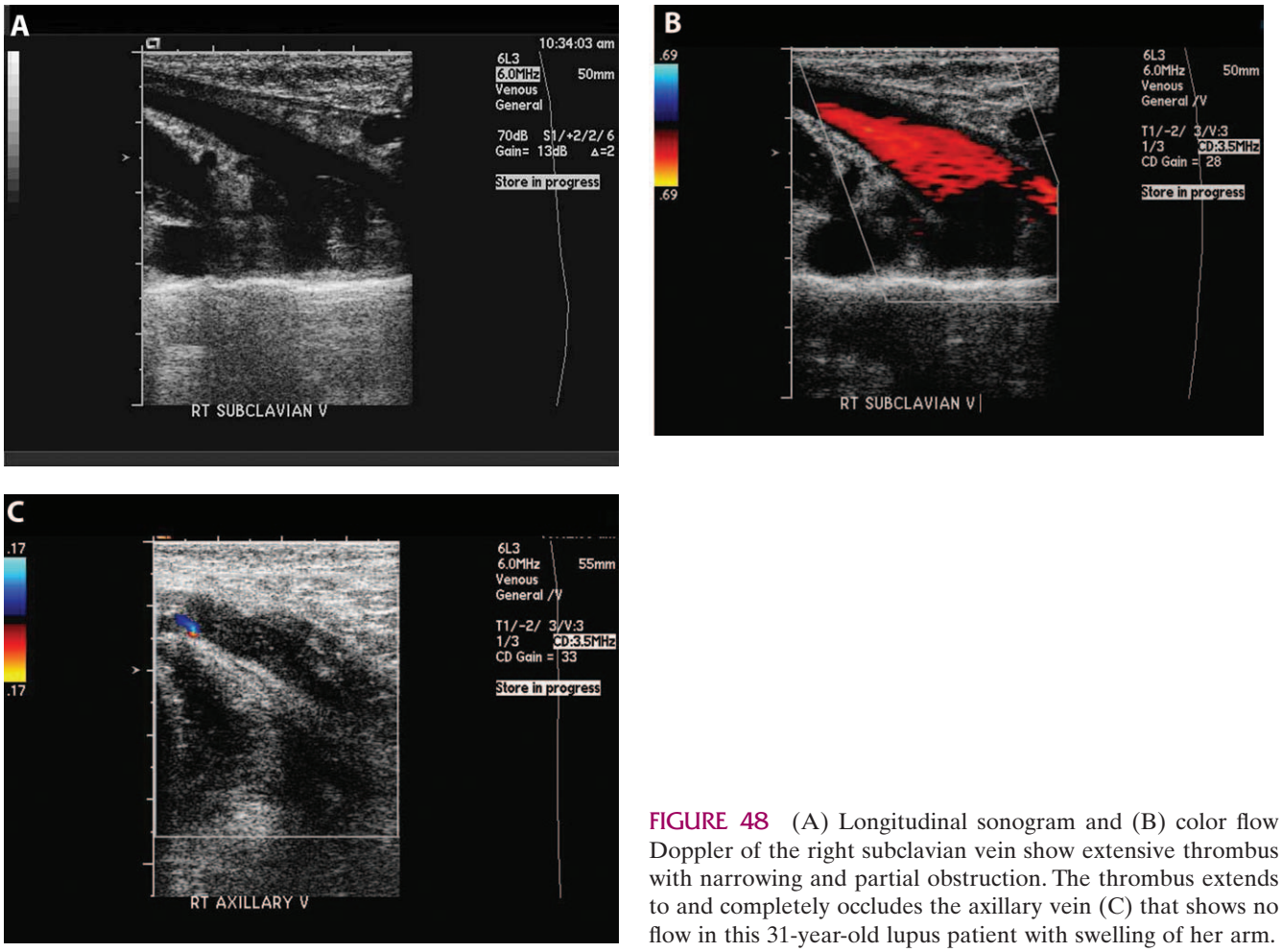


FIGURE 48 (A) Longitudinal sonogram and (B) color flow Doppler of the right subclavian vein show extensive thrombus with narrowing and partial obstruction. The thrombus extends to and completely occludes the axillary vein (C) that shows no flow in this 31-year-old lupus patient with swelling of her arm.

sonography (Fig. 49) and, more recently, power Doppler [33c], have replaced venography as the preferred initial test for deep venous thrombosis [69, 293–295] by demonstrating the noncompressible, thrombosed vein, the presence or absence of flow, and the areas of vein recanalization, when present. MRA should be reserved for situations where there is discordance between clinical and sonographic diagnoses.

In summary, a multimodality approach permits the best evaluation of cardiovascular disease in SLE. Echocardiography and color flow Doppler/duplex sonography should be the initial tests for evaluating cardiac anatomic and functional abnormalities, proximal aortic disease, and venous occlusive disease. Myocardial ischemia and perfusion can be evaluated by ^{201}thallium/^{99m}Tc sestamibi stress tests and potentially with dynamic contrast-enhanced MRI. EBCT can demonstrate coronary artery calcification. MRA has shown potential for evaluating noninvasively the coronary arteries and myocardial function. The rare aortic or coronary artery aneurysm and central venous thrombosis or dissecting aneurysm can be visualized at both CT and MRI, and conventional angiography and MRA can confirm the diagnosis, when necessary.

IMAGING OF GASTROINTESTINAL SLE

In 1885, William Osler [296] reported 11 patients with erythema exuditavum multiforme who had "gastrointestinal crises, colic, usually with vomiting and diarrhoea." Symptoms referable to the gastrointestinal tract are quite common in patients with SLE. Gastrointestinal upset may develop secondary to vasculitis, concurrent uremia, corticosteroid therapy, immunosuppressive or antimetabolite therapy, CNS involvement, or coincidental gastritis, hepatitis, pancreatitis, or peritonitis. Thus, at some time during the course of their illness, most patients with SLE report anorexia (47–82%) and nausea and vomiting (11–53.2%) and some have diar-

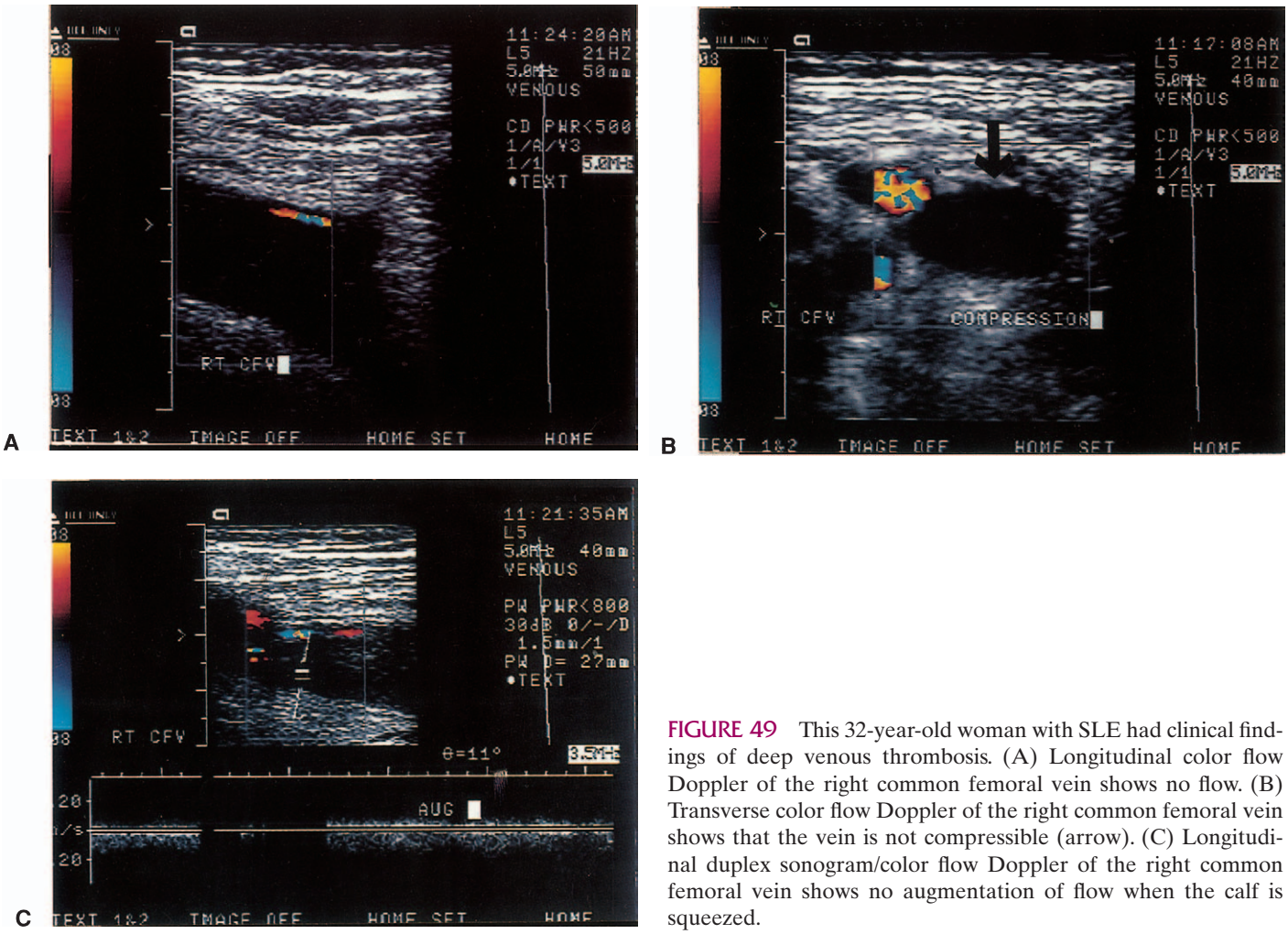


FIGURE 49 This 32-year-old woman with SLE had clinical findings of deep venous thrombosis. (A) Longitudinal color flow Doppler of the right common femoral vein shows no flow. (B) Transverse color flow Doppler of the right common femoral vein shows that the vein is not compressible (arrow). (C) Longitudinal duplex sonogram/color flow Doppler of the right common femoral vein shows no augmentation of flow when the calf is squeezed.

rhea (5.9–21%), bowel hemorrhage (5–6.3%), and abdominal pain (10–37%) [77, 80, 297–299].

Acute Reversible Lupus Gastrointestinal Ischemia

Acute reversible lupus ischemia of the gastrointestinal tract is a recognized but frequently misdiagnosed manifestation of SLE. A clinical picture consistent with reversible ischemia suggestive of vasculitis has been reported [80, 300–303]. It may be mistaken for various entities, as detailed earlier. In their series of 141 patients, Shapeero *et al.* [303] found 20 patients with ischemic disease compatible with a vasculitis that was reversed by corticosteroids. Nine had radiographic findings consistent with an ischemia: pseudo-obstruction of the gastric outlet (Fig. 50), duodenal stasis, effacement of mucosal folds, and thumbprinting (Fig. 51). Because the most common area of involvement is the small bowel, it is important to obtain a small bowel series as well as an upper gastrointestinal study to evaluate for changes of ischemia. The radiographic manifestations of gastrointestinal ischemia in SLE reflect the underlying pathologic process of a decrease in the blood supply and are similar to those of other types of ischemic bowel disease. The different layers of the bowel are not equally sensitive to a decrease in their blood supply [303–305]. The mucosa is most vulnerable to vascular compromise, followed by the muscular layers; the submucosa and serosa are least sensitive. The first changes to develop are superficial mucosal ulcerations that are radiographically not visible on small bowel series. In the colon, a fine pattern of ulceration may simulate ulcerative colitis [306–308]. When the muscular layer is impaired, an ileus may develop with fluid accumulation in the lumen of the gut. Unfortunately, at this stage, these changes are nonspecific. When submucosal hemorrhage and edema develop, as they frequently do, diffuse nodularity and

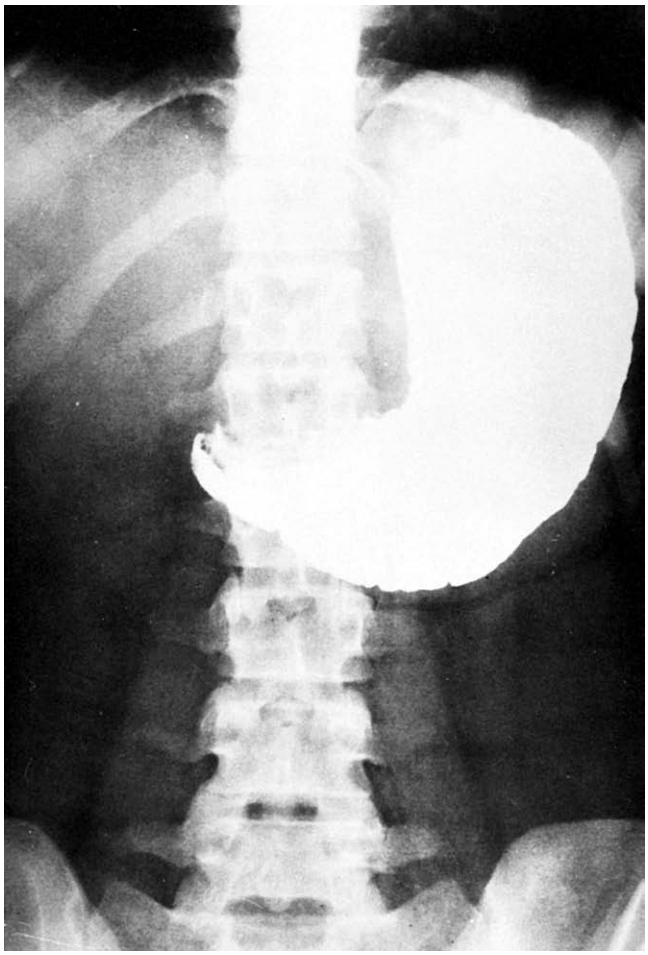


FIGURE 50 Upper gastrointestinal series shows a mildly dilated stomach with pseudo-obstruction of the gastric outlet in a 23-year-old woman with SLE. From Shapeero *et al.* [303], with permission.

thickening of the mucosal folds and the wall of the small bowel [303, 309] and colon strongly suggest the diagnosis of ischemia (Figs. 51 and 52). These findings may simulate other disease such as regional enteritis or submucosal hemorrhages from some other cause than ischemia; however, in the lupus patient, ischemia is the most probable diagnosis.

Phillips and Howland [310] described the angiographic findings of lupus arteritis as irregularities of the small mesenteric vessels. Asherson *et al.* [311] described mesenteric vascular occlusive disease at angiography and bowel infarction in lupus patients who have elevated antiphospholipid antibodies (Fig. 53). Although not yet reported in the lupus patient, MRA noninvasively can delineate the thromboses and irregularities of mesenteric vessels.

Ischemia secondary to vasculitis may have an acute or insidious onset. Chronic lupus vasculitis of the colon can rarely lead to stricture [312]. However, when ischemia is unrecognized, massive infarction, penetrating ulcers, intussusception, peritonitis, abscess formation, and perforation of the small and large bowel may result [312–320, 321, 321a] with the radiographic findings of ischemia, intramural air (pneumatosis intestinalis), air in the portal vein, and pneumoperitoneum. CT can demonstrate the findings of thickened ischemic bowel and its complications: pneumatosis intestinalis, air in the portal vein, abscess formation, intussusception, and ascites [322–324]. Management of the acute abdominal manifestations of SLE is a difficult therapeutic and surgical problem. Although some patients with ischemic episodes may respond to medical therapy and show improvement on barium studies and at CT [302, 324], the progression of clinical symptoms and signs, despite therapy, should indicate the need for laparotomy [320, 321].

Pneumatosis intestinalis does not always imply necrotizing lupus enterocolitis; a benign form [325–327]

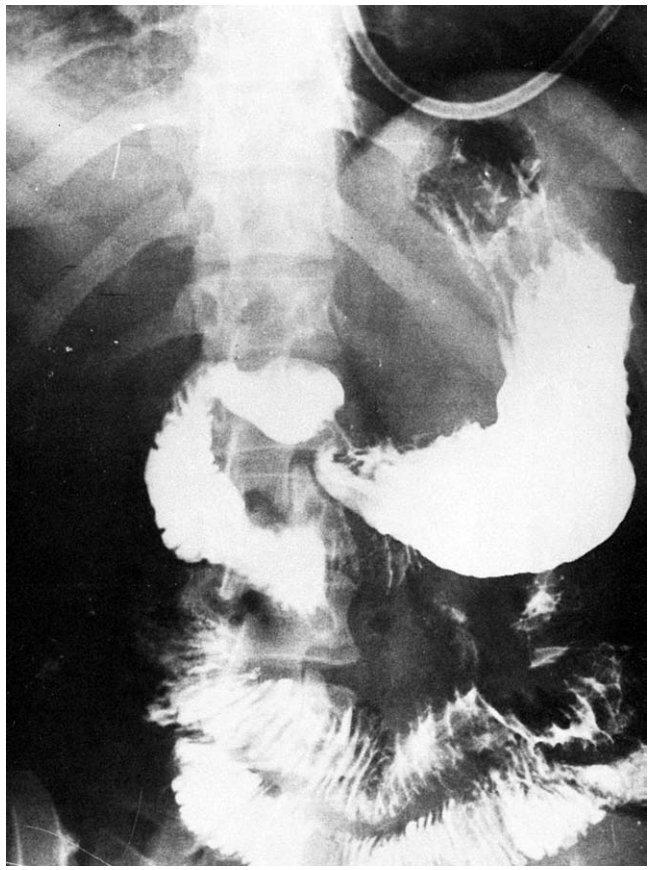


FIGURE 51 Small bowel series shows thickening and effacement of jejunal mucosal folds and thumbprinting in a 23-year-old woman with SLE. From Shapeero *et al.* [303], with permission.

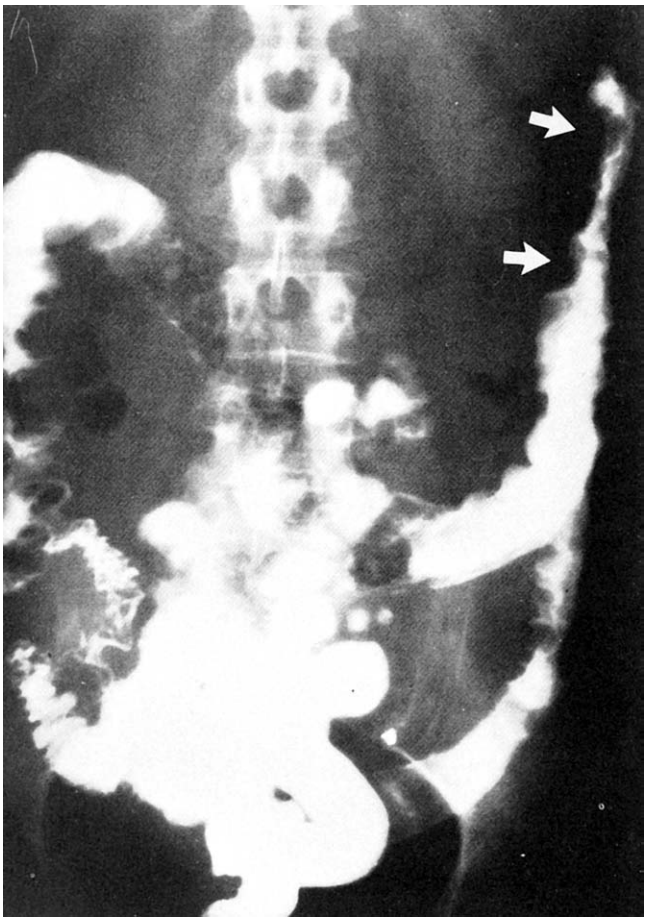


FIGURE 52 Barium enema shows thumbprinting of the entire colon (*arrow*) in a 26-year-old woman with ischemia lupus colitis. From Kistin *et al*. [307], with permission.

occurs and may resolve spontaneously (Fig. 54). In the benign form, the pathogenesis remains unclear and is not always associated with ischemia.

A rare gastrointestinal complication in SLE is colitis associated with neutropenia, a complication of immunosuppressive therapy [328]. In neutropenic colitis, barium studies should be avoided because of the risk for perforation. When cautiously performed, they show a thick-walled cecum with thumbprinting. CT, after oral contrast, also defines the cecal wall thickening and sometimes thickening of the ascending colon and the proximal portion of the transverse colon. Pneumatosis intestinalis, pericolonic fluid, and thickening of adjacent mesenteric fat may also be identified. As with acute reversible lupus gastrointestinal ischemia, bowel wall thickening in necrotizing enterocolitis can resolve with effective therapy. Bowel wall thickening and thumbprinting of neutropenic colitis cannot be differentiated from ischemia associated with SLE; however, the distribution of involvement in the cecum and right side of the colon should suggest neutropenic colitis in the appropriate clinical setting.

Chronic Gastrointestinal Lupus

Chronic gastrointestinal changes in lupus patients include esophageal dysfunction, gastric polyps, malabsorption syndrome, and protein-losing enteropathy [68, 329–341].

Esophageal abnormalities in SLE, which occur in 13–32% of patients [298, 329–334], more commonly affect the lower one-third of the esophagus and have been associated with Raynaud's phenomenon. Although the barium swallow may reveal aperistalsis, hypomotility, or esophageal dilatation, manometric

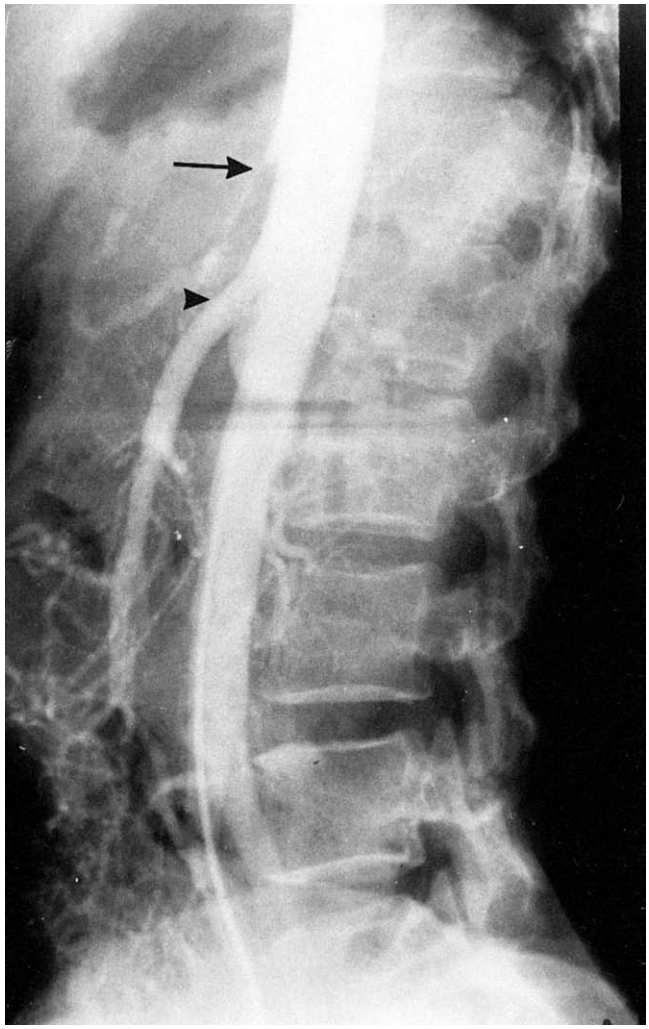


FIGURE 53 Lateral aortogram shows occluded celiac axis, patent superior mesenteric artery, and nonopacified inferior mesenteric artery in a 44–year-old patient with SLE and elevated antiphospholipid antibodies. From Asherson *et al*. [311], with permission.

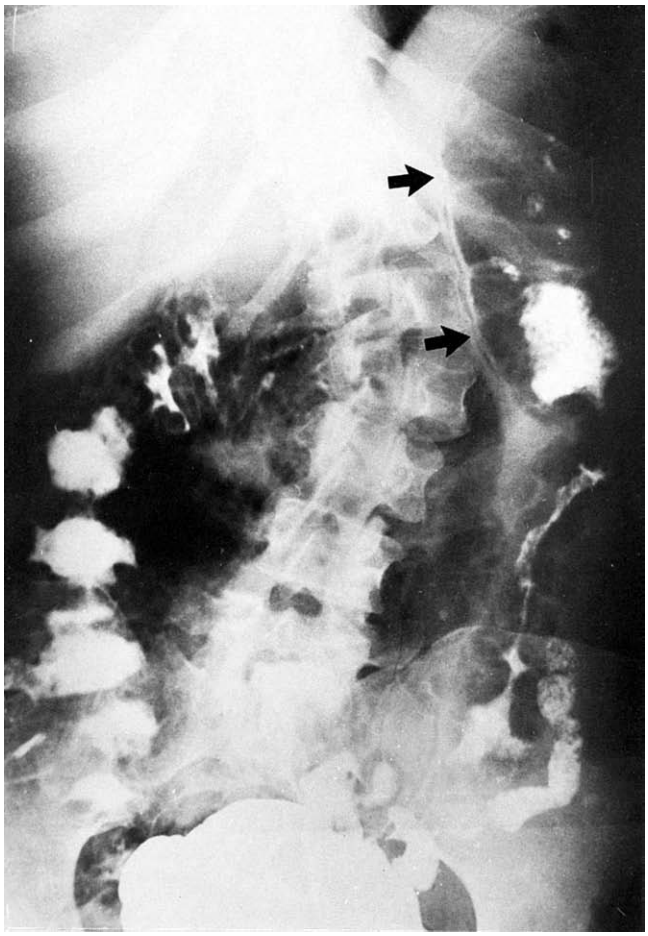


FIGURE 54 Postevacuation radiograph of barium enema shows linear air collections (*arrow*) in the splenic flexure and air bubbles in the wall of the ascending colon and in the mesocolon typical for pneumatosis intestinalis. From Freiman *et al*. [325], with permission.

studies are reported to be more sensitive for defining these changes [331, 332]. Esophageal motility disorders are less severe and less frequent in lupus patients as compared with scleroderma, mixed connective tissue disease, and polymyositis [331, 334].

Gastric polyps, seen as nodular-filling defects on gastrointestinal series, are a rare finding in lupus. Microscopic examination shows areas of chronic and acute inflammation with fibrinoid necrosis in the arteriolar walls associated with vasculitis [335].

Malabsorption was associated with SLE in several studies [336, 337, 337a]. Patients usually have abnormal d-xylose studies and/or increased fecal fat excretion [336, 337, 337a]. The radiographic pattern is similar to malabsorption in other entities with spiculation, fragmentation, and clumping of small intestinal loops. Biopsies may be normal or show blunting and loss of normal villi.

Lupus patients may also manifest a protein-losing enteropathy with coarsened and thickened intestinal folds (Fig. 55) and multiple smooth and nodular lesions predominantly in the proximal small bowel but occasionally in the stomach and colon [338–340, 340a, 341]. Jejunal endoscopy may show dilated lymphatics and submucosal edema [340a].

Ascites

Ascites may develop in patients with the nephrotic syndrome or with peritoneal inflammation secondary to primary lupus serositis, gastrointestinal lupus vasculitis, or infection. Ascitic fluid is easily identified on sonograms, CT, and MR images (Fig. 56) [342]. At sonography, thickened mesentery associated with serositis may appear as a "target sign" (echogenic rim with hypoechoic center) [343]. In addition to ascites, CT shows the significantly thickened bowel wall [342a].

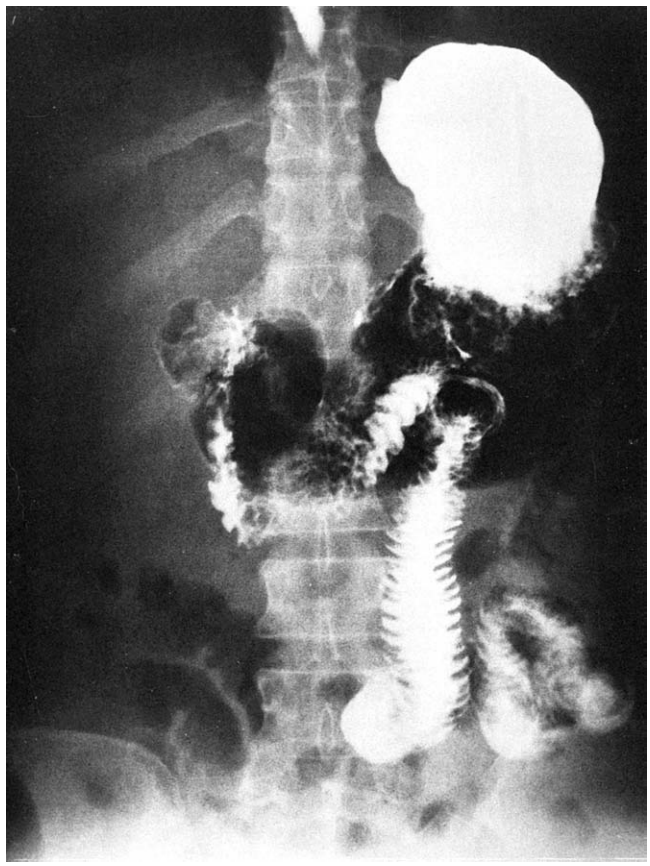


FIGURE 55 Small bowel series shows coarsened and thickened intestinal folds in a 25-year-old woman with SLE and protein-losing enteropathy. From Trentham and Masi [338], with permission.

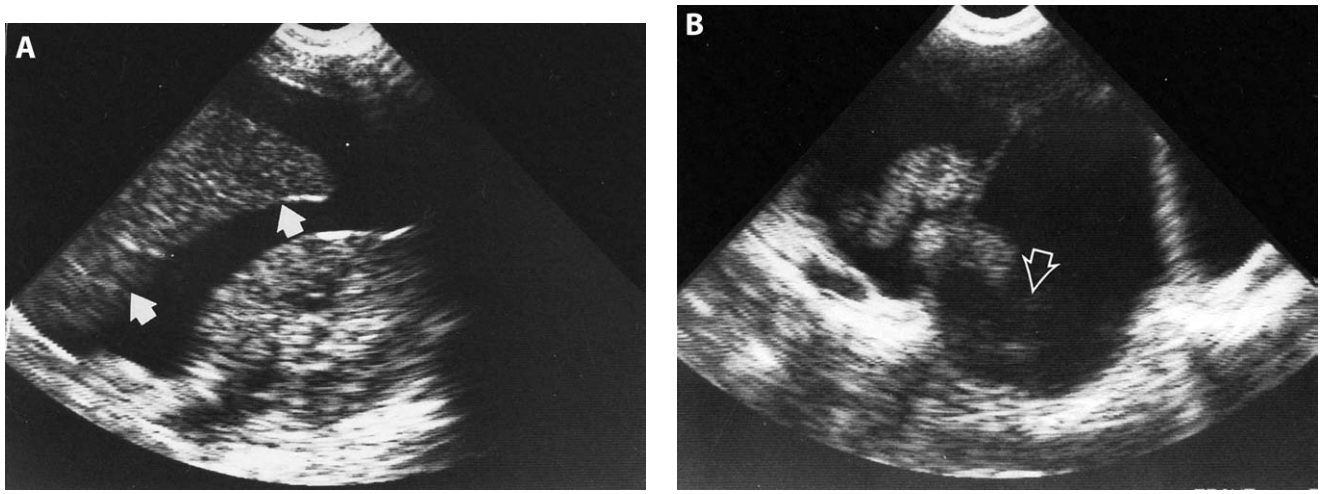


FIGURE 56 Longitudinal abdominal sonogram shows anechoic ascites (A) in Morison's pouch (*arrows*) and surrounding the liver and (B) in the pelvis (*open arrow*).

Hepatic Abnormalities in SLE

The cross-sectional imaging modalities (MRI, MRA, CT, power Doppler, and duplex sonography) [287, 346–353] can play a major role in detecting thrombosis of the portal and hepatic veins as in the Budd–Chiari syndrome in SLE and for demonstrating the rare hepatic infarction secondary to hepatic artery occlusion. Occlusion of these vessels is associated with antiphospholipid antibodies and lupus anticoagulants [344–347, 353]. These modalities can delineate the thrombus in the affected vein, which is frequently enlarged, and the level and length of occlusion of the vein and the collateral vessels (Fig. 57). Conventional angiography [354] MRI and MRA not only identify the venous occlusions and the collateral circulation but also show abnormalities of the hepatic, mesenteric, and splenic arteries and veins (Figs. 58 and 59). Aneurysms, although rare (Fig. 60), may develop in hepatic artery branches and may rupture with resultant hematomas [354, 355]. CT, sonography, or MRI can localize the associated hematoma, and interventional angiographic procedures are used to embolize the leaking vessel to induce hemostasis.

In contrast to their definitive role for evaluating hepatic and mesenteric vasculature, the different imaging techniques cannot demonstrate characteristic parenchymal hepatic changes in SLE. Fatty liver, which may be found in lupus patients on corticosteroid therapy, appears echogenic on sonograms and low attenuation on CT. Generalized hepatomegaly without focal mass or changes in architecture on cross-sectional imaging, but particularly with ascites, has been described in SLE but has also been reported in a rare case of primary lymphoma in the SLE [80, 356].

An unusual, although important, complication of treatment for SLE is the development of hepatic adenomas and even hepatocellular carcinoma in patients on longterm danazol therapy [357]. Weill *et al.* [357] reported a lupus patient who had received danazol therapy for 4 years and developed anterior chest pain and right hypochondrial pain. Echocardiography showed no pericardial effusion, but two large hypoechoic hepatic masses were incidentally discovered (Fig. 61). α-fetaprotein was negative. CT and angiography confirmed the presence of the masses but could not exclude malignancy. A left lobectomy and segmental resection of the right lobe revealed a hepatocellular carcinoma in the left lobe and an adenoma in the right lobe. The patient is clinically well 5 years postoperatively. The clinician should be aware of this possible complication and consider screening sonography in patients on longterm danazol therapy.

IMAGING OF THE RETICULOENDOTHELIAL SYSTEM IN SLE

Lymphadenopathy

Generalized lymphadenopathy [360, 361] occurs in 36–57% of lupus patients and most commonly involves the cervical, mesenteric, axillary, and inguinal and rarely the hilar regions. Lymph nodes can show changes of necrosis and hematoxylin bodies.

Development of lymphadenopathy in SLE also may herald the onset of lymphoma [362–368]. For more than 15 years, CT and MRI with standard sequences have been used to identify the extent of the disease and to

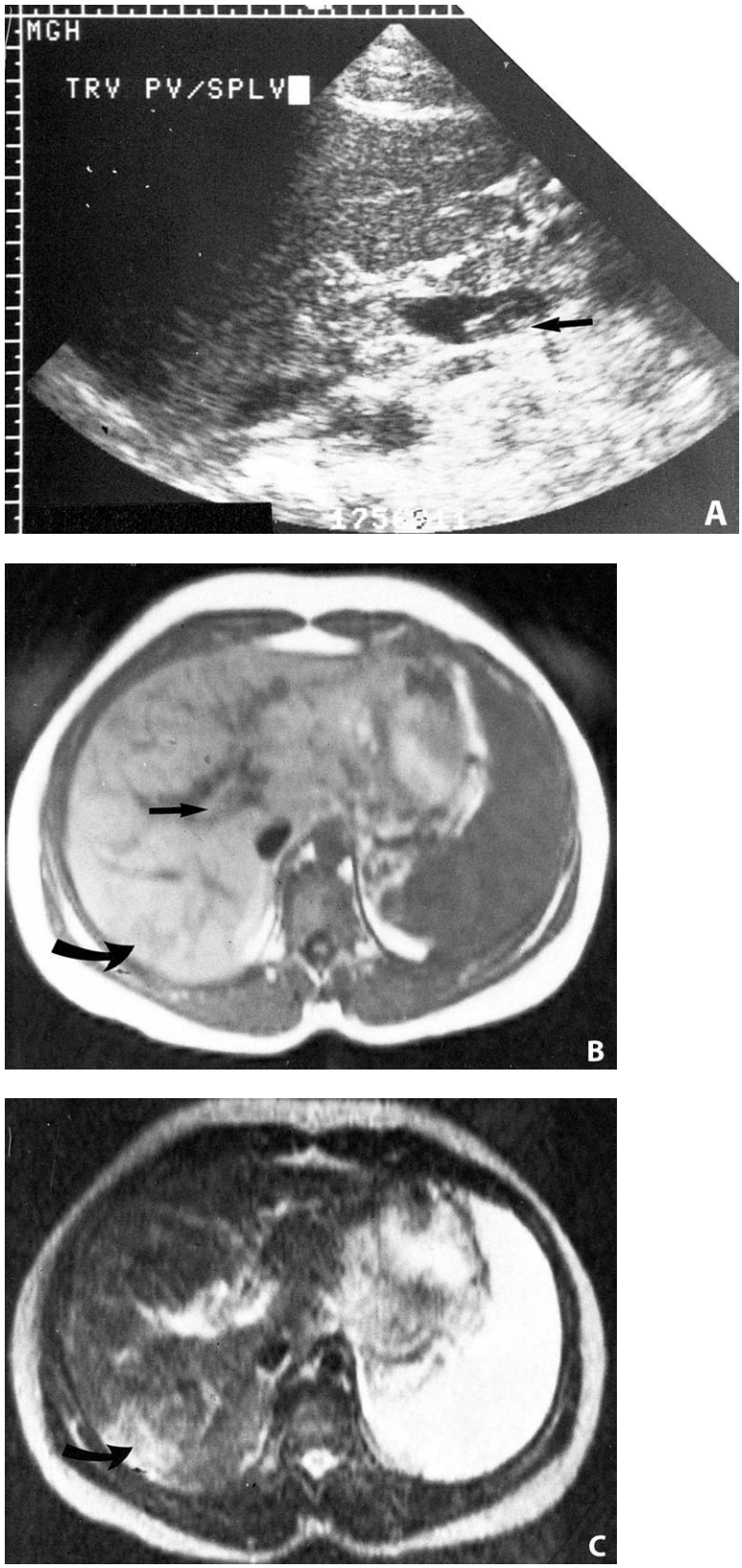


FIGURE 57 (A) Transverse abdominal sonogram shows echogenic thrombus (*arrow*) in the splenomesenteric confluence and occlusion of the intrahepatic portal vein. (B) Axial T1-weighted MR image shows the medium-signal-intensity thrombus in the portal vein (*arrow*) and a low-signal-intensity mass in the right lobe of the liver (*curved arrow*). (C) Axial T2-weighted MR image shows the high-signal-intensity thrombosed portal vein and the heterogeneous high-signal intensity right lobe lesion that probably represents hepatic infarction (*curved arrow*). From Scully [348], with permission.

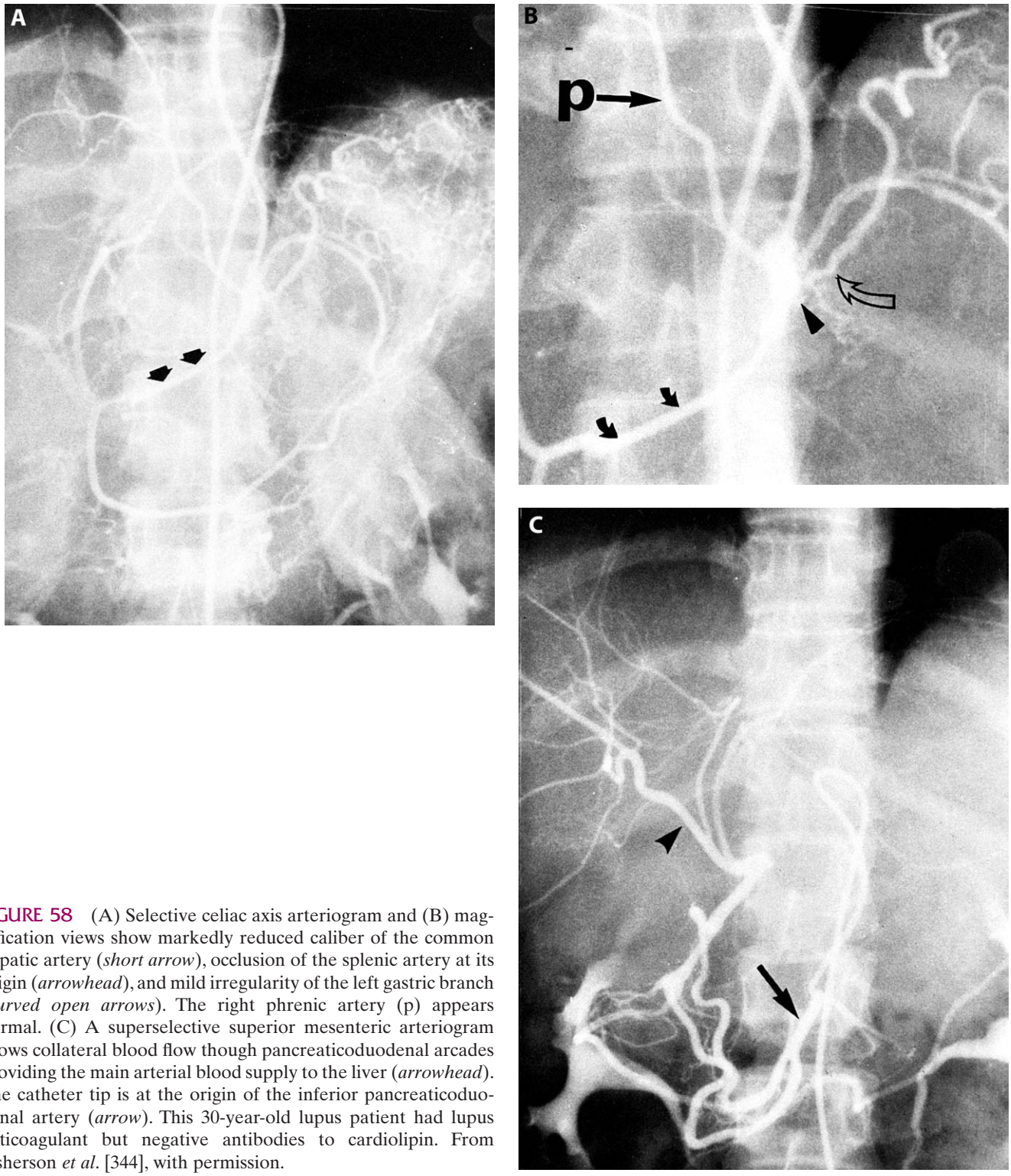


FIGURE 58 (A) Selective celiac axis arteriogram and (B) magnification views show markedly reduced caliber of the common hepatic artery (*short arrow*), occlusion of the splenic artery at its origin (*arrowhead*), and mild irregularity of the left gastric branch (*curved open arrows*). The right phrenic artery (p) appears normal. (C) A superselective superior mesenteric arteriogram shows collateral blood flow though pancreaticoduodenal arcades providing the main arterial blood supply to the liver (*arrowhead*). The catheter tip is at the origin of the inferior pancreaticoduodenal artery (*arrow*). This 30-year-old lupus patient had lupus anticoagulant but negative antibodies to cardiolipin. From Asherson *et al*. [344], with permission.

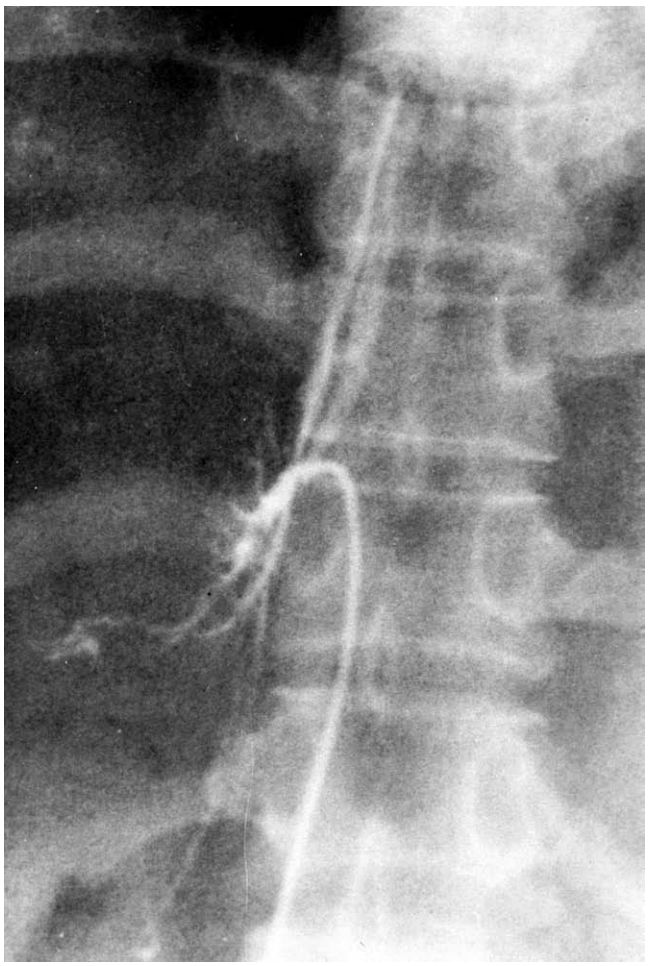


FIGURE 59 Hepatic venography shows the right hepatic vein replaced by a network of abnormal, spidery vessels in a patient with SLE, lupus anticoagulant, and Budd–Chiari syndrome. From Asherson *et al*. [344], with permission.

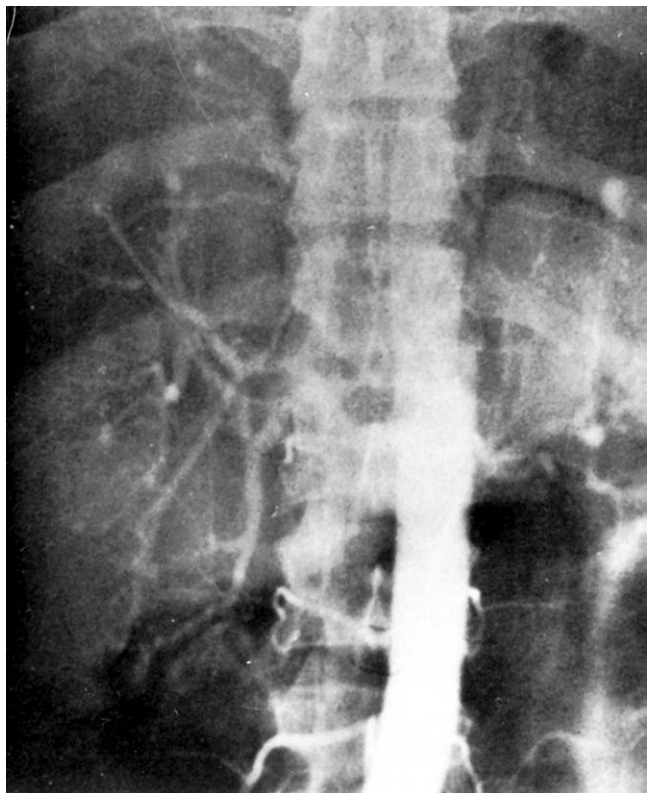


FIGURE 60 Abdominal aortogram shows multiple small pools of contrast media in the distribution of the hepatic and right renal arteries, which are consistent with microaneurysms. From Longstreth *et al*. [354], with permission.

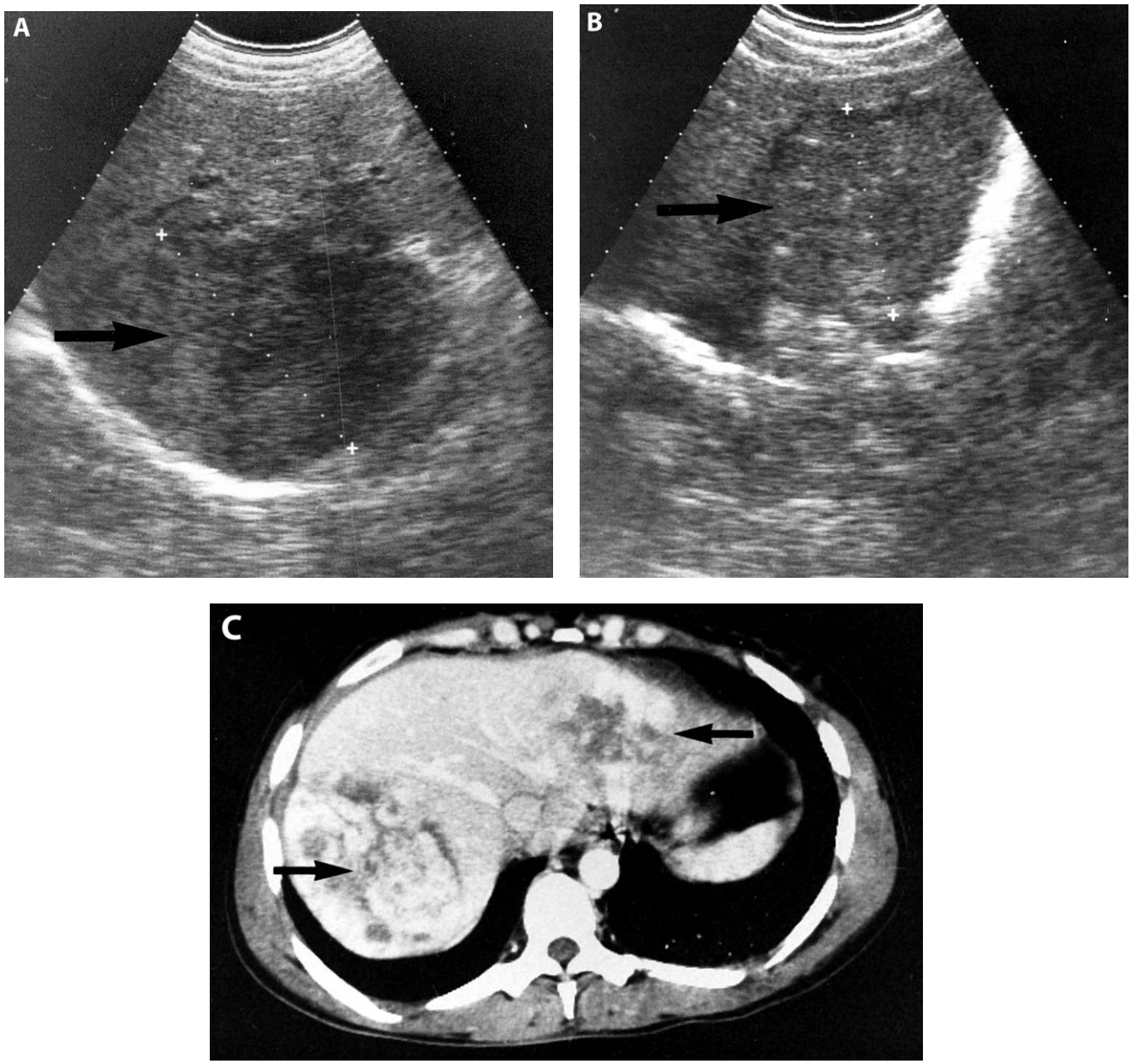


FIGURE 61 A 30-year old lupus patient on long-term danazol therapy developed hypochondrial and anterior chest pain. Echocardiocardiography showed no cardiac abnormality but incidentally showed echogenic hepatic masses (*arrows*) of the right (A) and left (B) lobes of the liver. (C) An axial contrast-enhanced CT of the liver shows heterogeneously enhancing masses of the right and left lobes (*arrows*). Surgical exploration showed a hepatocellular carcinoma in the left lobe and an adenoma in the right lobe. Courtesy of Dr. Bernard Weill, University of Paris.

follow therapeutic response. MRI is the recommended modality for assessing cerebral lymphoma because of its excellent soft tissue contrast. In the spine, MRI can differentiate lymphomatous infiltration from normal marrow [368a]. The development of new fast MRI sequences allows total body imaging in 45 minutes and could be useful for rapidly evaluating the extent of lymphomatous involvement of both soft tissues and bones [368a, 368b]. In addition, FDG-PET has shown increasing utility for staging patients with lymphoma and following their response to treatment.

Splenic Abnormalities

Splenomegaly itself is a nonspecific finding in SLE and may be also a primary manifestation of lupus or be

associated with idiopathic thrombocytopenic purpura or neoplastic infiltration.

Functional asplenia/atrophy in SLE [369–372] is characterized by decreased radiopharmaceutical uptake at scintigraphy and atrophy at sonography, CT, and MRI. However, duplex sonography/color flow Doppler, CT, and MRA also may show splenic artery and vein and flow dynamics. A decreased radiopharmaceutical splenic uptake correlates with a depressed IgM level [369]. In SLE, functional asplenia seems to predispose to significant septic complications. In one study, 6 of 10 patients with SLE and functional asplenia developed pneumoccocal or salmonella sepsis [370, 371]. Only one of these patients survived the sepsis with return of splenic function [371].

In lupus patients who have lymphomatous involvement of the spleen, neither MRI nor CT is sufficiently sensitive to detect splenic involvement. Patients with splenic lymphoma may have a small or normally sized spleen as well as splenomegaly. Splenic infiltration and its resolution can be shown on sequential FDG-PET studies.

IMAGING OF GENITOURINARY SLE

Renal Manifestations

Most patients with SLE have normal urograms and sonograms. Significant findings are found in lupus patients with infarcts, chronic lupus nephritis, superimposed infection (both acute and chronic), and renal vein thrombosis.

When chronic renal failure develops, poor renal function precludes adequate urographic visualization of the kidneys. Contrast medium is contraindicated because it may aggravate renal failure. Sonography is the preferred imaging modality for evaluating renal size, echogenicity, and obstruction. In lupus nephritis, the kidney may be normal in size and echogenicity (equal to or less than that of the liver) for even months and years after diagnosis. However, in chronic lupus nephritis, the parenchyma becomes more echogenic [373] than the hepatic parenchyma (Fig. 62). The echogenic kidney is not specific for lupus nephritis and may be seen in other chronic medical renal disease, including chronic amyloidosis, a complication of SLE. Moreover, the increase in echogenicity does not correlate with the severity of the renal compromise [374, 375]. One report [375] described multiple focal hypoechoic areas within the renal cortex in diffuse active proliferative membranous lupus glomerulonephritis, but this has not been verified by other investigators. In chronic lupus nephritis, MRI may show loss of the normal corticomedullary differentiation on T1-weighted images (Fig. 63). As with sonography, these findings are not specific for SLE and may be seen in chronic renal disease and obstruction.

When renal function suddenly worsens, obstruction must be excluded, and sonography should be carried out (Fig. 64). When sonography shows no separation of the pelvocalyceal echoes, hydronephrosis can be excluded in 95% of the patients. When sonography demonstrates

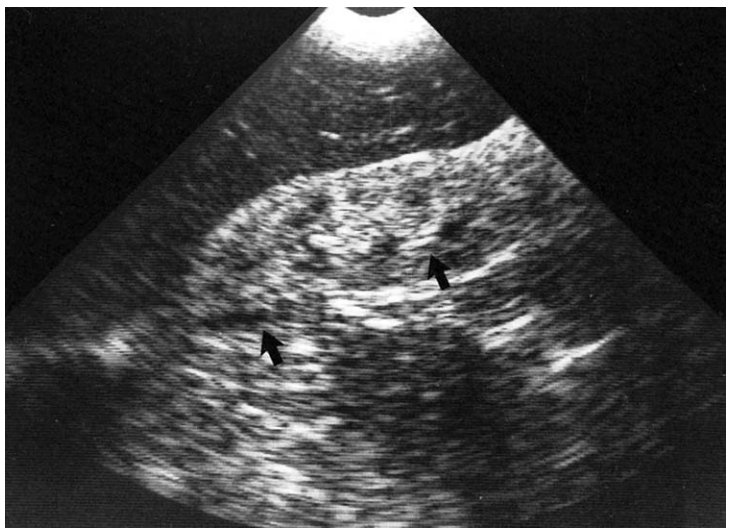


FIGURE 62 Longitudinal sonogram of the right kidney shows increased echogenicity of the kidney (*arrows*) as compared to the liver in a 27-year-old woman with SLE and chronic renal failure.

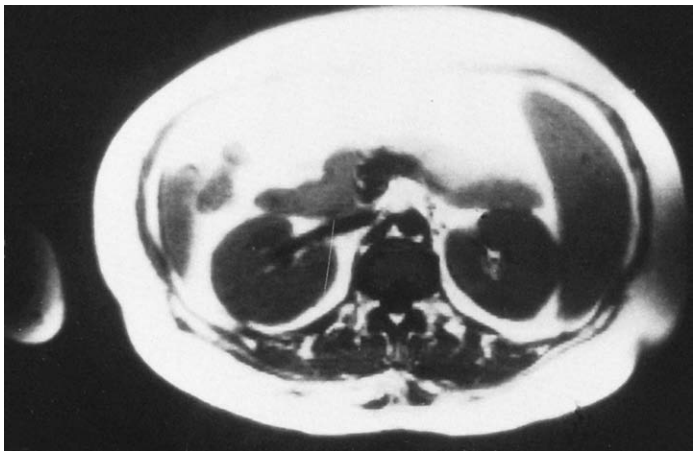


FIGURE 63 Axial T1-weighted MR image (500/30) shows loss of corticomedullary differentiation in chronic lupus nephritis.

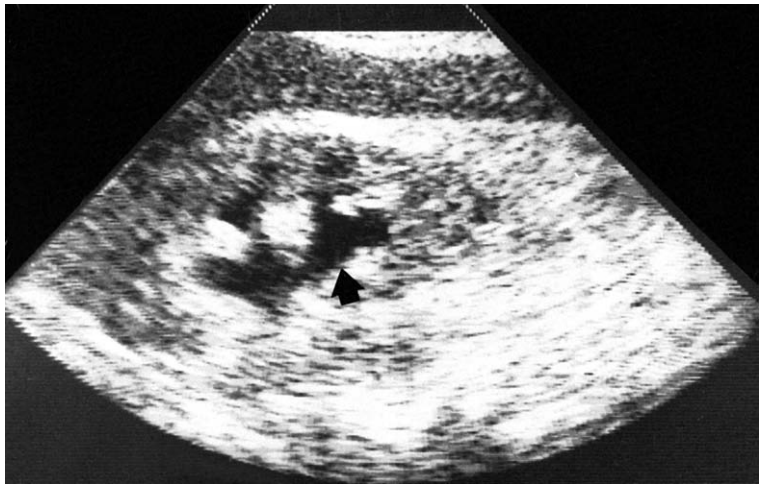


FIGURE 64 Longitudinal sonogram of an echogenic right kidney (*arrows*) shows hydronephrosis with separation of the pelvocalyceal echoes.

dilatation of the pelvocalyceal, 80% of patients will have hydronephrosis. False-positive diagnoses occur with the overdistended bladder, reflux, extrarenal pelvis, diuresis, and parapelvic cysts. When dilatation is present, renal scintigraphy after Lasix can differentiate obstructive from nonobstructive hydronephrosis.

Acute infarcts are hypoechoic, often wedge-shaped areas extending to the cortex at sonography. The peripheral wedge-shaped infarcts can also be identified at CT and MRI and appear as peripheral defects on renal Tc99m DMSA scans but are defined more clearly by the tomographic imaging of SPECT. With chronic infarcts and scarring, sonography, CT, MRI, and intravenous urography (IVU) demonstrate the cortical loss and renal contour deformities. Renal infarcts have been associated with anticardiolipin antibodies [376].

Because of their susceptibility to infection, patients with SLE may develop acute, generalized pyelonephritis. Usually, the kidneys are normal in size and echogenicity (sonography) and density (IVU). Occasionally, the IVU or sonogram may demonstrate a large edematous kidney [377]. IVU may also show diminished nephrographic density, delayed calyceal appearance time, faint visualization of the collecting systems, pyelocaliectasis, and ureterectasis. The focal form of acute pyelonephritis or lobar nephronia appears as a mass at IVU and may be indistinguishable from an abscess or even tumor. Sonography and CT aid in establishing the appropriate diagnosis [378–380]. Sonography, particularly with the newer techniques of tissue harmonic imaging sonography for improved resolution [33a], extended field of view [33b], and power Doppler,

depicts the focal pyelonephritis as a poorly marginated, hypoechoic mass with increased vascularity consistent with inflammation. Contrast-enhanced CT delineates a mass with ill-defined borders without frank liquefaction; noncontrast CT scans, however, may fail to disclose a lesion when it is isoattenuation with the renal parenchyma. When intrarenal abscesses evolve, CT, sonography, and MRI show well-circumscribed, often thick-walled intraparenchymal collections. Sonographically, the abscesses usually appear fluid-filled but may have internal echoes or even be echogenic. The presence of through transmission and refractive shadowing characterize the fluid nature of the abscess. At contrast-enhanced CT, abscesses show enhancing rims and central low attenuation necrosis [378]. At MRI, the abscess, when proteinacious, will be hyperintense on T1- and T2-weighted MR images, but, when nonproteinacious, the abscess is hypointense on T1-weighted MR images and hyperintense on T2-weighted MR images.

Infectious extension beyond the renal capsule causes pararenal and perirenal abscesses with thickening of these spaces and the adjacent psoas muscle. Both CT and MRI define the retroperitoneal extent of the abscess, but CT better identifies air in the abscess. Air bubbles in these soft tissues should alert the clinician to the possibility of a gas-forming organism. Sonography may be misleading because small air bubbles in the perirenal space are echogenic and may simulate fat and obscure the underlying small abscess.

In chronic pyelonephritis, all modalities show the small, atrophic kidney, but sonography is the most expeditious and least expensive. As with the acute process, the IVU may also demonstrate pyelocaliectasis and ureterectasis but is contraindicated in patients with compromised renal function.

In the lupus patient, the onset of renal vein thrombosis may be confusing. Frequently these patients have recurrent episodes of pleuritis. However, in the patient with a nephrotic syndrome, the sudden onset of chest pain may alert the clinician to the onset of pulmonary embolism secondary to thrombosis in the renal veins or veins of the extremities. Previously, angiography and venography [381] were (Fig. 65) and currently MRI [386] and MRA are the best tests for noninvasively studying thrombi in the renal vein and inferior vena cava and collateral circulation. Contrast-enhanced CT (Fig. 66A) can evaluate patency of the renal veins, but this study and standard venography are contraindicated with renal failure [381–385]. Renal veins can also be evaluated at duplex sonography (Fig. 66B) but may be obscured by bowel gas, particularly on the left side.

Renal arterial involvement in SLE affects primarily the small- and medium-sized arteries. At angiography, Ekelund and Lindholm [387] and Bookstein and coworkers [388] found impaired filling or nonvisualization of the interlobular arterioles on the arterial phase and absent glomerular granularity and indistinct corticomedullary junction on the nephrographic phase (Fig. 67) [387]. These angiographic changes reflect the histologic findings of diffuse glomerular involvement, partial obliteration of glomerular capillaries, and luminal narrowing of the interlobular arterioles by intimal and medial hyperplasia [388]. Occasionally lupus patients develop renal artery aneurysms (Fig. 68) [354]. Rupture of the aneurysms may result in acute retroperitoneal hemorrhage. The latter process may also develop as a complication of renal biopsy and presents as a perirenal and/or pararenal collection that may also involve the psoas muscle. As the hematoma changes from acute to chronic, the collection changes from hyperechoic to mixed to hypoechoic at sonography (Fig. 69) and from increased to normal to decreased attenuation at CT. At MRI, subacute hematomas are hyperintense on both T1- and T2-weighted MR images. Another complication of renal biopsies, arteriovenous (AV) fistulas, can be demonstrated at MRI (especially MRA) and power Doppler/duplex sonography. With AV fistulas, the renal vein develops an arterialized flow pattern.

Bladder Abnormalities

The differential diagnosis of bladder abnormalities of SLE includes lupus interstitial cystitis [389–393a], infectious cystitis, bladder tumor, and cystitis secondary to cyclophosphamide therapy [394, 395] and occasionally the neurogenic bladder [396]. IVU, sonography, and CT depict cystitis of various etiologies as bladders with thickened, sometimes irregular, walls with reduced capacity. Contrast-enhanced CT also shows an abnormally enhancing bladder wall [393]. Weisman *et al.* [389] described two patients with lupus cystitis who also showed pyelocaliectasis and ureterectasis (Fig. 70). Obstruction, when present, occurred at the ureterovesical junction. In one patient, immunofluorescent deposits were found in the blood vessel walls of the bladder, suggesting an immune etiology [389]. Recognition of this type of bladder involvement in SLE is important because it may be a partially reversible cause of renal failure. The acute radiographic and clinical findings usually improve with corticosteroid therapy. Bladder cancers, usually first evaluated at cystoscopy because of a history of hematuria, are staged at CT and MRI studies. The neurogenic bladder, as a complication of transverse myelitis, may be either hyperreflexic (upper motor neuron lesion) or areflexic (lower motor neuron lesions). Radiographic voiding cystourethrogram and urodynamic studies are the usual studies

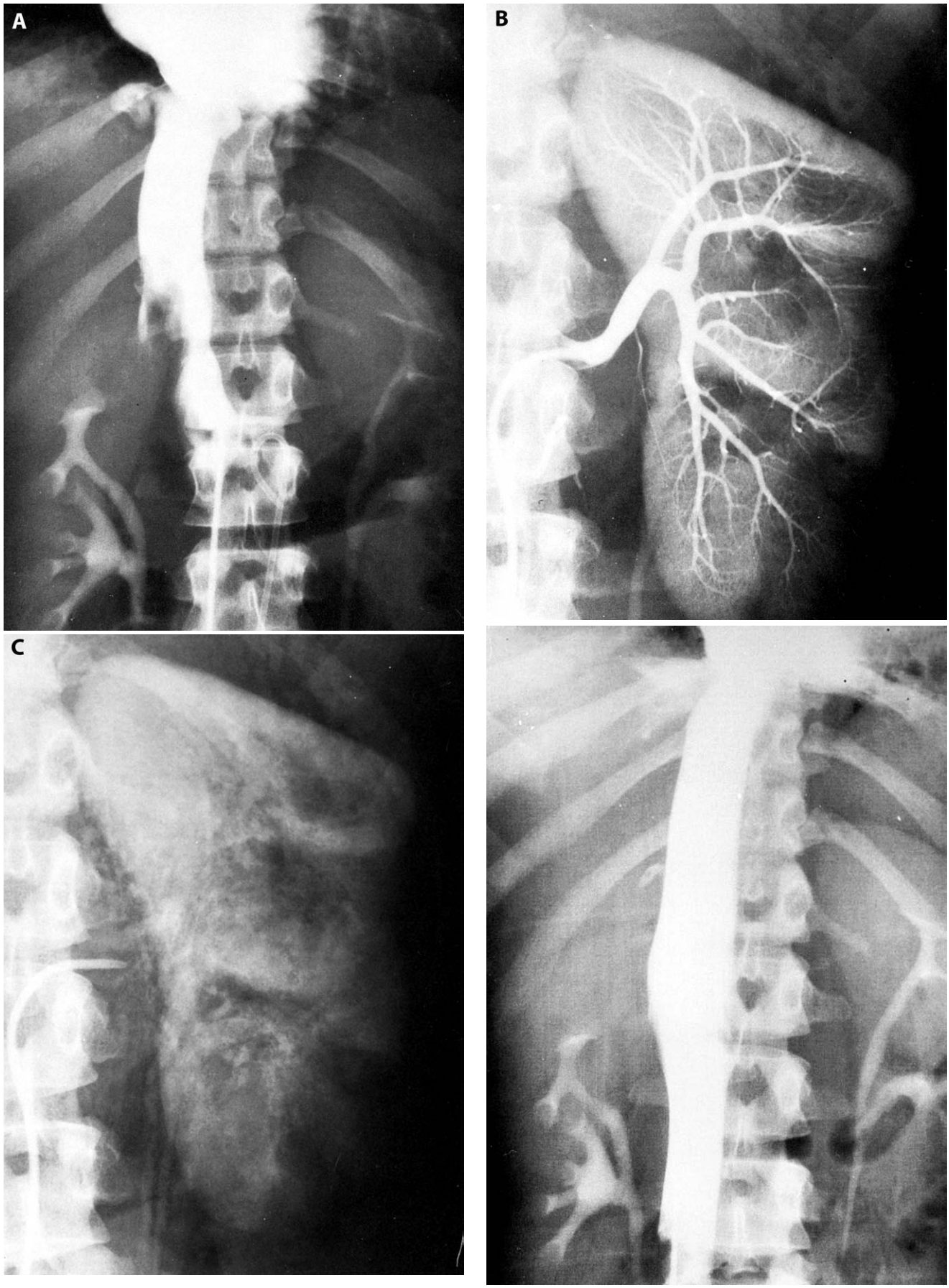
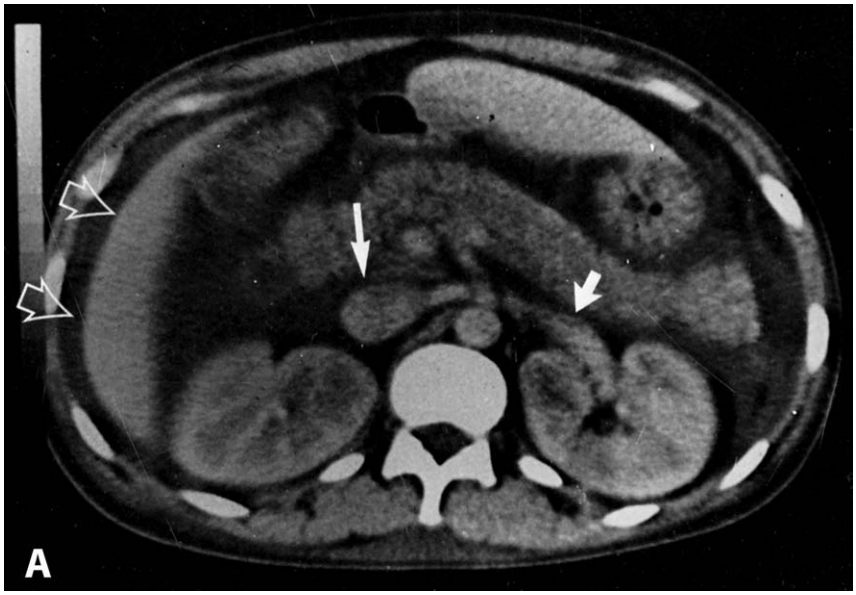


FIGURE 65 A 27-year-old woman with nephrotic syndrome and membranous lupus nephritis. (A) An inferior vena cavagram shows a thrombus in the IVC extending from the level of the renal veins. (B) Arterial phase of a left renal arteriogram shows an enlarged kidney with stretched branches. (C) Venous phase of a renal arteriogram shows primary drainage through venous collaterals and ovarian vein. (D) After treatment, the inferior vena cavagram shows a patient cava with normal washout of contrast media by "streaming" of blood flow from patent renal veins. From Appel *et al*. [381], with permission.



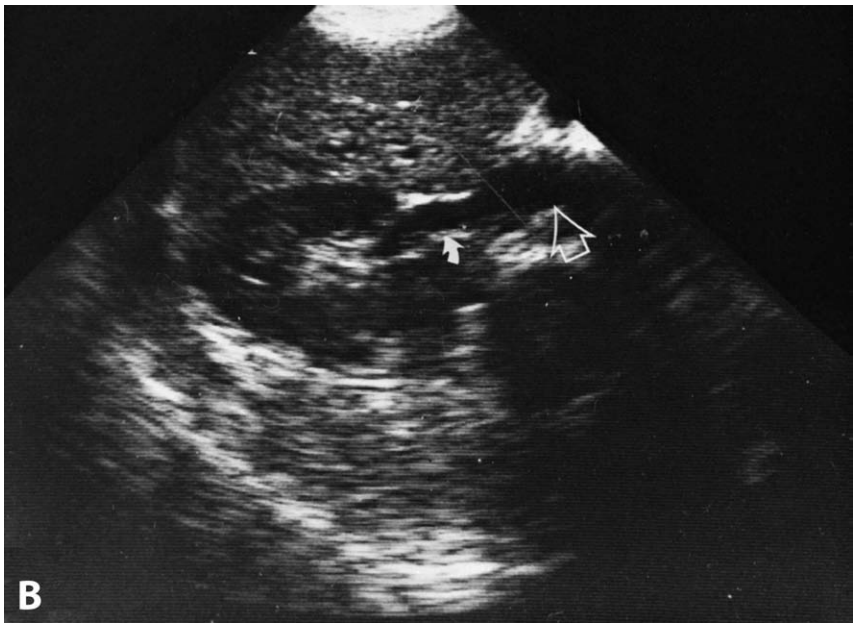


FIGURE 66 (A) Axial contrast-enhanced CT of the abdomen shows a patent left renal vein (*short arrow*) and inferior vena cava (*long arrow*) in a lupus patient with nephrotic syndrome without renal failure. The liver (*open arrows*) is pushed medially by ascites. From Strahlman *et al*. [382], with permission. (B) Transverse sonogram shows a patent right renal vein (*arrow*) entering a patent inferior vena cava (*arrowhead*) in a 23-year-old patient with SLE.

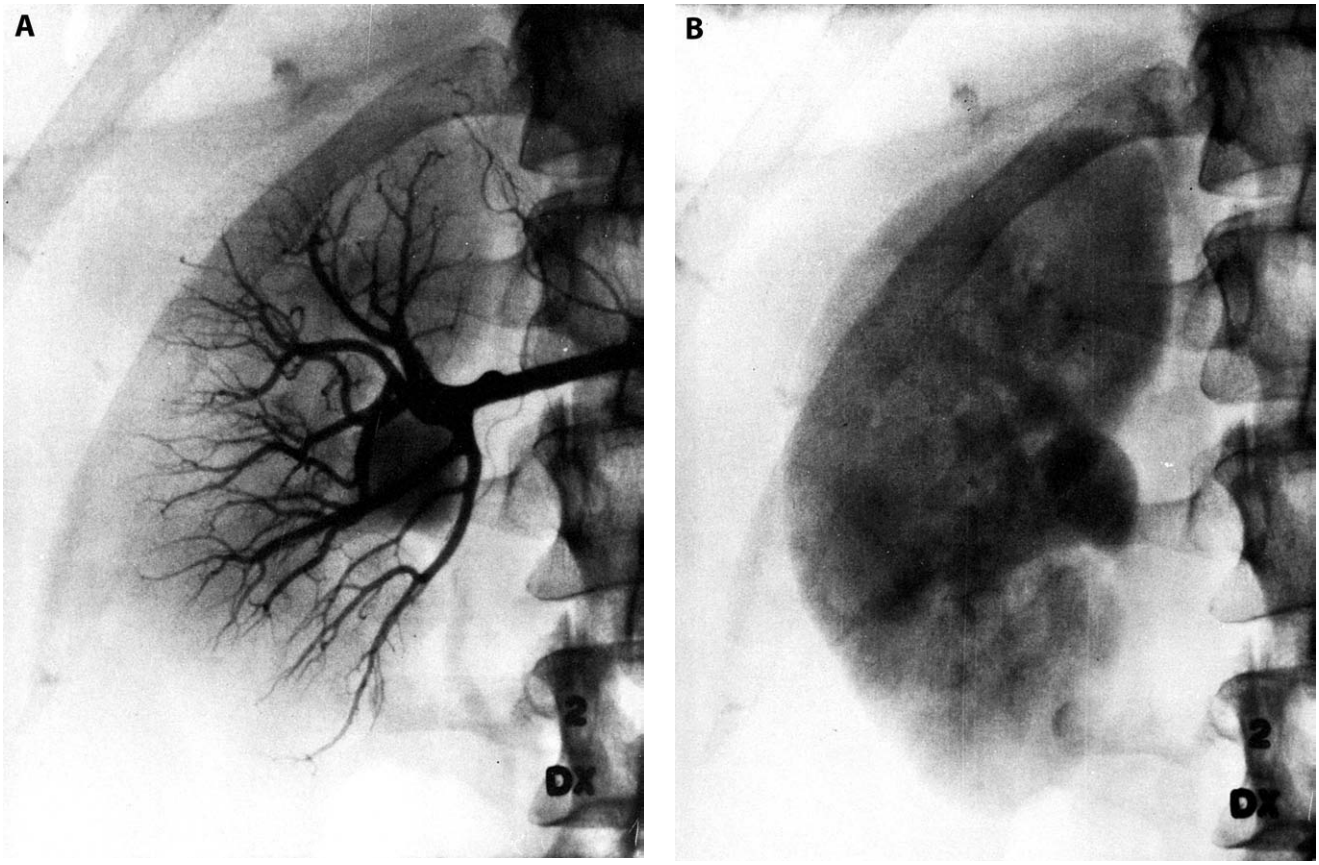


FIGURE 67 (A) Arteriographic and (B) nephrographic phases of selective angiograms of the right kidney reveal interlobar arteries narrowed toward the periphery, nonfilling of interlobular arteries, and indistinct boundary between the cortex and the medulla in arterial involvement in SLE. From Ekelund and Lindholm [387], with permission.

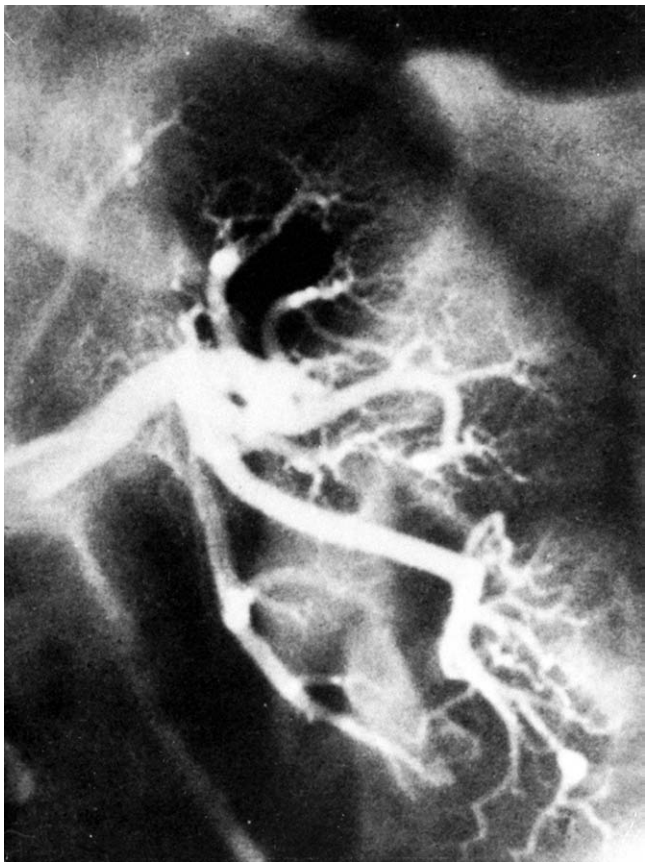


FIGURE 68 Selective arteriogram of the left kidney demonstrates a microaneurysm of a lower pole intralobar artery. From Longstreth *et al*. [354], with permission.

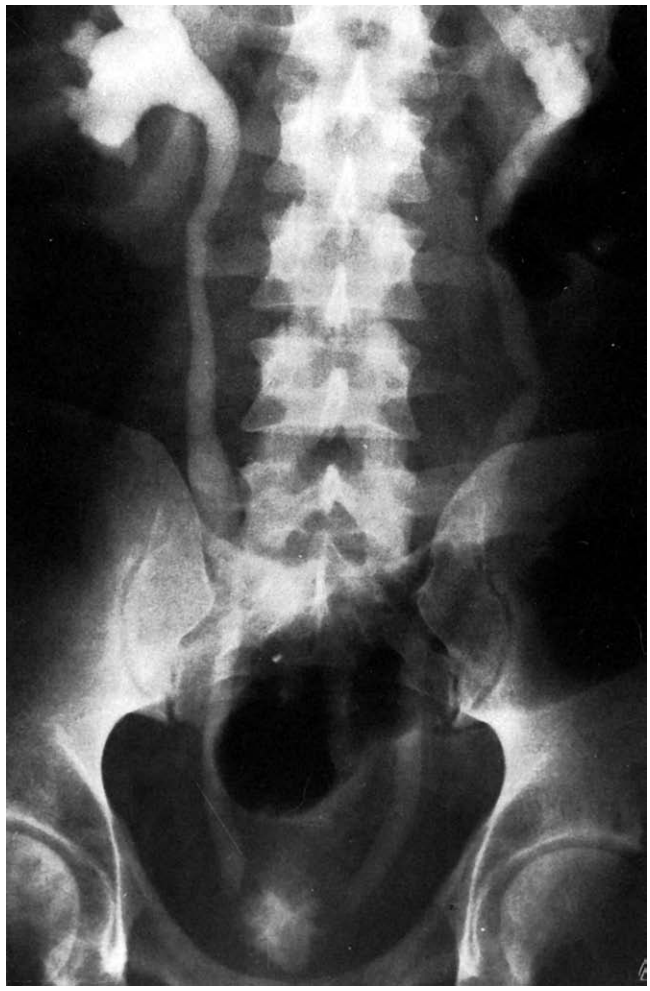


FIGURE 70 Intravenous urography demonstrates bilateral hydroureters, hydronephrosis, and a small contracted bladder in a 33-year-old man with lupus cystitis. From Weisman *et al*. [389], with permission.

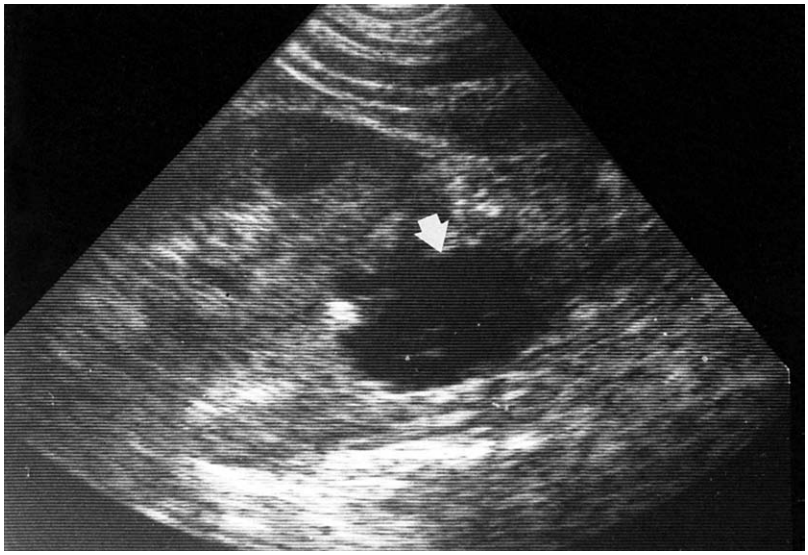


FIGURE 69 Longitudinal sonogram of the right kidney shows a chronic anechoic retroperitoneal hematoma posterior and inferior to the kidney in a 27-year-old woman with membranous lupus nephritis.

performed for evaluating dysfunction. Another technique, the endorectal sonographic voiding cystourethrogram, developed by Shapeero *et al*. [397], can also evaluate neuromuscular function of the bladder neck and urethra (Fig. 71) and could be applicable to the lupus patient. This technique requires no irradiation or catheterization with its attendant risk of infection. During the procedure, the patient can be monitored continuously, without irradiation, for subtle abnormalities of dyssynergia and for the effects of drugs. This technique can be particularly useful for younger patients who need multiple sequential examinations to evaluate for the efficacy of medical and surgical therapy.

Renal Allografts and Their Complications

William Amend (personal communication, University of California, San Francisco) found that lupus patients comprised 3% of their patients receiving cadaveric allografts and 8% of those waiting for cadaveric allografts. It is more difficult to match the lupus patient with a histocompatible allograft because of the more complex antibody profile in SLE. Improved histocompatibility matching, refined surgical technique, and more effective immunosuppressive therapy (azathioprine, prednisone, and cyclosporine) enhance graft survival [398–400]. However, lupus patients do not appear to do as well as patients with renal allografts with other end-stage renal disease [401]. Several groups of investigators have reported an increased risk of graft failure in lupus patients within the first year and frequently within the first month after transplantation, a prevalence as high as 35–40% [401–410]. The most important cause of early graft loss in the lupus patient appears to be allograft rejection. In addition, thrombosis of renal arteries and veins may lead to graft loss in renal transplant patients because allografts have no collateral vessels [401]. Antiphospholipid antibodies and lupus anticoagulants have been associated with thrombosis of allograft arteries and veins in SLE.

Although there is some overlap, major complications of renal allografts during three major time periods: the first 24 h after transplantation, the first postoperative week, and weeks to months after transplantation. Renal allografts are subject to both renal parenchymal insults ("medical" complications) [398, 400, 411] and mechanical injuries ("surgical" complications) [398–400, 412, 413].

The First 24 Hours after Transplantation

Sudden anuria should alert the clinician to the possibility of hyperacute rejection, renal arterial or venous occlusion, acute compression of the allograft by acute hematoma, or urinoma. When portable scintigraphy (^{99m}Tc DTPA or ^{131}IOH) and/or portable duplex sonography/power Doppler both show no flow, the patient returns to surgery immediately [398].

The First Week after Transplantation

When the transplant patient becomes oligoanuric, the most frequent medical complication is acute tubular necrosis (ATN) that remains stable or slowly improves over several days or even weeks provided that rejection or other insults do not complicate ischemic damage [398, 414, 415]. In previous years, renal scintigraphy was the first test used to document decreased perfusion and/or decreased parenchymal function [414, 415] (Fig. 72) but is nonspecific for differentiating ATN from acute rejection and cycloporine toxicity, which are less common during the first postoperative week [414–416]. Currently, during the first postoperative week, patients are followed with power Doppler/duplex sonography as the baseline study or for excluding accelerated rejection or surgical complications of urinary obstruction and perirenal collections (most commonly hematoma and urinoma). Some investigators empirically biopsy the renal allograft on the seventh day with sonographic guidance (personal communication, William Amend, University of California, San Francisco). Other investigators biopsy the allografts when resistance indices on duplex sonography remain elevated and accelerated rejection is the presumed diagnosis [398]. Obstruction, detected at sonography, is corrected either by direct surgical intervention or by fluoroscopically or sonographically-guided percutaneous nephrostomy or stents depending on the location of the obstruction.

Complications of Renal Allografts Weeks to Months after Surgery

The symptom complex of rejection (fever, oliguria, local tenderness, and hematuria) is diagnostically nonspecific and may also be seen in acute rejection, urinary tract obstruction or extravasation, primary vascular compromise, and infection. Appropriate imaging studies along with the clinical examination will permit the correct differentiation [398].

Medical Complications of Renal Allografts

Power Doppler/duplex sonography are particularly useful for diagnosing rejection. Measurement of the vascular resistance of the renal vascular bed is the most specific imaging technique for evaluating rejection. Maximum systole and minimum diastolic flow velocities are obtained from interlobar, segmental arteries, and arcuate arterioles [398, 417–420]. With rejection, the

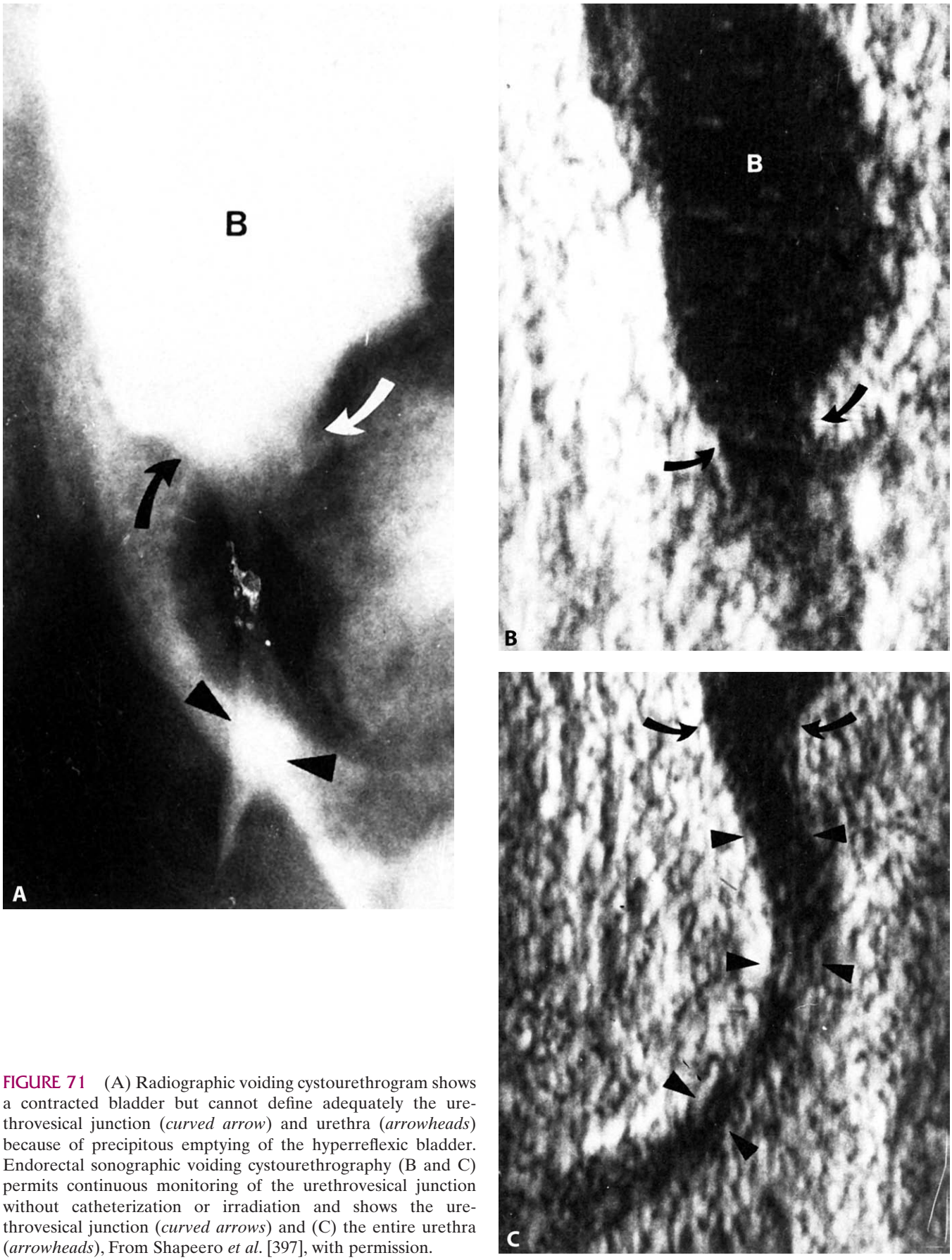


FIGURE 71 (A) Radiographic voiding cystourethrogram shows a contracted bladder but cannot define adequately the urethrovesical junction (*curved arrow*) and urethra (*arrowheads*) because of precipitous emptying of the hyperreflexic bladder. Endorectal sonographic voiding cystourethrography (B and C) permits continuous monitoring of the urethrovesical junction without catheterization or irradiation and shows the urethrovesical junction (*curved arrows*) and (C) the entire urethra (*arrowheads*), From Shapeero *et al*. [397], with permission.

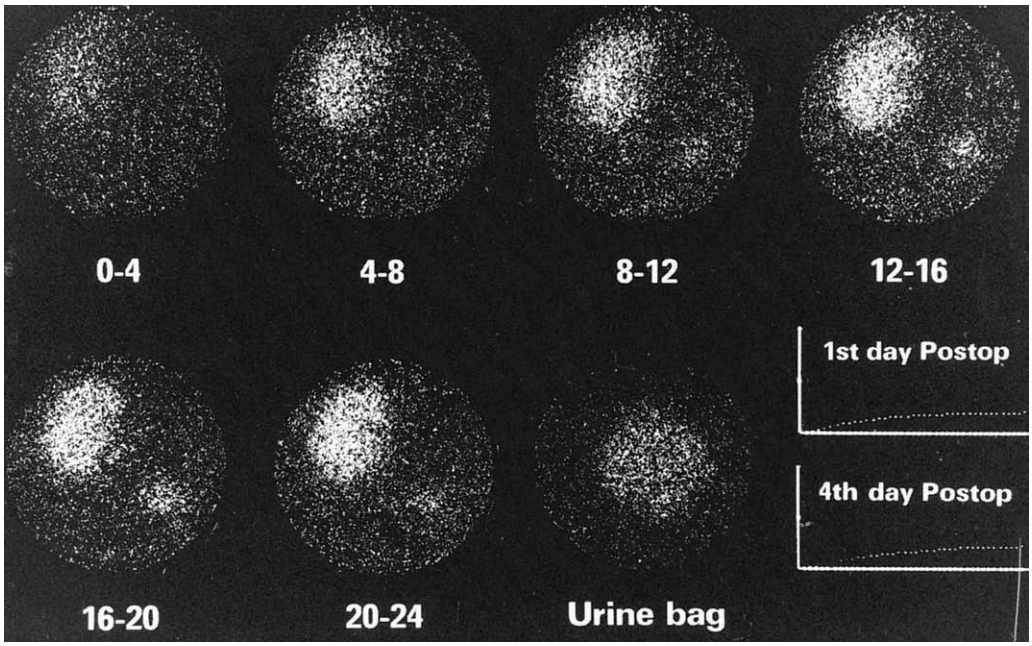


FIGURE 72 ^{131}I scintigraphy and computer-generated renogram (*right*) show delayed perfusion and delayed excretion in a renal allograft from the first to fourth postoperative day suggestive of acute tubular necrosis. From Shapeero [398], with permission.

resistance of the renal vascular bed increases as evidenced by a decrease in, absence of, or even reversal of diastolic flow and is measured by the resistive index (Fig. 73). The sonographic morphologic features of allograft swelling, medullary pyramid enlargement, pelvi-infundibular thickening, increased medullary conspicuity, and diminished renal sinus fat echogenicity are less sensitive than Doppler resistances but may be used in combination with the Doppler spectrum [417, 421–423]. When the clinical and sonographic findings of acute rejection correlate, biopsy is often done for confirmation. Although duplex sonography has a high predictive capability for diagnosing acute rejection, other pathologic processes, such as pyelonephritis, severe ATN, renal vein thrombosis, acute obstruction, and extrarenal compression of the graft secondary to perinephric collections, may occasionally cause elevated vascular resistive indices [419, 424, 425] and should be excluded at sonography [398] and scintigraphy. MRI can be performed when other modalities are equivocal.

Papillary necrosis [426, 427], although uncommon, can develop as early as 2 months after transplantation. Medullary ischemia has been suggested as the final common pathway for this process. Generalized papillary necrosis can result from fulminant acute rejection or may accompany long-term rejection and gradual loss of renal function. It may also be a sequela of acute pyelonephritis, renal vein thrombosis, ATN, or analgesic therapy. IVU is contraindicated in these patients because of deteriorating renal function. Hoffman *et al.* [427] and Shapeero and Vordermark [426] have described the sonographic features of papillary necrosis as multiple round and triangular anechoic spaces and/or echogenic necrosed papillae in the region of the papillae (Fig. 74).

Recurrent lupus nephritis (although relatively rare) [401] or *de novo* lupus nephritis may develop in the allograft and may show the same findings as the native kidney: increased echogenicity at sonography and loss of corticomedullary differentiation at MRI [428–430].

Another complication of renal allografts is segmental infarction. Power flow Doppler/duplex sonography shows decreased perfusion of the infarcted region and a difference in echogenicity between infarcted and normal renal parenchyma. On T2-weighted MR images, the *normal* cortex increases in signal intensity as compared with the cortex on T1-weighted images. However, the infarcted segments of the kidney do not show the signal intensity increase and display loss of corticomedullary differentiation [431].

Because lupus patients with allografts receive immunosuppressive therapy, they are more susceptible to infection. Focal or diffuse pyelonephritis, abscess, and emphysema of the renal allograft show similar sonographic, CT, and MR findings as with the native kidney (see earlier discussion on the native kidney).

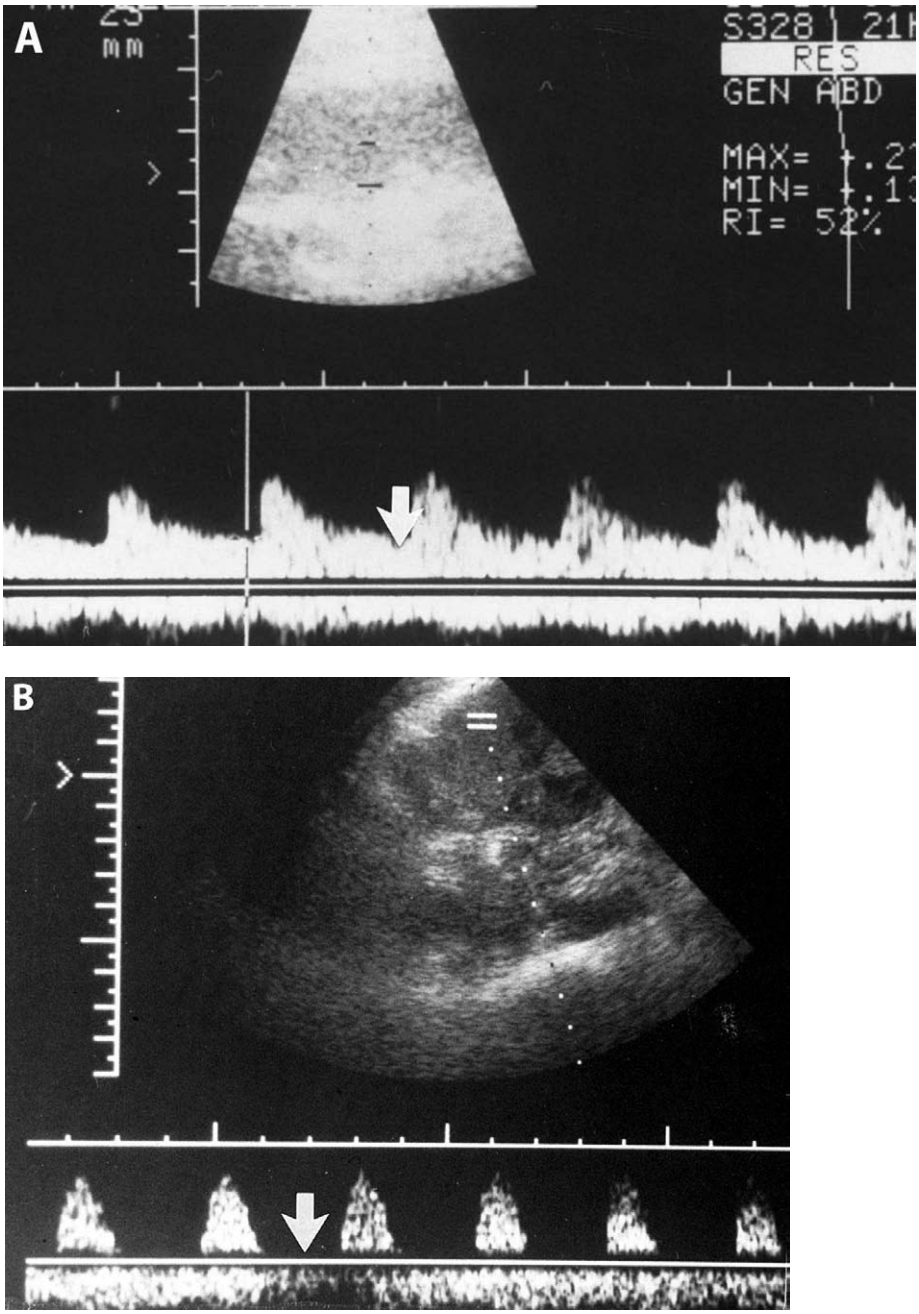


FIGURE 73 (A) Doppler spectrum in a normal renal allograft shows normal diastolic flow (*arrow*). (B) Doppler spectrum in an allograft undergoing rejection shows absent diastolic flow (*arrow*).

Another complication of the renal allograft is the potential development of malignancy. Renal transplant patients, in general, who are on cyclosporine therapy, have 350 times the incidence of lymphoproliferative disorders as the general population [432]. When cyclosporine regimens are compared with those based on azathioprine and cyclophosphamide, lymphomas comprise 20% of neoplasms in the former group and only 11% in the latter group [433]. In the lupus transplant patient, several investigators have found an increased risk of various cancers (Hodgkin's lymphoma, non-Hodgkin's lymphoma, cervical and prostate cancer) [433a, 433b, 433], although the overall risk for malignancy does not appear to be increased [433b, 433c]. Thus, patients with SLE should have careful long-term follow-up with MRI and CT studies when needed.

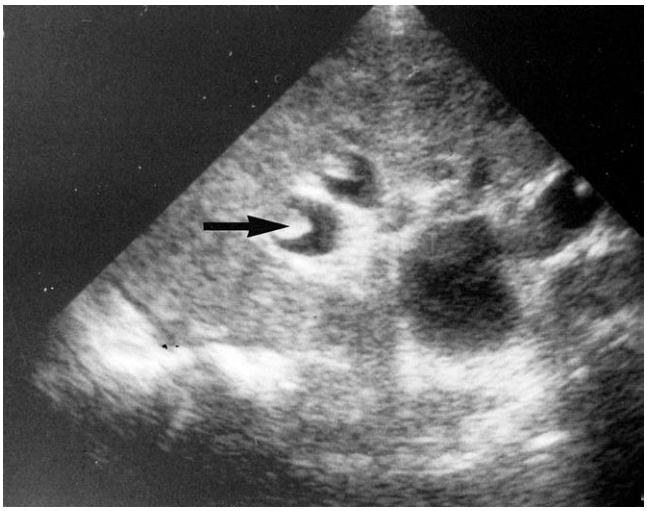


FIGURE 74 Longitudinal sonogram of renal allograft demonstrates necrotic papillae (*arrows*) in papillary necrosis.

Surgical Complications of Renal Allografts

Urinary obstruction is the most common surgical complication of allografts (Fig. 75) and can be divided into three types: (1) intraluminal obstruction from calculus, blood clot, fungus ball, or necrotic papillae; (2) intrinsic ureteral obstruction due to edema, necrosis, stricture secondary to ischemia or kinking; and (3) extrinsic ureteral compression from perinephric collections, retroperitoneal fibrosis, and arterial anastomoses [398–400, 434–439]. Sonography demonstrates hydronephrosis [398] and some of the causes of obstruction: echogenic calculus, fungus ball, blood clot or necrotic papilla, and the perinephric collection. Separation of the pelvocalyceal echoes on sonography, however, does not always signify a urologic complication and can be seen in allograft rejection. When the bladder is overdistended or atonic (the neurogenic bladder in the lupus patient), hydronephrosis can be functional. When sonography demonstrates obstruction and renal function is deteriorating, antegrade pyelography can be performed with Whitaker flow measurements, as needed [400]. Interventional radiographic techniques not only identify the location and type of obstruction but also provide the definitive therapy such as stone or papilla extraction and balloon dilatation and stenting of ureteral strictures by antegrade and retrograde techniques [439, 440].

Extravasation occurs in 1–2% of allografts [436, 440]. Although scintigraphy may suggest extravasation, sonography, CT, and MRI confirm the location of the perirenal collection [398, 411, 440–443], and antegrade or retrograde pyelography and the voiding cystourethrogram show the exact point of leak [443]. The most common locations are the ureteroneocystotomy site or the cystotomy through which the ureterocystotomy was created.

Perinephric collections have been reported in up to 51% of allografts [399, 400, 440–444]. The majority of urinomas and hematomas develop in the first postoperative week, whereas most lymphoceles and abscesses occur after the first week [436]. Both urinomas and lymphoceles conform to the anatomic spaces, whereas abscesses and hematomas may not [399]. Sonography, CT, and MRI can define the extent of the perirenal collections as with the native kidney. Sonography and CT are not specific for characterizing the type of collection. Urinomas and lymphoceles are often cystic masses with or without septations. Hematomas show variable echogenicity depending on their age, changing from echogenic in the acute stage to hypoechoic in the chronic stage. Abscesses may be anechoic or echogenic and show through transmission. As with the native kidney, MRI can help differentiate the lymphocele or urinoma (hypointense on T1-weighted MR images and hyperintense on T2-weighted MR images) from the subacute hematoma or proteinaceous abscess (hyperintense on both T1- and T2-weighted MR images) (Fig. 76). When patients are afebrile and have normal renal function, small collections are usually followed sonographically. When patients are symptomatic (febrile, edematous, or tender over the allograft) or when they have increasing creatinine, fine-needle aspiration should

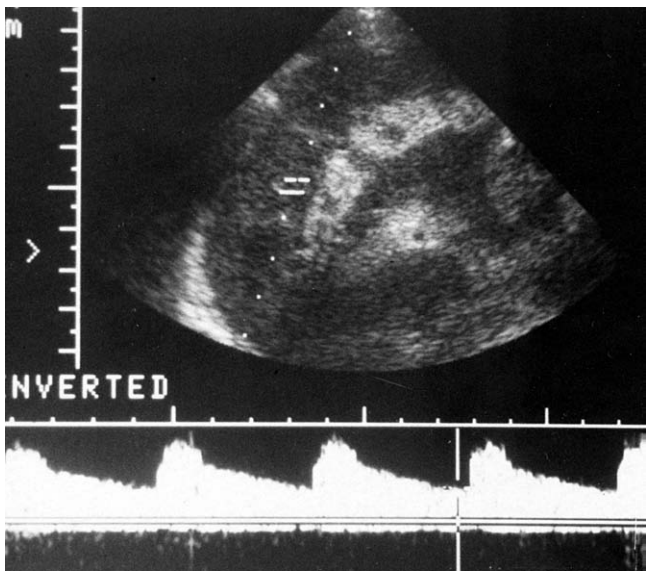


FIGURE 75 Longitudinal sonogram of renal allograft shows separation of the pelvocalyceal echoes and hydroureter consistent with hydronephrosis. The Doppler spectrum demonstrates normal diastolic flow. This 33-year-old lupus patient had decreasing renal function.

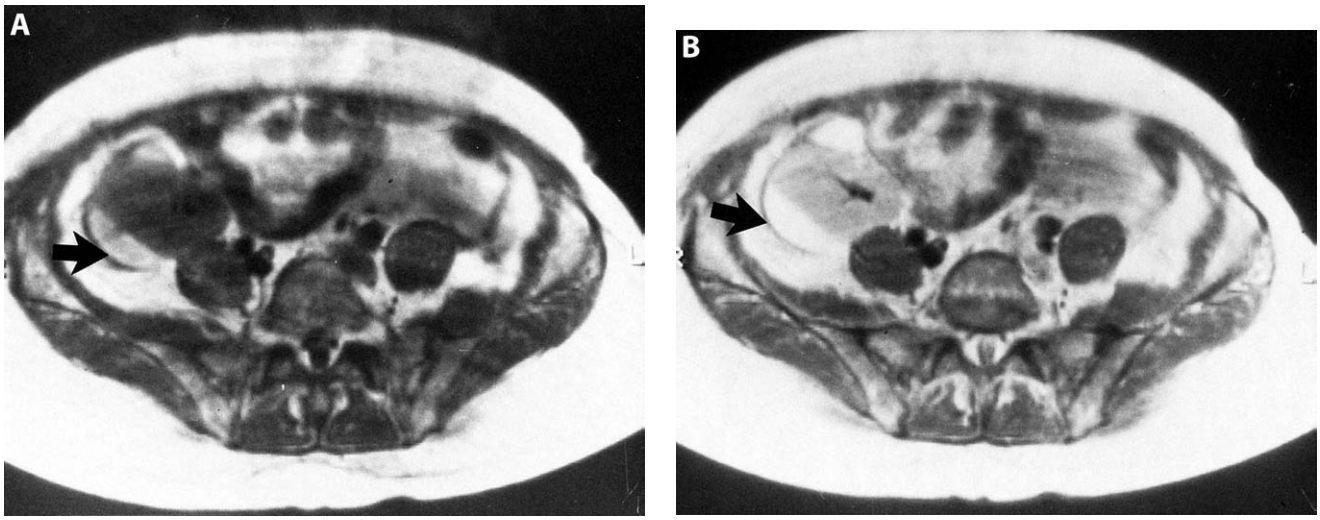


FIGURE 76 (A) Axial T1-weighted MR image (500/30) and (B) T2-weighted MR image (2000/60) show a high-signal-intensity, subacute perirenal hematoma surrounding a renal allograft after biopsy.

be performed with CT or sonographic guidance to determine the etiology. Urinomas and hematomas will require immediate surgical intervention and evacuation. Lymphoceles can be marsupialized internally via a peritoneal window, and abscesses are drained surgically or percutaneously under sonographic or CT guidance.

Vascular Complications of Renal Allografts

Renal artery stenosis is the most frequent surgical vascular complication in renal allografts and occurs in 7–23% of patients [413, 445, 446]. It may be caused by intimal hyperplasia at the anastomotic site, intraoperative trauma to the allograft artery, or arteriosclerosis of the donor vessel. Rarely vascular occlusion can result from torsion of the renal allograft in the intraperitoneal renal allograft [445]. Arterial stenosis should be suspected when patients present with severe and refractory hypertension and/or deterioration of renal function. Previously, conventional angiography or digital subtraction angiography [445a–448] was performed to show the stenotic area. Currently, three-dimensional MRA provides vascular information without ionizing radiation, iodinated contrast medium, or arterial catheterization. MRA improves the accuracy and sensitivity for detecting and grading the severity of the stenosis and provides flow measurements [445]. Eight-one percent of stenoses in renal transplant patients are treated successfully with percutaneous transluminal angioplasty [448]. Other vascular complications identified at angiography, particularly MRA, are intimal dissections, pseudoaneurysms, complete occlusion of the renal artery, renal vein thrombosis, and arteriovenous fistulae resulting from biopsy [398, 399, 445]. Power Doppler/duplex sonography can identify the A-V fistula and renal vein thrombosis [449–453]. MRA can produce excellent images of fistulae and thromboses but, because of expense, may be reserved for equivocal sonographic studies. Interventional radiologic techniques are used for the treatment of fistulae and vascular occlusions. A-V fistulae are occluded with coil placement, and acute vascular thrombi can be dissolved with the infusion of fibrinolytic drugs [454].

IMAGING OF OBSTETRIC SLE

Echocardiography, color flow Doppler, and duplex sonography identify the important fetal and placental abnormalities associated with lupus pregnancies: cardiac-related abnormalities (congenital heart block and other arrhythmias, cardiac dysfunction secondary to myocarditis and rarely pancarditis), intrauterine growth retardation, and placental abnormalities.

Intrauterine Cardiac Abnormalities of Neonatal Lupus

Isolated A-V block occurs in 1 in 20,000 births [455, 456]. Several clinical studies have reported that an isolated congenital A-V heart block develops more frequently in children of mothers with SLE or other connective tissue disease [455–461]. A multicenter evaluation [455] of 7200 pregnant women found that 19 of 55 fetuses with complete AV block had no associated complex cardiac structural defects and were born to mothers who had connective tissue disease or who had tested positive for ANAs, most frequently SS-A/Ro. Autoantibodies, particularly to the SS-A/Ro or SS-B/La

antigens, have been found not only in the serum of mothers, but also in the serum and fetal cardiac tissue of their affected newborns [462–473], suggesting a causal relationship. Although the risk of a patient with SLE having a complete A-V block appears to be low, the risk increases considerably when anti-SS-A antibodies are present [462].

Echocardiography of the fetal heart [455, 460, 471] can depict the anatomic and functional dynamics of the fetal heart: congenital heart block (Fig. 77), other arrhythmias, systolic ejection fractions, chamber enlargement, patent ductus arteriosus, and atrial septal defect. Also, duplex sonography can show associated extracardiac abnormalities associated with cardiovascular disease (hydrops fetalis and effusions).

Investigators comment that mothers with risk factors for neonatal lupus could be prospectively monitored with serial fetal echocardiograms beginning at 16–18 weeks of pregnancy, which is the earliest time for adequate visualization of the fetal heart and fetal bradyarrhythmias [460, 460a]. It was suggested that treatment with dexamethasone might benefit fetuses with incomplete or unstable block or myocarditis by helping prevent permanent fibrosis [460a].

Placental Abnormalities and Intrauterine Growth Retardation in SLE

In SLE, fetal death results from spontaneous abortion, intrauterine prematurity, and intrauterine death [472]. These complications of pregnancy are associated with circulating lupus anticoagulants, thrombocytopenia, and antiphospholipid antibodies and placental abnormalities in the lupus patient [472–475]. Histologic examination

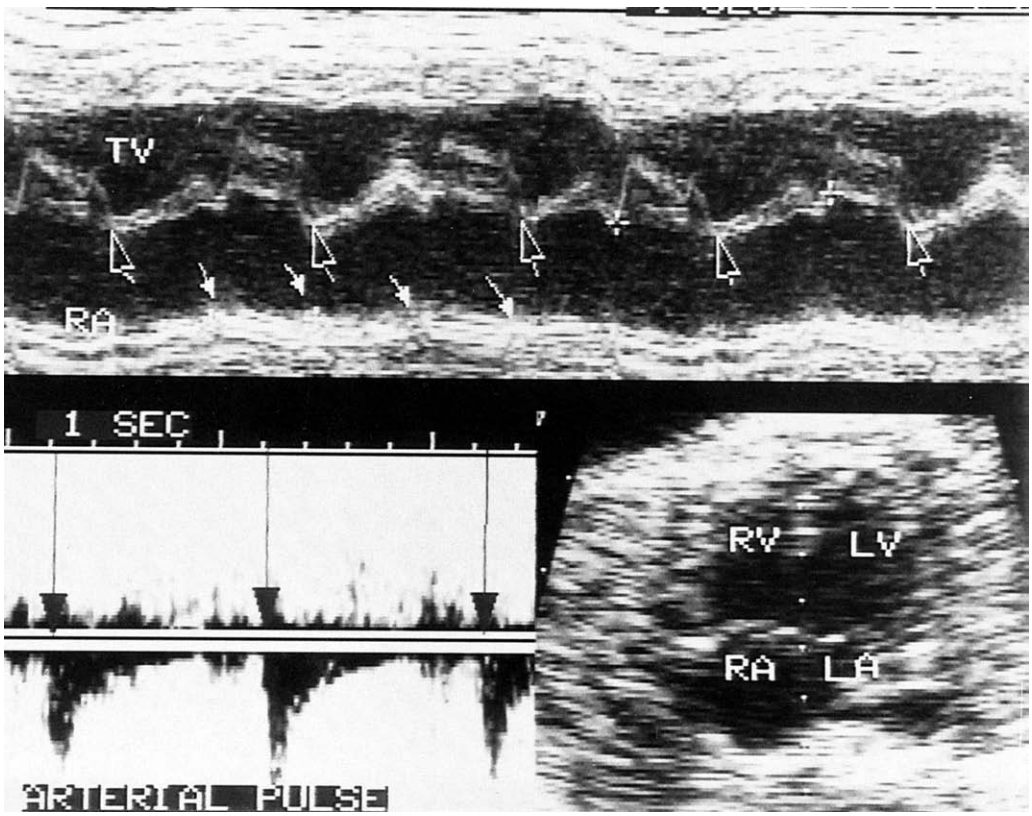


FIGURE 77 M-mode and two-dimensional echocardiography of a 30-week-old fetus of a mother with SLE show complete heart block. The top frame shows the tricuspid valve (TV) moving between the ventricle above and the atrium behind. The point of tricuspid closure is indicated by open arrows and indicates the point of the onset of systole. The ventricular rate is 120 beats/min. Posteriorly, the right atrial wall (RA) is displayed; the onset of contraction is indicated by small arrows; the atrial rate is 60 beats/min. Thus the atrial rate is approximately twice the ventricular rate consistent with a complete heart block. The lower left-hand frame shows a Doppler signal taken from the aortic root at a rate of 60 beats/min that correlates with the echocardiographic findings of a complete heart block. The lower right-hand image shows a normal four-chamber echocardiographic image, suggesting no structural cardiac disease. Courtesy of Dr. N.A. Silverman, University of California, San Francisco.

of the placenta in patients with SLE show extensive changes, including placental infarction, intraplacental hematoma, deposition of immunoglobulin and complement, decreased placental size, thickening of trophoblastic basement membrane, and decidual vasculopathy [467, 468, 472, 476, 478]. Color flow Doppler/duplex sonography also defines placental abnormalities, such as abruptio placenta and placental insufficiency. The sonographic findings of retroplacental hemorrhage, a rounded, thickened placenta, and echogenic blood in the amniotic cavity all suggest the diagnosis of abruptio placenta. At duplex sonography, placental insufficiency is manifested as increased vascular resistance in the fetal umbilical and sometimes uterine arteries [479–482]. When the resistance of these vessels increases, the diastolic flow decreases and the ratio of systolic/diastolic flows increases (Fig. 78A), similar to the increased vascular resistance in the rejecting renal allograft [479–482]. Furthermore, when mothers, particularly those with lupus anticoagulant syndrome, are treated successfully with corticosteroids and aspirin in infant doses, duplex sonography can detect the improvement in vascular resistance, i.e., increased diastolic flow and decreased systolic/diastolic ratio (Fig. 78B) [479]. Placental insufficiency and increased vascular resistance are also associated with intrauterine growth retardation. Duplex sonography provides sequential documentation of the fetal growth and helps determine the need for early delivery [479–482].

IMAGING OF NERVOUS SYSTEM SLE

Nervous system involvement in SLE is both central and peripheral, transient and permanent, and occurs in 30–75% of patients with SLE. It is a major contribu-

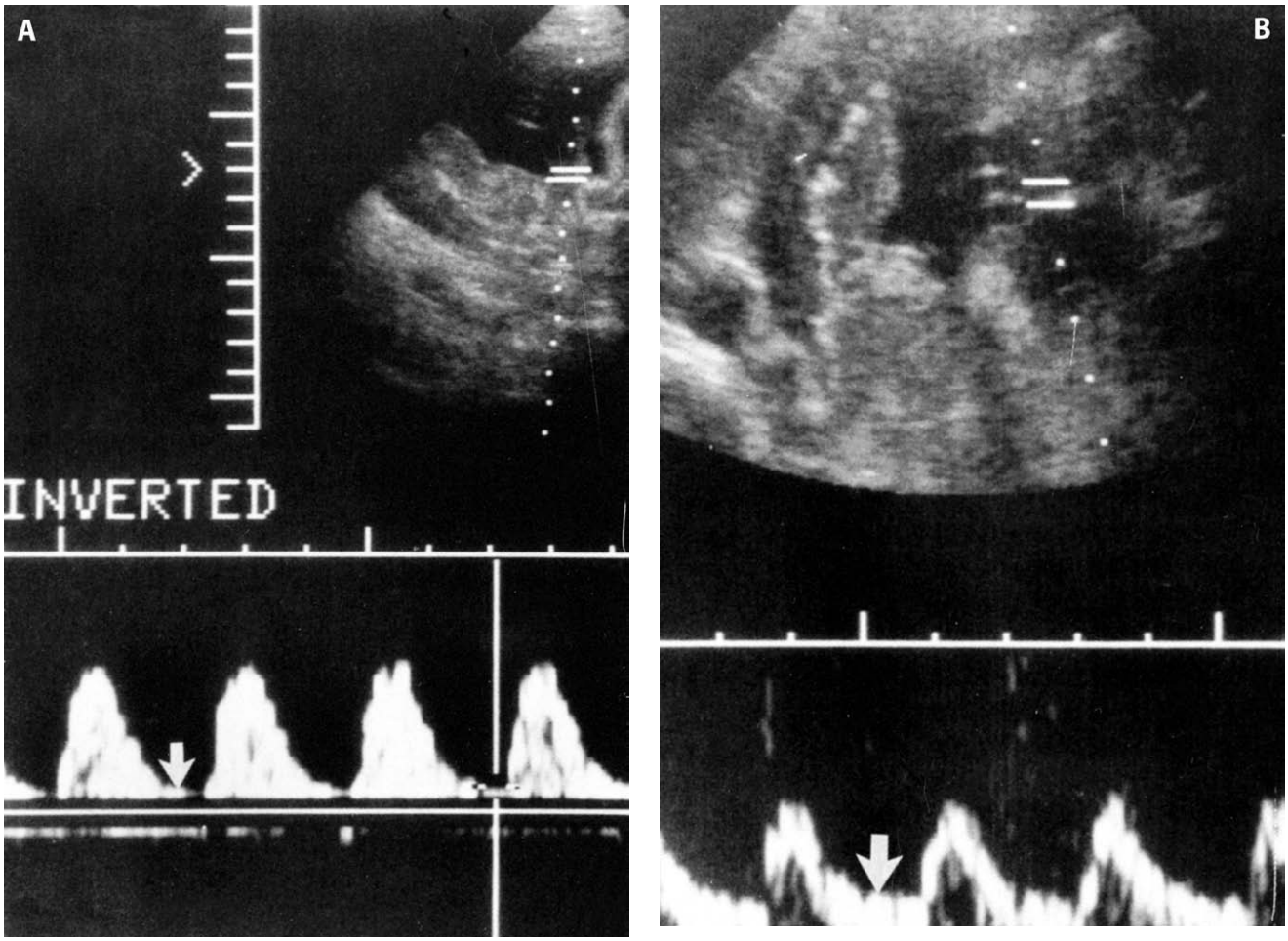


FIGURE 78 (A) Doppler spectrum of a patient with lupus anticoagulant syndrome at 33 weeks gestation demonstrates a markedly decreased end diastolic flow (*arrow*). (B) Twenty-four hours after the initial treatment with high-dose aspirin for infants, the end diastolic flow has improved (*arrow*). From Carroll, [479], with permission.

tor to morbidity and mortality in lupus [483–488]. Nervous system SLE results from a combination of factors related to the autoimmune response: vasculopathy, *in situ* vascular thrombosis, thromboembolism, antiphospholipid antibodies, lupus anticoagulant, circulating inflammatory factors, and antineuronal antibodies [484–501]. In SLE, neurologic impairment may also be associated with uremia, hypertension, infection, and corticosteroid and immunosuppressive therapy.

Central Nervous System Lupus

Cerebral imaging of the lupus patient has changed dramatically since the mid-1990s. MRI, SPECT, and FDG-PET have replaced conventional brain scintigraphy and CT as the primary imaging modalities for evaluating cerebral involvement in SLE [488, 500–512]. MRI, with its contrast resolution, defines focal morphologic lesions and anatomy [500, 502–506], and SPECT provides important information about cerebral blood flow [507–513]. PET [508, 514–524] shows regional blood flow and metabolism. Another diagnostic tool for brain metabolism, MRS, also enhances our understanding of cerebral neuropsychiatric SLE (NPSLE), particularly of inaccessible, deep structures and subclinical disease. MRS quantitates cerebral metabolites and defines brain energetics (ATP, ADP, phosphocreatine). Conventional scintigraphy, the first imaging technique used for lupus encephalopathy, is now considered to be an insensitive test that lacks specificity [483, 529–531]. Prior to the development of MRI, PET, and SPECT, CT was the principal imaging modality for evaluating cerebral lupus [532–539] and is still used for the acutely ill, unstable patient [483] because of its speed and ability to recognize acute hemorrhage (whether intracerebral, subarachnoid, subdural, and epidural hematomas) [483, 540–545], large infarcts and areas of edema [537, 547–549], ventricular dilatation [483, 550], and infectious complications of therapy such as abscesses [483, 551], meningitis [552–556], or mycotic aneurysms [557].

Cerebral and Subdural Hemorrhage in SLE

At CT, acute hemorrhage displays high attenuation because proteinaceous blood highly attenuates the X-ray beam as it passes through the involved region. With age, the protein denatures, and the attenuation characteristics of a clot resemble those of normal brain and may even decrease in attenuation similar to the water attenuation of brain edema [541, 560] (Fig. 79A). As described with other organ systems (see cardiovascular and genitourinary section), MRI is more specific than CT for detecting subacute hemorrhage because subacute clots are high-signal intensity on both T1- and T2-weighted MR images (Figs 79B and 79C).

Cerebral Atrophy in SLE

The most frequent cerebral CT finding [532, 560–562], perisutural atrophy, with or without ventricular dilatation, may be present in 50% of lupus patients [542] and rarely develops rapidly [539]. McCune *et al.* [563] and Sibbitt *et al.* [500] found that MRI, like CT, showed that mild cerebral atrophy occurred frequently in SLE. Atrophy tends to be superficial rather than deep, and ventricular enlargement was uncommon.

There are conflicting opinions as to whether cerebral atrophy is secondary to corticosteroid therapy or to primary lupus cerebritis. Carette *et al.* [535] found no correlation between the presence of cerebral atrophy on CT and lupus cerebritis and suggested that the degree of atrophy was related to the patients' sensitivity to corticosteroid therapy. However, Ostrov *et al.* [564] reported that 10 of 14 patients with primary lupus cerebritis had moderate cerebral atrophy, 4 had minimal atrophy, and none was normal; of 18 lupus patients on corticosteroids who did not have clinical cerebritis, none had moderate atrophy, 6 showed mild atrophy, and 12 had normal scans. In addition, Miguel *et al.* [561] also found that atrophy was related neither to neuropsychiatric manifestations nor to corticosteroid use and suggested that widened sulci could result from subclinical SLE activity. Using MRI computer-generated quantification procedure, Koroza *et al*. [504] found increased mean ventricle-to-brain ratios (VBR) and increased number of hyperintense white matter lesions in 35% of lupus patients who had no history of central nervous system disease. However, no correlation was found between the VBR, total number of hyperintense lesions and cognitive scores. The investigators suggested that MRI is not likely to contribute clinically pertinent information in the evaluation of lupus patients who have cognitive dysfunction but no other overt manifestations of CNS-SLE.

The newest technique for elucidating cerebral metabolism, MRS, combined with MRI, has helped expand our understanding of cerebral atrophy [565]. Using the neuronal marker *N*-acetylaspartic acid (NAA), Sibbitt and associates [546] measured the ratio of NAA-to creatine (C)/phosphocreatine(PC) ratio in 10 lupus patients with cerebral atrophy and 10 lupus patients with no significant atrophy. The NAA-C/PC ratio was decreased with patients with significant atrophy as compared to lupus patients with normal brain volumes. The authors [565] suggested that cerebral atrophy in SLE is caused by neuronal and axonal dropout or damage as manifested by a relative decrease in NAA [565]. Chinn and co-

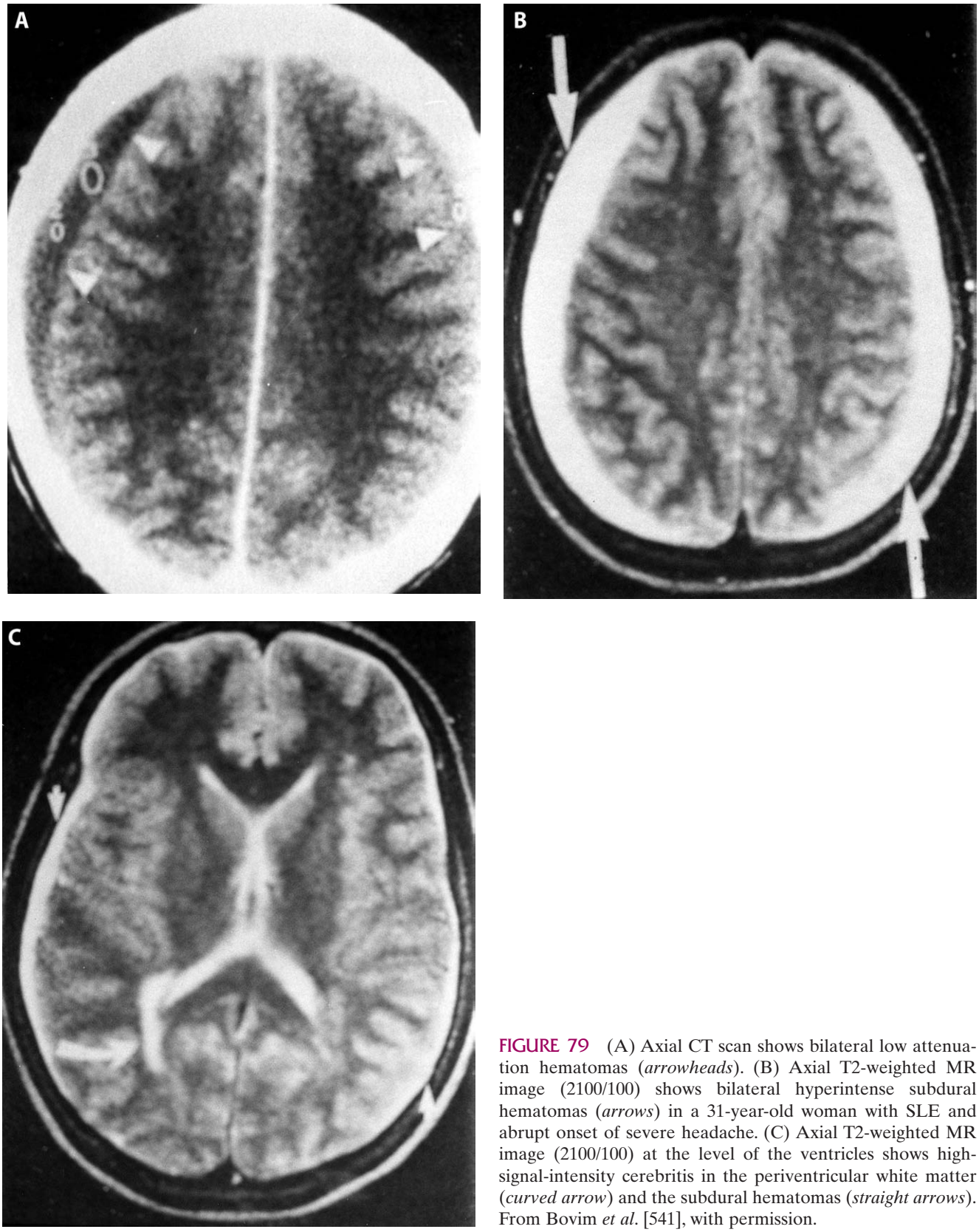


FIGURE 79 (A) Axial CT scan shows bilateral low attenuation hematomas (*arrowheads*). (B) Axial T2-weighted MR image (2100/100) shows bilateral hyperintense subdural hematomas (*arrows*) in a 31-year-old woman with SLE and abrupt onset of severe headache. (C) Axial T2-weighted MR image (2100/100) at the level of the ventricles shows high-signal-intensity cerebritis in the periventricular white matter (*curved arrow*) and the subdural hematomas (*straight arrows*). From Bovim *et al*. [541], with permission.

investigators [566] also found a relative reduction in *N*-acetyl-containing compounds, particularly *N*-acetylaspartate and to a lesser extent *N*-acetyl aspartyl glutamate in lupus patients with cerebral atrophy and showed that the presence of atrophy was not related to current or prior corticosteroid therapy.

Cerebral Calcifications in SLE

CT shows the periventricular, basal ganglia, and cerebellar calcifications in SLE that develop in 1–30% of lupus patients with major or minor neuropsychiatric symptoms (Fig. 80) [561, 567–572]. On a series of 27 patients, one group of researchers showed no correlation between the presence or extent of calcification and age of the patients or duration or type of neurologic presentation. Other investigators found calcifications more frequently in patients with major psychiatric manifestations [561].

Lupus Cerebritis

Compared with contrast-enhanced CT, MRI better defines the presence, number, location, extent, and clarity of noncalcified lesions in lupus cerebritis [483, 500, 573–581] because of its multiplanar imaging and excellent soft tissue contrast (Fig. 81). Unlike CT, MRI does not produce bone-hardening artifacts that obscure parenchymal detail, particularly in the posterior fossa (cerebellum and brainstem) (Fig. 82) [483, 500, 573]. Cerebral lesions in SLE are best detected on T2-weighted MR images as either reversible focal areas of high-signal intensity (ischemia or edema) or fixed areas of high-signal intensity (infarct or residual injury) in white or gray matter or both [483, 500] (Figs 83 and 84). Labile lesions may be superimposed on fixed lesions [499]. Abnormalities that are isolated to gray or white matter are usually not visible on CT [483]. After the acute neurologic event, it is important to obtain an MRI promptly because labile lesions can disappear rapidly with or without corticosteroid therapy. In SLE patients at risk for neuropsychiatric diseases, it can be useful to obtain a baseline MRI to distinguish the chronic lesion from a subsequent acute insult [500].

On MRI studies, quantitative measurement of the T2 of lupus lesions may help distinguish among the different types of cerebral lupus [582]. Tissues that are edematous or infarcted will demonstrate longer T2 (i.e., they appear hyperintense on T2-weighted images). Sibbitt *et al.* [582] found that T2 values were increased in all types of cerebral lesions in SLE and even in normal-appearing brain in patients with SLE as compared with the brains of nonlupus controls. Their SLE patients were subdivided into five subgroups: (1) quiescent minor neuropsychiatric (NP) SLE (such as previous headache, minor cognitive dysfunction), (2) quiescent major (such as previous seizure, coma, psychosis), (3) NPSLE headache without significant focal neurologic finding or major cognitive dysfunction, (4) diffuse NPSLE with acute diffuse neurologic findings and major cognitive deficits (seizures, psychosis, coma), and (5) NPSLE stroke with focal neurologic deficit and positive anticardiolipin antibodies. The increased T2 of gray matter in lupus patients could be mainly attributed to a large increase in T2 of the subgroup of patients who had diffuse NPSLE. These patients showed either a multifocal pattern of T2 increase localized to a few brain segments or a generalized accentuation of T2 affecting cortical segments. The mean age of these patients with diffuse NPSLE was younger than that of other lupus patients. In contrast, the increase in T2 of the white matter was mainly associated with lupus patients with headache, stroke, or prior major NPSLE and not with diffuse findings or quiescent minor NPSLE. Furthermore, T2 values for infarcts were greater than T2 for reversible lesions, which, in turn, were greater than T2 for small nonreversible lesions. It was concluded, therefore, that T2 of reversible and nonreversible focal lesions and infarcts differed significantly, implying that the microenvironments of these lesions differed. The reversible lesions should represent focal edema resulting from the vasculopathy, whereas the focal nonreversible lesions would signify small infarctions with secondary gliosis [582]. Thus, T2 measurements of lupus lesions may be useful in identifying subtle changes that are not evident on conventional MRI.

It was reported that MRI detected abnormalities in 38 of 100 lupus patients with neurologically asymptomatic [503]. SPECT was done on 23 of these patients with positive MRIs and showed areas of hypoperfusion in 14. In addition, the SLE activity index was significantly greater in patients with brain lesions that in those without. This is a preliminary result and further longitudinal prospective studies of asymptomatic lupus patients with cerebral lesions are needed.

In addition to standard T1- and T2-weighted and contrast-enhanced MRI, various investigators have studied magnetization transfer MRI for evaluating neuropsychiatric NPSLE. The contrast created by this technique is different from T1- and T2-weighted MR imaging because of the dipole–dipole interaction between the protons of water and large macromolecules. This transfer of energy from macromolecules to water results in the magnetization transfer effect, i.e., water appears as low-signal intensity. Those tissues that have increased water content will display low-signal intensity on magnetization transfer images. Two studies [505, 506] have

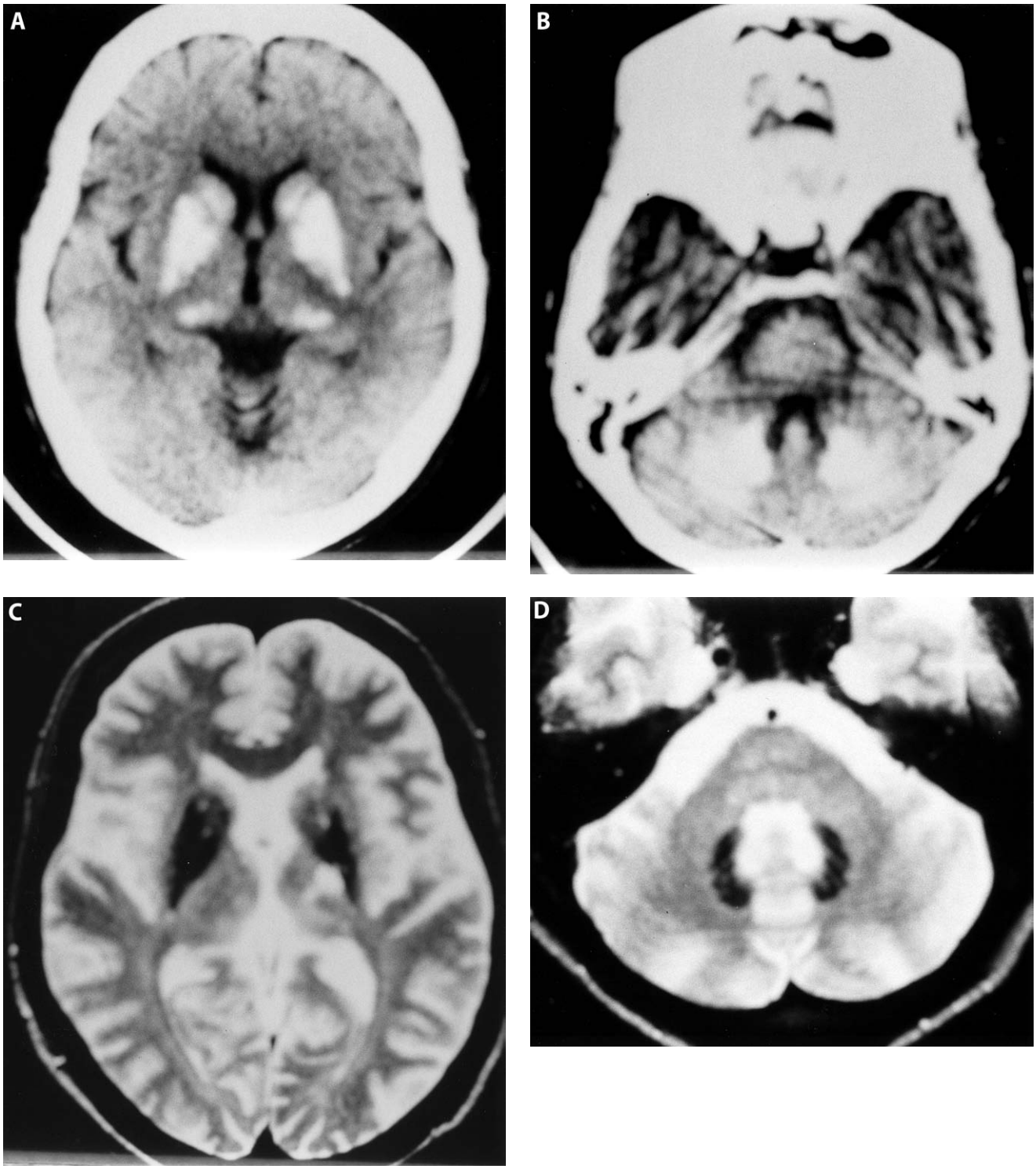


FIGURE 80 (A and B) Axial CT scans of 32-year-old woman with SLE and right hemiparesis and dysarthria show bilateral calcifications in the lentiform nuclei, caudate nuclei, thalamus, dentate nuclei, and the white matter of the cerebellar hemispheres. (C and D) Axial T2-weighted MR scans 19 months later show abnormal low-signal-intensity areas in the basal ganglia and cerebellum in the region of the calcifications. From Yamamoto *et al*. [570], with permission.

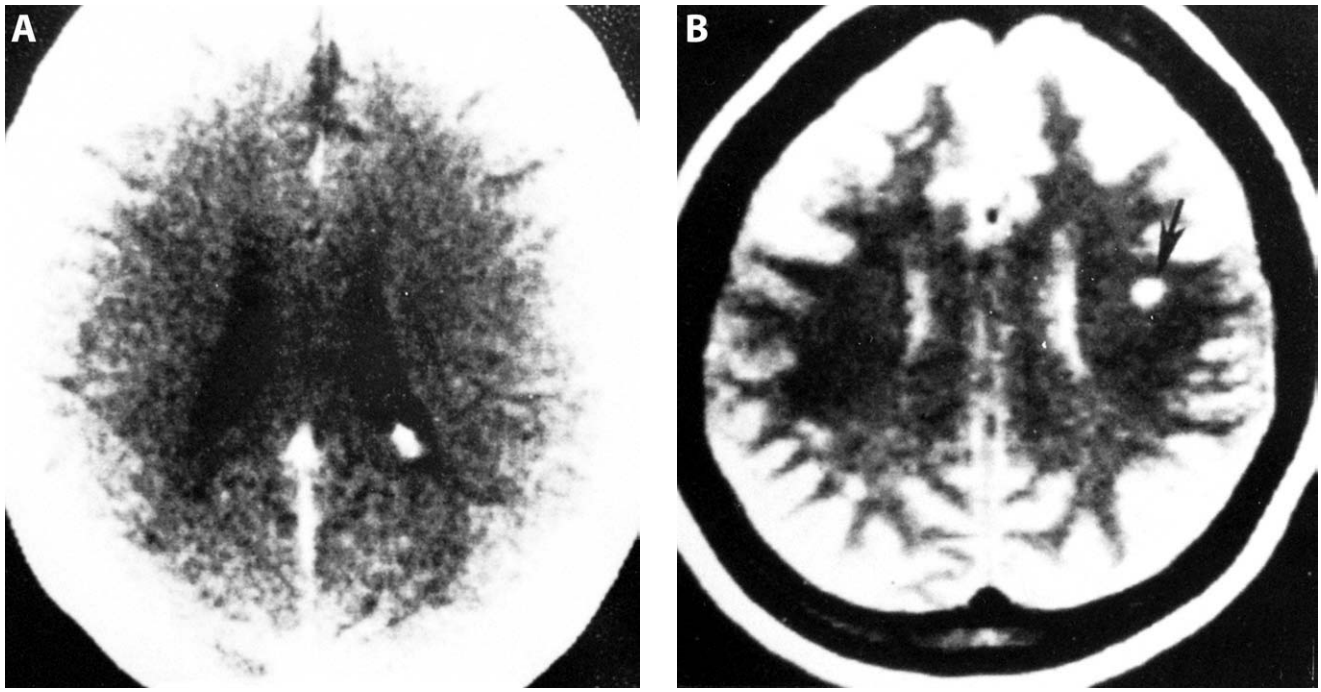


FIGURE 81 (A) Axial contrast-enhanced CT of patient with SLE shows no abnormality. (B) Axial T2-weighted MR image (TR/TE 2000/56) shows a focal area of high signal intensity in the left frontoparietal white matter (*arrow*) in a patient with lupus cerebritis. From Aisen *et al*. [573], with permission.

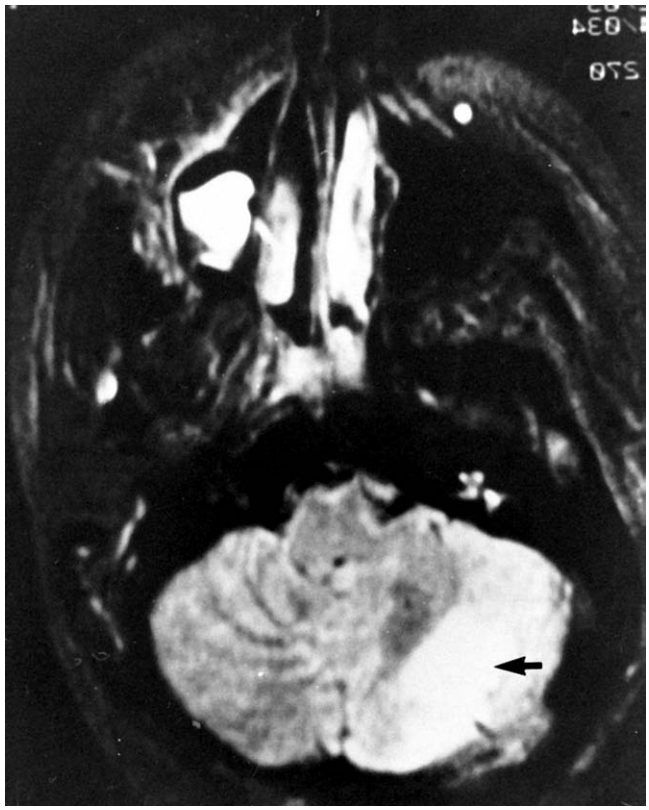


FIGURE 82 Axial proton-density MR image (2000/20) shows a large high-signal-intensity lesion in the cerebellum representing focal edema or ischemia in the cerebellum, but not infarction because it resolved after 8 days of corticosteroid therapy. CT usually misses this type of posterior fossa lesion. From Sibbitt *et al*. [500], with permission.

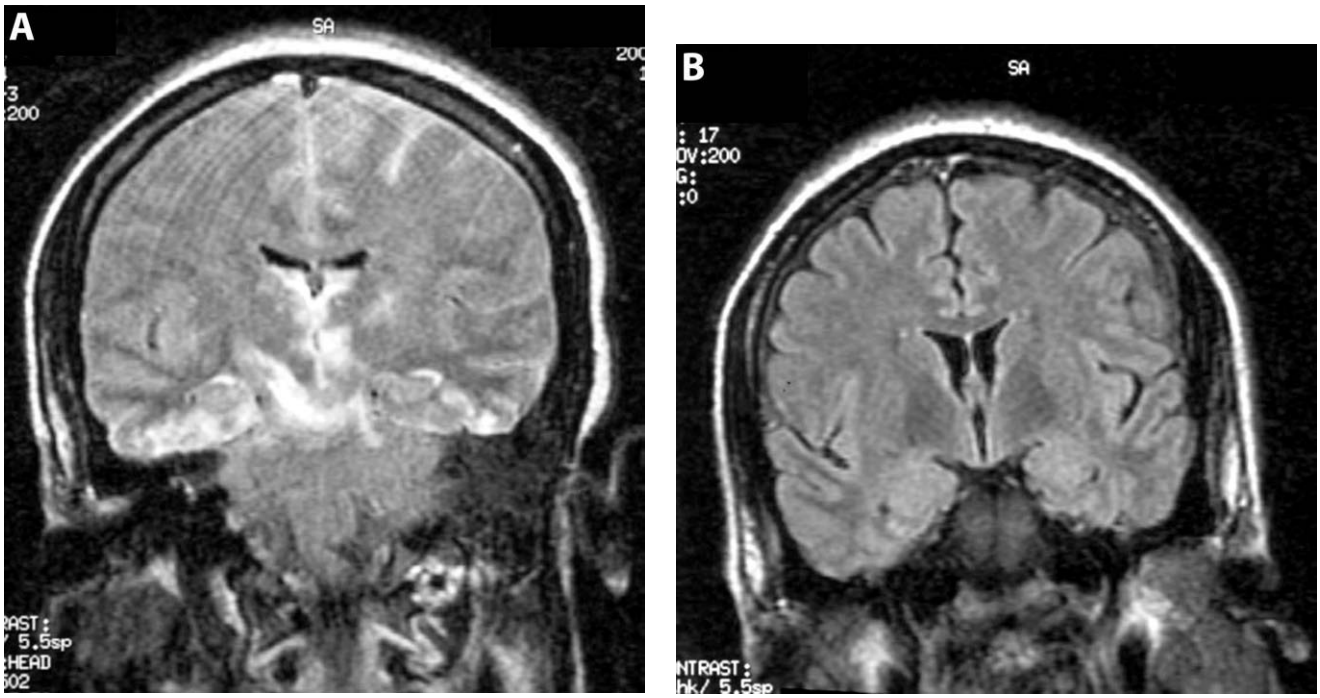


FIGURE 83 Sequential MR images of a 31-year-old woman with acute lupus cerebritis and seizures. (A) Coronal fluid attenuation inversion recovery (FLAIR) MR image (9502/147) shows areas of high signal intensity in the right temporal lobe, basal ganglia, and thalami. (B) After 10 days of corticosteroid therapy, coronal FLAIR MR image (9502/147) shows marked improvement.

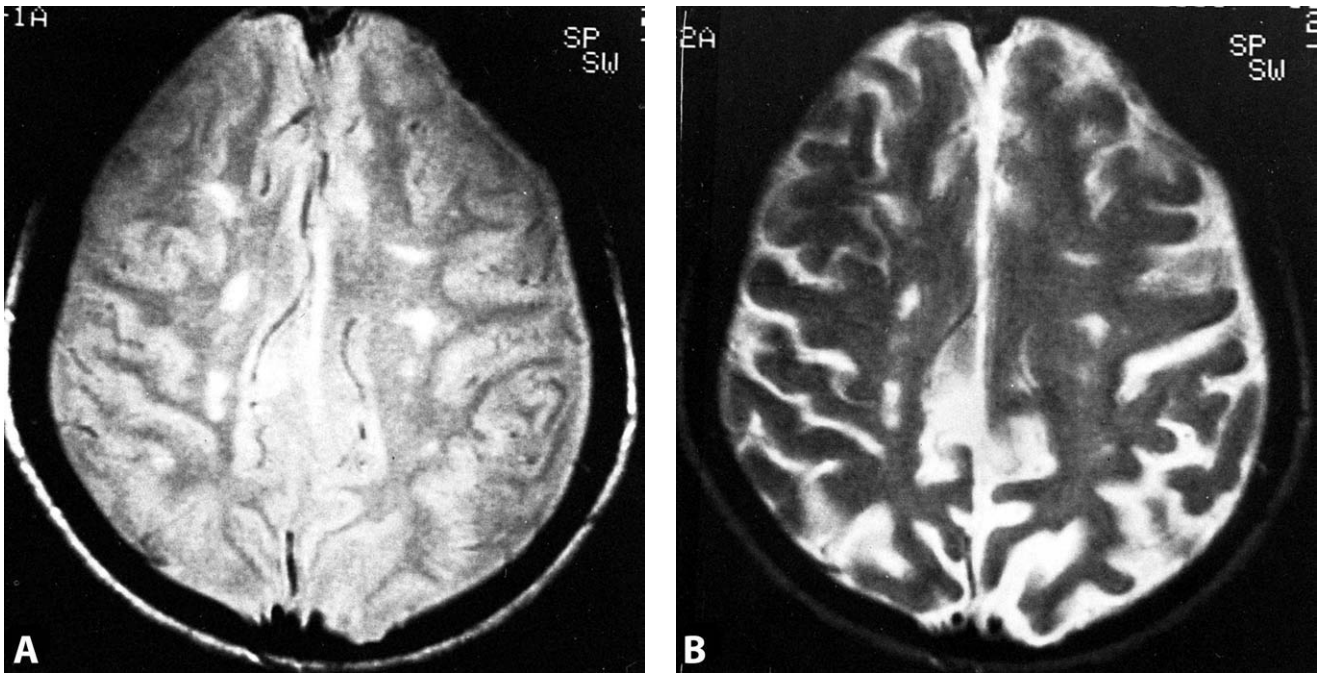


FIGURE 84 (A) Axial proton density MR image (2800/20) and (B) T2-weighted MR image (2800/80) show multiple high-signal-intensity periventricular lesions in a patient with long-standing lupus cerebritis. Courtesy of Dr. M.-F. Kahn, University of Paris.

showed that the magnetization transfer MR imaging detected, with more specificity than standard MRI, microsopic brain tissue damage in NPSLE similar to multiple sclerosis, whereas patients with SLE without NPSLE had findings similar to those of normal patients.

Bell *et al.* [583] found that a combined MRI–serologic approach may have utility in predicting NPSLE responsiveness to corticosteroid therapy. In their study, patients with diffuse clinical disease and symmetrically increased signal intensity in the subcortical white matter on T2-weighted MR images had elevated levels of antineurofilament antibodies and responded to high-dose corticosteroid therapy with resolution of their findings. In contrast, patients with focal central nervous system lupus had hyperintense areas and atrophic changes corresponding to major cerebral vessels and had normal levels of antineurofilament antibodies but elevated levels of anticardiolipin antibodies and lupus anticoagulant and did not respond to corticosteroids.

High-signal-intensity lesions seen in cerebral lupus, when isolated from the clinical history, are nonspecific and may mimic the plaques of multiple sclerosis or the microinfarcts in hypertensive encephalopathy on MRI images [483, 584]. However, when hyperintense lesions are present in both cerebral peduncles and along perivenous channels along the corpus callosum, multiple sclerosis would be the more likely diagnosis.

In recent years, SPECT and FDG-PET have been used to attempt to unravel the complex neuropsychiatric manifestations of SLE and the response to therapy [510, 519, 523]. Both modalities detect lesions not visible on MRI in adults and children [484, 498, 512, 515, 585–590] although MRI will better delineate the focal morphologic changes in a particular area because of its greater spatial resolution [505].

Because FDG-PET was expensive and required rapidly decaying radiopharmaceuticals that were produced by cyclotrons and was not available at most centers, SPECT initially was used more extensively in the evaluation of NPSLE. However, since the late 1990s, the increased proliferation and availability of PET scans have increased the use of FDG-PET [517–522, 524] as well as SPECT [507–513] for evaluating cerebral disease in SLE.

In SPECT studies on patients with SLE, several groups of investigators have reported an increased prevalence of blood flow impairment not only in lupus patients with overt neuropsychiatric symptomatology [507, 512, 588–592], but also in those lupus patients without symptoms [507, 508, 511, 517, 588, 589, 592]. Colamussi *et al.* [507] found that areas of hypoperfusion were more frequent in patients with more extensive NPSLE manifestations. Rubbert *et al.* [592] found that the majority of patients without neuropsychiatric symptoms had normal cerebral perfusion, but patients with minor symptoms (headache, anxiety, cognitive disturbances) frequently had abnormal SPECT studies (73.3%) with two-thirds showing focal areas of hypoperfusion and one-third showing diffuse hypoperfusion. Their patients with predominantly focal uptake defects at SPECT had long-standing disease (>5 years), whereas most patients with normal SPECT or with diffuse hypoperfusion abnormalities had disease of shorter duration. The addition of acetazolamide to SPECT may help test cerebral reserve in the lupus patient. In one report, one lupus patient with no abnormalities at FDG-PET and only minor changes at SPECT showed marked reduction in the cortical perfusion reserve when challenged with acetazolamide [593].

On addition to cerebral perfusion, FDG-PET can evaluate cerebral metabolism. In early ^{15}O-oxygen PET studies, Pinching *et al*. [515] reported that lupus patients with mild cerebral signs, as well as those with severe symptoms, had marked changes on cerebral blood flow and oxygen utilization (Fig. 85). Sequential follow-up scans demonstrated a therapeutic response to corticosteroids. Current FDG-PET show improved resolution as compared with earlier scans (Fig. 86). Pinching *et al.* [515] and Awada *et al*. [516] who used FDG-PET identified a multicentric patchwork during the metabolic phase that correlated with abnormal glucose metabolism. They suggested that cerebrovascular alteration may be more common and widespread than previously thought and may account for the diverse manifestations of cerebral lupus. Volkov *et al*. [594] reported that, in patients with NPSLE, the severity of blood flow and metabolic abnormalities at FDG-PET appeared to correlate with the severity of neuropsychiatric symptomatology. In a longitudinal study of three patients, Carbotte *et al.* [595] showed that FDG-PET abnormalities correlated with focal cognitive deficits; changes in each patient's cognitive profile paralleled changes at FDG-PET. In another FDG-PET study, Weiner *et al.* [524] found hypometabolism in at least one brain region in all patients with severe or mild CNS symptomatology as compared with 40% of patients without symptomatology. The most commonly involved region was the parieto-occipital region (96%) followed by the parietal region (32%). The improvement, stability, or deterioration of symptoms correlated with improvement persistence or worsening of hypometabolism [524].

Another method for evaluating cerebral metabolism in SLE is MRS, which can measure brain metabolites in both chronic and acute NPSLE [525–528, 576]. Sibbitt *et al.* [528] and Griffey *et al.* [525] showed that ATP and PCr were depressed in the white matter in all lupus patients with symptomatic central nervous system disease whether focal (neurologic deficit) or

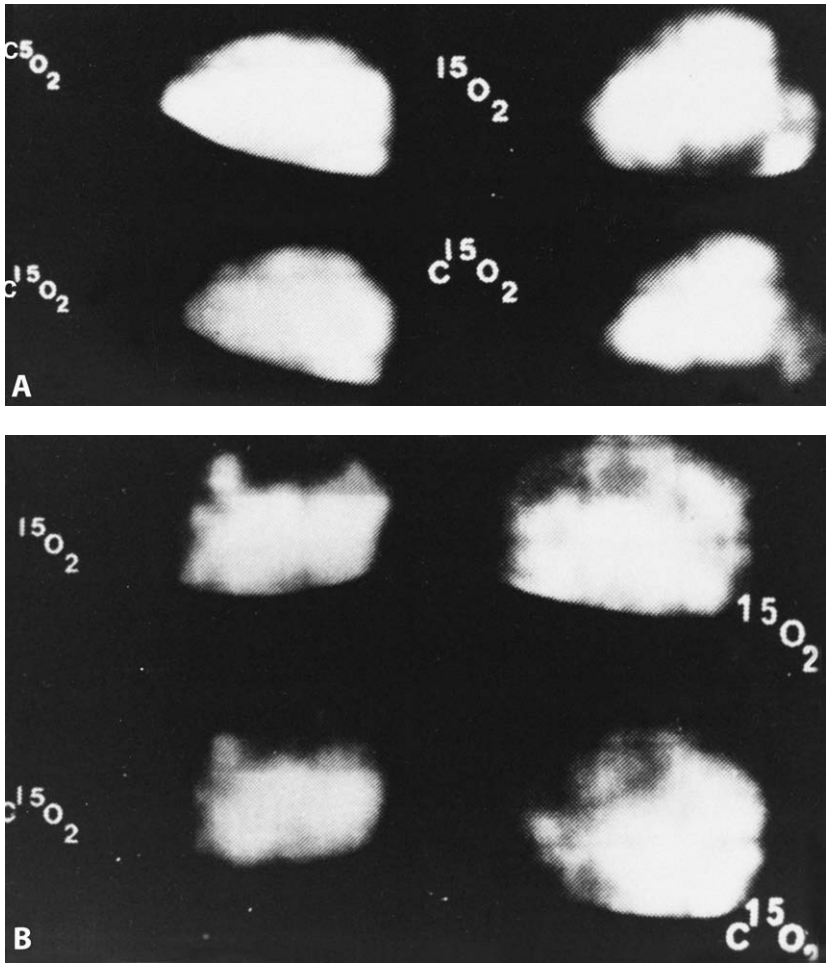


FIGURE 85 (A) Lateral brain PET images with $^{15}O_2$ (metabolism), and $C^{15}O_2$ (blood flow) in a normal patient. (B–D) PET scans of the right hemisphere show several large defects that are more extensive on the metabolism than on the blood flood images in a 52-year-old lupus patient with gross paranoid psychosis. From Pinching *et al*. [515], with permission.

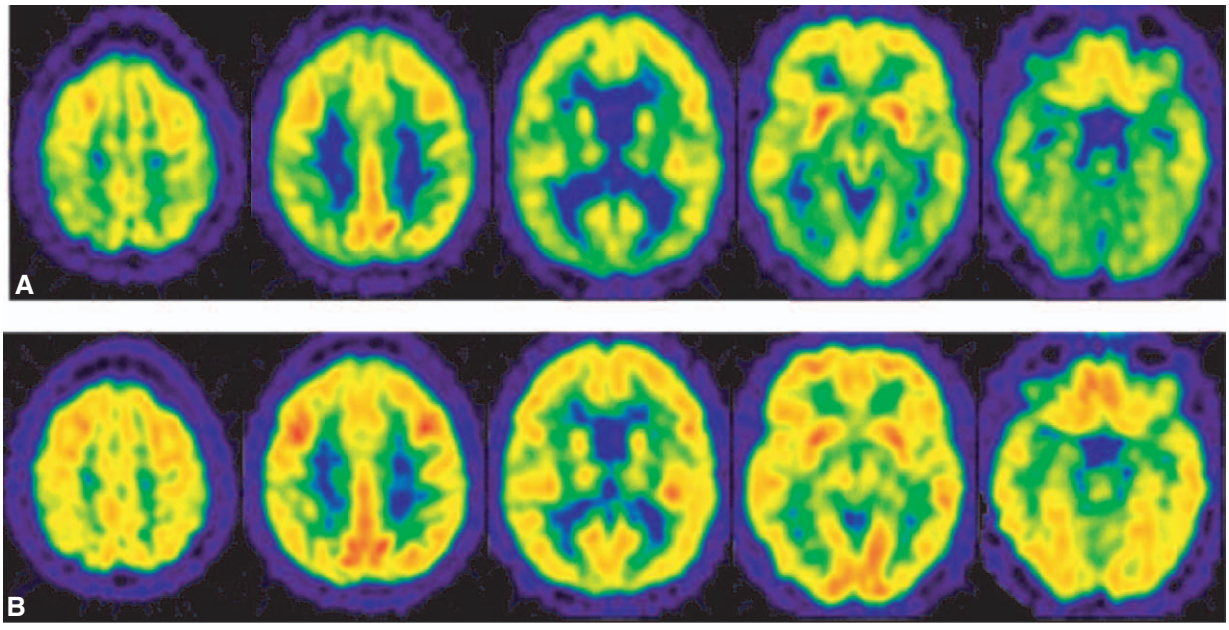


FIGURE 86 (A) Axial FDG-PET scans show multiple defects before treatment in this SLE patient. (B) Therapy with corticosteroids produced improvement on FDG-PET images. Courtesy of Professor E. Nitzsche, Basel, Switzerland.

nonlocalized (headache, confusion) (Fig. 87). The areas of high-energy phosphate depletion did not correlate with the high-signal intensity lesions on MR images. In fact, some patients had normal MR images yet significantly depressed high-energy phosphates that returned to normal with corticosteroid therapy. Thus, depressed high-energy phosphate levels may reflect a more diffuse metabolic process than the focal MR lesion [525]. In addition, lactate and lipid peaks increased in active NPSLE and returned to normal with corticosteroid therapy. Davie *et al.* [526] have also evaluated the application of MRS to SLE, using neuronal marker *N*-acetylaspartic acid markers to cerebral lesions, although its utility is as yet unproved. MRS of these cerebral lesions showed a reduction in the *N*-acetyl aspartate creatine ratio as compared to the normal ratio in lupus patients and control patients with normal-appearing white matter. Twelve of the 13 patients with abnormal MRS had recent or recurrent psychiatric involvement, although there was no correlation between the presence of current neuropsychiatric and/or cognitive dysfunction and the abnormalities. The abnormal NAA/creatine ratio within the cerebral lesions, however, is nonspecific and currently does not allow differentiation of lupus cerebritis from chronic plaques of multiple sclerosis [520].

Thus, for the acutely ill lupus patient, CT allows prompt diagnosis of the bleed or massive infarct. For diagnosis and therapeutic responses in NPSLE, MRI can evaluate the focal infarcted and ischemic brain and the global effects of neuronal damage or loss, and SPECT, FDG-PET, and MRS depict the metabolic and perfusion abnormalities and neuronal loss.

Vasculitis of Cerebral Lupus

When the small- and medium-sized cerebral arteries are affected in SLE, MRA and conventional angiography show a vasculitis with irregular, beaded arteries and areas of narrowing interspersed with zones of aneurys-

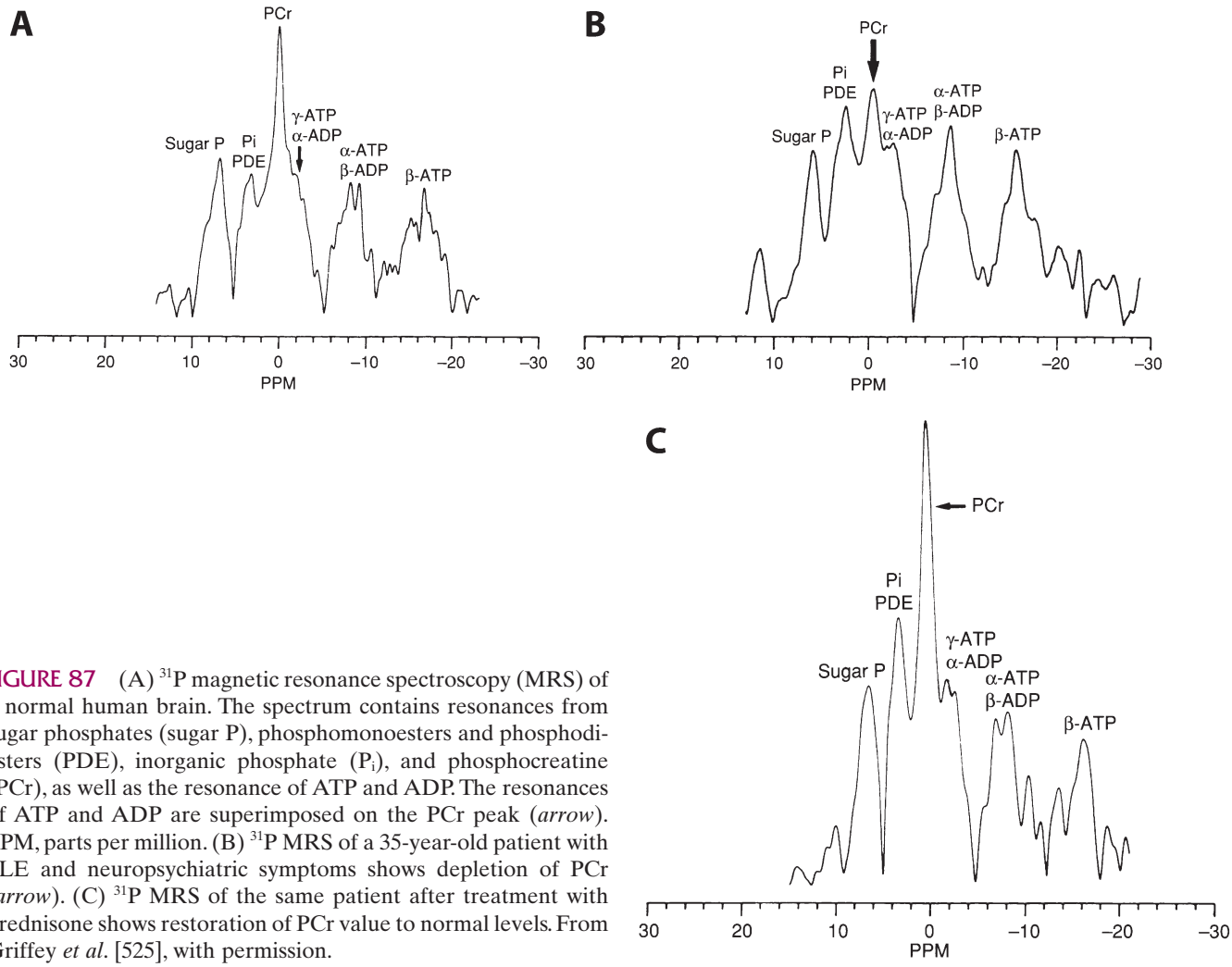


FIGURE 87 (A) ^{31}P magnetic resonance spectroscopy (MRS) of a normal human brain. The spectrum contains resonances from sugar phosphates (sugar P), phosphomonoesters and phosphodiesters (PDE), inorganic phosphate (P_i), and phosphocreatine (PCr), as well as the resonance of ATP and ADP. The resonances of ATP and ADP are superimposed on the PCr peak (*arrow*). PPM, parts per million. (B) ^{31}P MRS of a 35-year-old patient with SLE and neuropsychiatric symptoms shows depletion of PCr (*arrow*). (C) ^{31}P MRS of the same patient after treatment with prednisone shows restoration of PCr value to normal levels. From Griffey *et al.* [525], with permission.

mal dilatation (Fig. 88). Rarely saccular aneurysms develop in the cerebral arteries as in the kidney or liver [596–606]. Major vascular occlusion is less common and may be associated with circulating lupus anticoagulant and more commonly affects the anterior and middle cerebral arteries and, rarely, the vertebrobasilar arteries [607–609]. Venous sinus thrombosis and thrombophlebitis, often manifested by severe, unrelenting headaches, have also been reported in cerebral SLE [537, 589–619]. MRA is currently the primary study used to evaluate the extent of thrombi, nonvisualization of the sinus, and associated cerebral infarcts and edema. Conventional angiography [612–614], is no longer necessary for diagnosing venous thromboses. Occasionally, dural sinus thrombosis clinically presents as patients with increased intracranial pressure and pseudotumor cerebri [614–616].

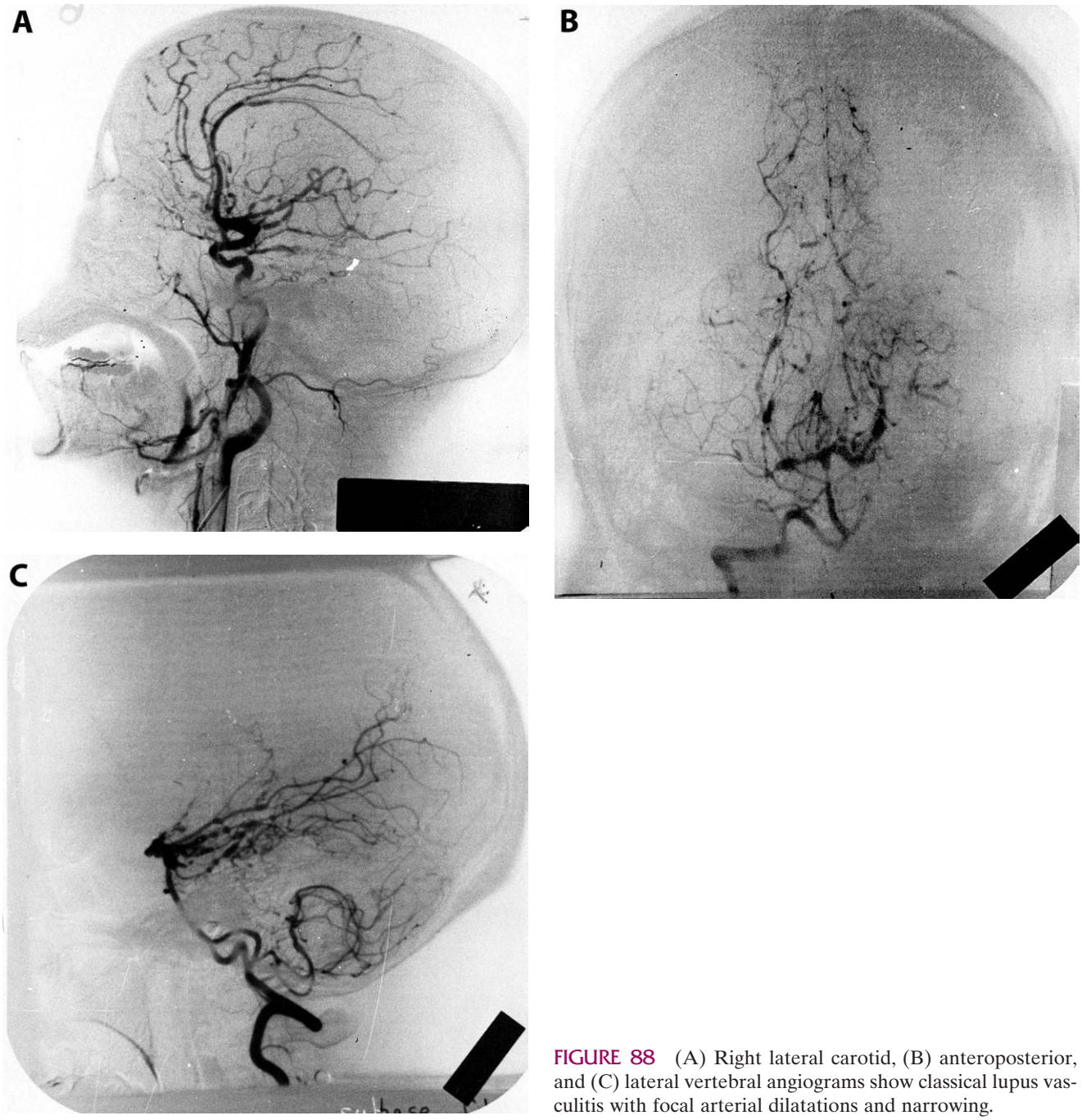


FIGURE 88 (A) Right lateral carotid, (B) anteroposterior, and (C) lateral vertebral angiograms show classical lupus vasculitis with focal arterial dilatations and narrowing.

Parkinsonism in SLE

Parkinsonism, as a manifestation of SLE, is rare and has been reported in nine lupus patients who presented with extrapyramidal symptoms (e.g., bradyphrenia, bradykinesia, hypophonia, rigidity, and abnormal gait). One-third of these patients had MRI studies. T2-weighted MR sequences showed high-signal intensity lesions in the basal ganglia sometimes with associated lesions of the brain stem and cerebral cortex, including the subcortical regions. Contrast-enhanced MRI may show enhancement of the lesions. With corticosteroid therapy, MRI can return to normal, and patients clinically have partial or complete improvement (Fig. 89) [620–622].

Lupus Cerebritis and Progressive Multifocal Leukoencephalopathy

Progressive multifocal leukoencephalopathy (PML) and cerebral involvement in SLE may occasionally occur synchronously [623–625] or lupus cerebritis occasionally may mimic PML both in the clinical course and on CT and MR images [626, 627]. PML is a rare viral infection that may develop in immunosuppressed patients, including the lupus patient. CT and MRI depict PML as areas of patchy or confluent demyelination (low attenuation on CT and high-signal intensity on T2-weighted MR images) in the white matter without mass effect. Histologic examination shows intranuclear viral inclusions and abnormalities of the oligodendrocytes. Electron microscopy and immunohistochemistry demonstrate the JC virus [625, 627].

Lupus cerebritis may rarely display similar MR features as PML. Marsteller *et al.* [626] described a lupus patient with extensive nonenhancing, low-attenuation white matter lesions at CT. Kaye *et al.* [627] reported T2-weighted MR findings in a lupus patient with progressive neurologic and MR findings. Initially MRI demonstrated confluent high-signal intensity areas in the deep white matter (centrum semiovale, corona radiata) consistent with demyelination associated with ventricular dilatation. Rapid progression of the disease ensued over 9 months with extension into the internal capsule, the pons, and cerebral peduncles [627]. In both patients, PML, a nontreatable disease, was considered. Biopsies were performed and revealed no evidence of PML but findings compatible with lupus cerebritis. Thus, in these cases, florid NPSLE, with rapid white matter changes, clinically simulated PML, and a biopsy was required for definitive diagnosis and treatment [627].

Ophthalmic and Orbital Involvement in SLE

Unilateral and bilateral internuclear ophthalmoplegia [628–632] is an unusual manifestation of SLE. MRI can identify the increased signal intensity in the mesencephalon and pons and along the medial longitudinal

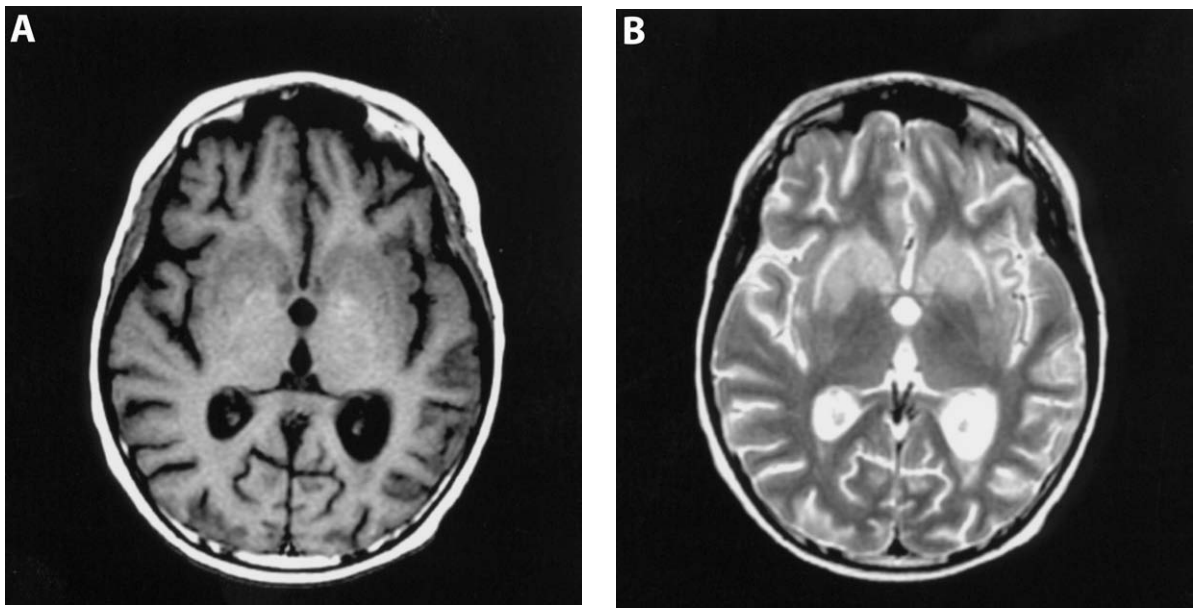


FIGURE 89 (A) Axial T1-weighted MR image shows areas of low signal intensity in bilateral caudate nuclei, putamen, and external capsule that become high signal intensity on T2-weighted MR image (B). This 9-year-old girl had CNS lupus and findings of parkinsonism. (From Kwong *et al*. [621], with permission).

fasciculus (Fig. 90). Postmortem examination reveals pontine infarcts, perivascular adventitial inflammation, intimal proliferation, and thrombosis of the vessels of the midbrain and brain stem and vascular degeneration of the medial longitudinal fasciculus.

Acute optic neuropathy in SLE is characterized by a sudden loss of vision in one or both eyes that may be associated with optic disk swelling [633]. Contrast-enhanced T1-weighted MRI delineates the enhancing, enlarged optic nerves, chiasm, and segment of the anterior visual pathway (Fig. 91).

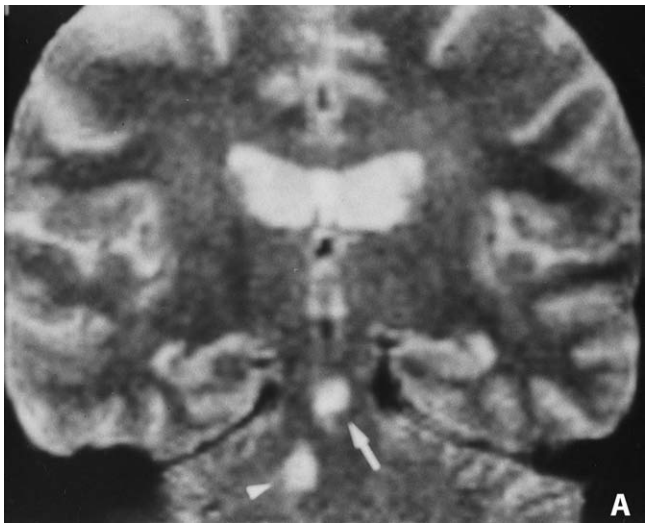
Orbital involvement is uncommon in SLE and may be the presenting manifestation or develop at some time during the course of the disease [634, 635]. On contrast-enhanced MR and CT images, inflamed retroorbital and periorbital tissues enhance as does myositis when present. Both panniculitis and myositis display high-signal intensity on T2-weighted MR images. Microscopic examination of the biopsied inflamed fat reveals fibroadipose tissue infiltrated by inflammatory infiltrate, multinucleated giant cells, and areas of vasculitis; cultures are negative. In all patients on immunosuppressive therapy [634, 635], acute cellulitis should be excluded.

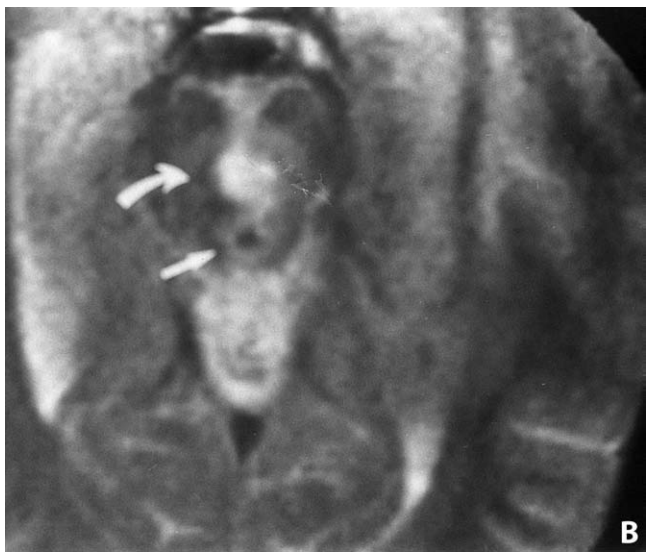
Cerebral Lymphoma in SLE

Cerebral lymphoma has also been reported in systemic lupus [636, 637] but no CT or MRI findings have been described. However, in persons without lupus, primary lymphomas are enhancing masses at CT and hyperintense masses at T2-weighted MRI [638–640].

Complications of Therapy in SLE

Because of their increased susceptibility to infection, lupus patients may develop cerebral or rarely intramedullary abscesses, mycotic aneurysms, and





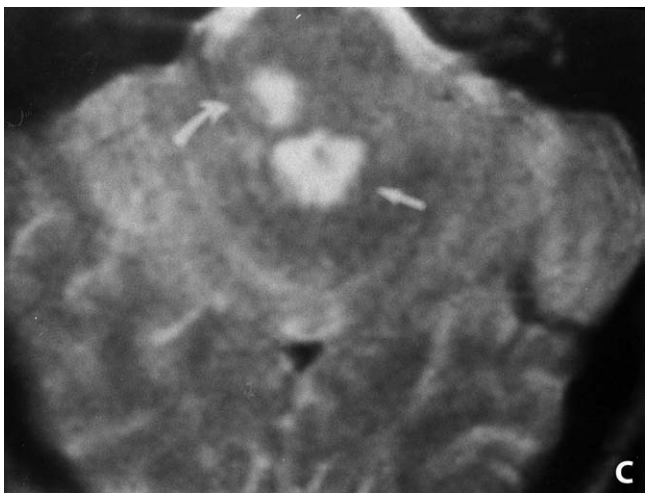


FIGURE 90 (A) Coronal T2-weighted MR image of the brainstem (2000/100) shows a high-signal-intensity lesion in the mesencephalon (*arrow*) and in the pons (*arrowhead*) in a 23-year-old woman with SLE and severe right-sided headaches and diplopia and diagnosis of ophthalmoplegia. (B) Axial T2-weighted MR image (2000/100) at the levels at the mesenephalon shows a high-signal-intensity lesion in the region of the medial longitudinal fasciculi (*curved arrow*). The cerebral aqueduct (*straight arrow*) appears low signal intensity because of cerebrospinal fluid pulsations. (C) Axial T2-weighted MR image of the pons shows a high-signal-intensity lesion (*curved arrow*) in the region of the right medial longitudinal fasciculus, which follows the high signal intensity of cerebrospinal fluid in the fourth ventricle (*straight arrow*). From Cogen *et al.* [628], with permission.

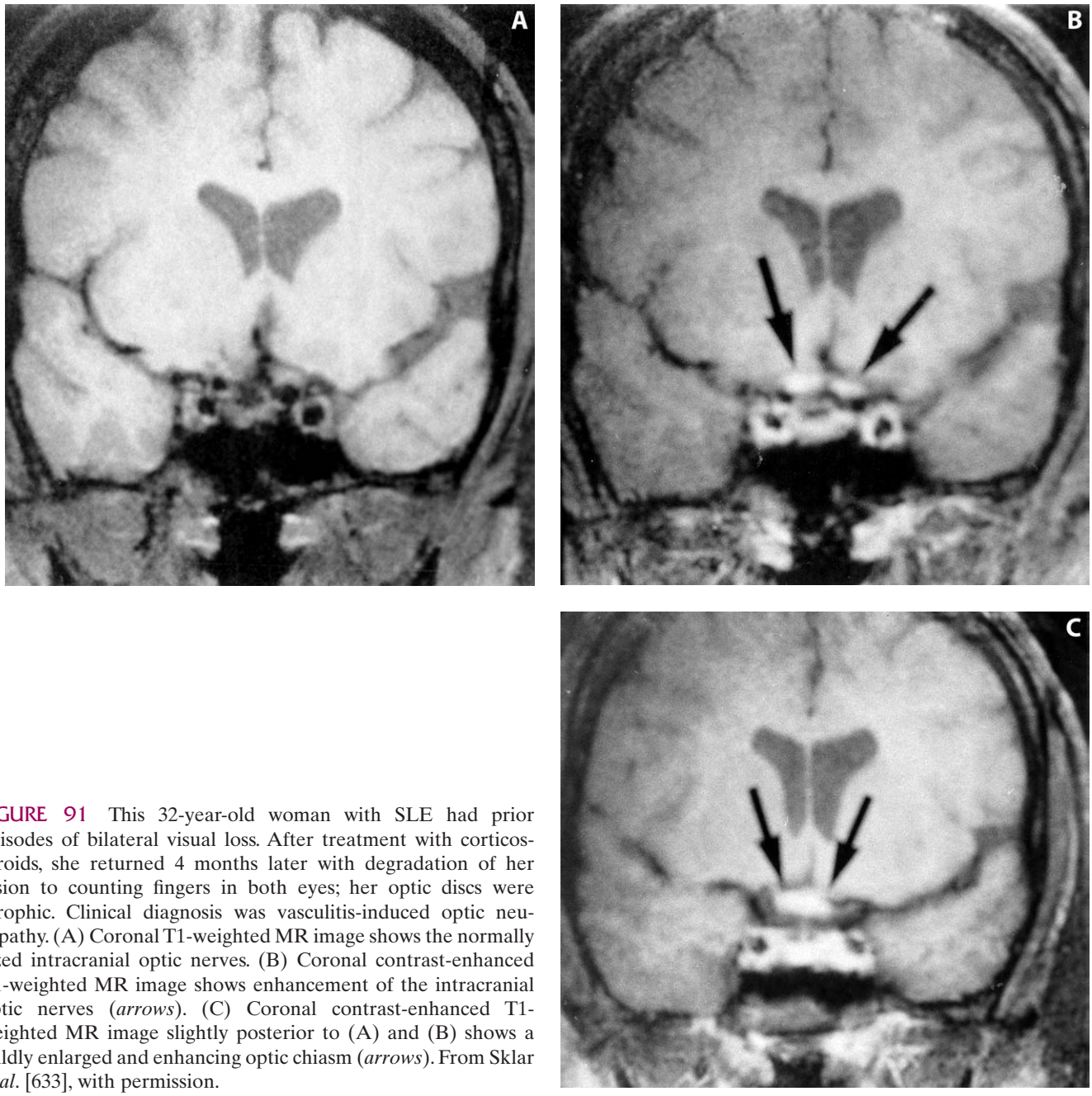


FIGURE 91 This 32-year-old woman with SLE had prior episodes of bilateral visual loss. After treatment with corticosteroids, she returned 4 months later with degradation of her vision to counting fingers in both eyes; her optic discs were atrophic. Clinical diagnosis was vasculitis-induced optic neuropathy. (A) Coronal T1-weighted MR image shows the normally sized intracranial optic nerves. (B) Coronal contrast-enhanced T1-weighted MR image shows enhancement of the intracranial optic nerves (*arrows*). (C) Coronal contrast-enhanced T1-weighted MR image slightly posterior to (A) and (B) shows a mildly enlarged and enhancing optic chiasm (*arrows*). From Sklar *et al*. [633], with permission.

meningitis [483, 554–558]. CT and MRI can identify all three entities. Abscesses appear as low-attenuation masses with well-defined, enhancing walls at contrast-enhanced CT. On T2-weighted MR images, intracerebral abscesses display high signal intensity. On contrast-enhanced T1-weighted MR sequences, abscesses are hypointense centrally with peripheral-enhancing walls [641]. Mycotic aneurysms are rare. When not thrombosed, they present, at contrast-enhanced CT and MRI, as enhancing masses and appear as low-signal intensity masses on T2-weighted MR images because of flowing blood. Conventional angiography and, more recently, MRA confirm the diagnosis (Fig. 92) [559]. With infectious meningitis, contrast-enhanced MRI and CT show intensely enhancing leptomeninges with a gyral pattern. Aseptic meningitis may develop as a complication of immunosuppressive therapy such as ibuprofen or azathioprine and as a manifestation of SLE [642–644] and shows similar features to its infectious counterpart. Rarely subdural fluid col-

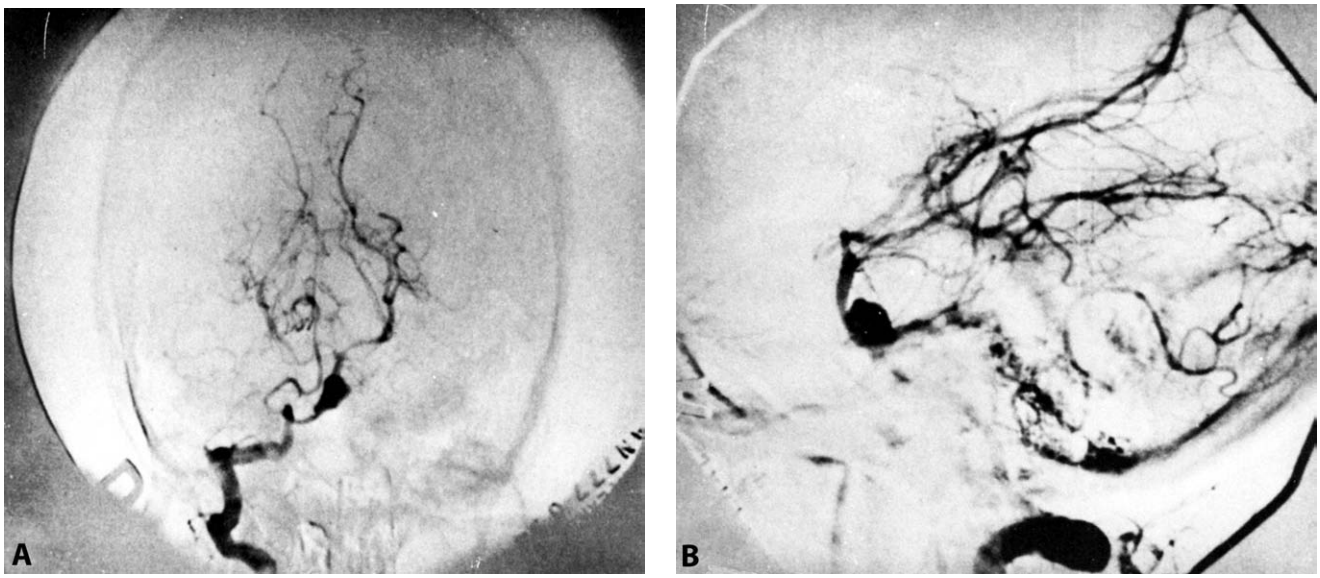


FIGURE 92 (A) Anteroposterior and (B) lateral subtraction vertebral angiograms show a large proximal basilar artery aneurysm. CT shows an enhancing mass anterior to the brain stem compatible with an aneurysm. From Goldman *et al*. [557], with permission.

lections may accompany aseptic meningitis and appear as lenticular hyperintense collections around the brain on T2-weighted MR images [645] and low-attenuation collections at CT.

Spinal Cord and Associated Dura in SLE

Transverse Myelitis in SLE

As compared to the incidence of lupus cerebritis, transverse myelitis is infrequent but may be the presenting manifestation of lupus or may develop at any time during the course of the disease [646–666]. Transverse myelitis sometimes may be associated with lupus anticoagulant and/or antiphospholipid antibodies [646, 648, 654, 655]. Rarely it may manifest as a Brown–Sequard syndrome [667] or anterior spinal artery syndrome [668, 669]. The clinical course can be variable. The onset may be acute, or myelitis gradually may evolve during days to weeks [617, 620]. T2-weighted MRI defines the focal or diffuse (Fig. 93) hyperintense edema of the spinal cord and the cord enlargement [646, 654, 657–659]. These lesions sometimes enhance on contrast-enhanced T1-weighted MR images [646, 663]. With successful therapy (Fig. 94), they resolve. Relapses, however, can occur with similar MR imaging features. Occasionally, well-defined areas of myelomalacia with cavitation or atrophy may result [660–662].

Although multiple sclerosis may also manifest focal hyperintense lesions of the spinal cord, these two disease may differ in location and extent of disease on MRI studies. Although both diseases may produce foci of high-signal intensity, lesions in SLE are fewer in number and extend over several vertebrae, whereas multiple sclerosis shows multiple simultaneous lesions over a shorter length of spinal cord [658, 670, 671].

Meningitis in SLE

As discussed in the section on the complications of central nervous system lupus, meningitis, either infectious or aseptic, is best evaluated on MR images. Rarely, aseptic meninogoencephalitis may be an early manifestation of SLE and disappears with treatment of lupus [672].

Epidural Lipomatosis in SLE

In lupus or other rheumatologic patients [673, 674], epidural lipomatosis, an unusual complication of corticosteroid therapy, is the abnormal deposition of unencapsulated fat around the dura, sometimes causing compression of the thecal sac. Fat accumulation occurs most commonly in the thoracic region but has also been found in the lumbar and sacral regions. The diagnosis should be suspected when a patient, who is receiving corticosteroids, develops signs or symptoms of spinal cord or cauda equina compression. Mild trauma combined with the epidural lipomatosis may further compromise the cord with resultant symptomatology [674].

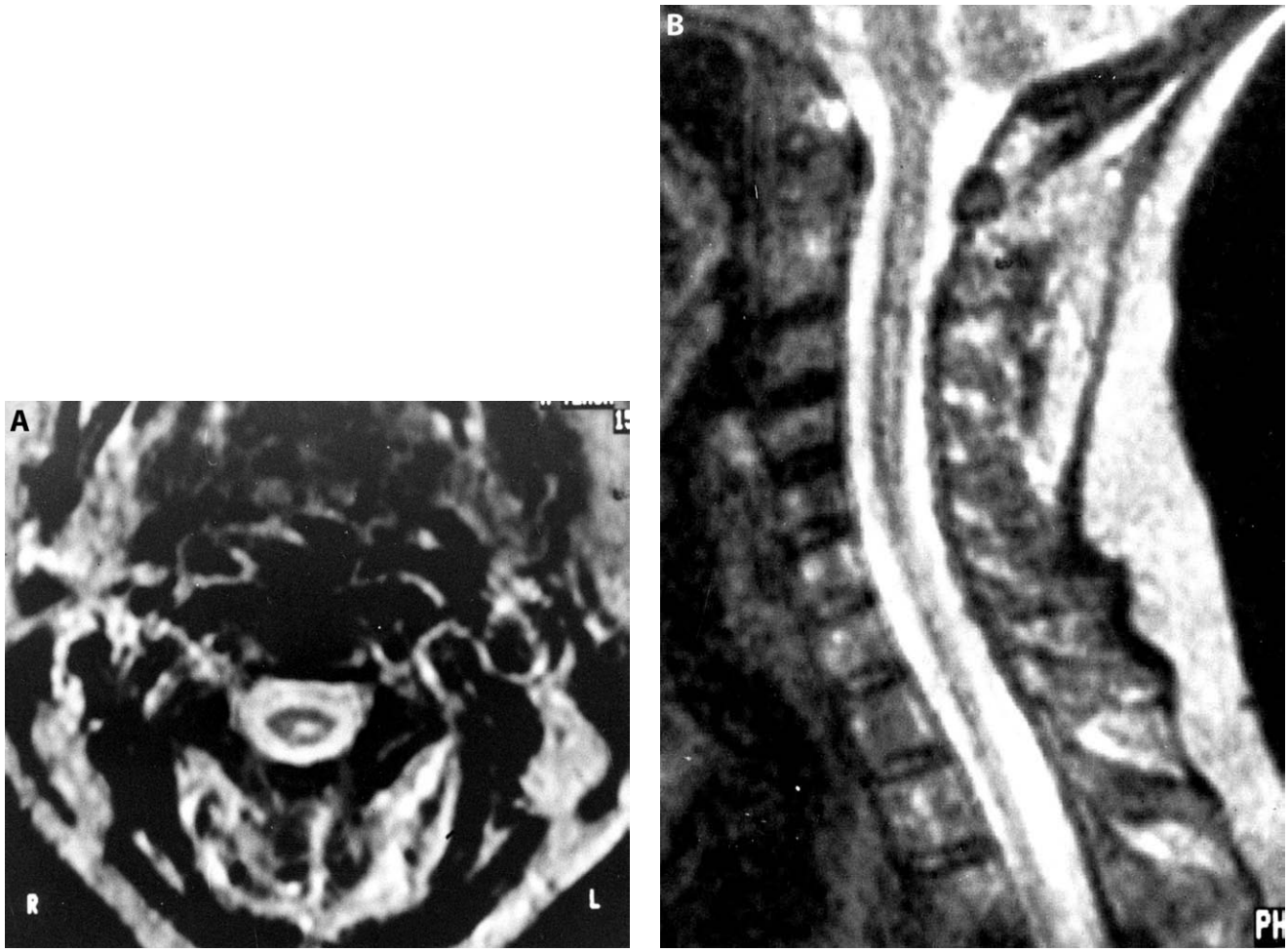


FIGURE 93 Axial (A) and sagittal (B) T2-weighted (1800/100) MR images of cervical spinal cord show enlargement and focal areas of high signal intensity in a 45-year-old patient with SLE and transverse myelitis. Courtesy of Dr. M.-F. Kahn, University of Paris, and Dr. J.-D. Laredo, University of Paris.

Both MRI and CT show the lipomatosis (hyperintense on T1-weighted MR images and on, T_2-weighted MR images and low attenuation on CT) surrounding the spinal cord. In addition, these modalities exclude other causes of thecal sac compromise, such as a herniated disc or a large epidural hematoma.

Epidural Hematoma in SLE

A rare complication of lupus is the spinal epidural hematoma. Patients may present with transverse myelopathy or develop back pain followed by radicular pain and paresis secondary to spinal cord compression [675, 676]. In lupus patients on immunosuppressive therapy, infection must be excluded. With subacute hemorrhage, MRI demonstrates the medium-to-high-signal intensity epidural collection on the T1-weighted sequence, which becomes high signal intensity on the T2-weighted sequence. Epidural abscesses are hyperintense masses on T2-weighted MR images and show peripheral rim enhancement without central enhancement on contrast-enhanced T1-weighted MR images.

CONCLUSIONS

Conventional radiography continues to be an important modality for the initial evaluation of the lupus patient and, in some instances, for the documentation of response to therapy. For example, radiographic studies are essential for studying and following pleuropulmonary disease (pneumonitis, hemorrhage, pleural effusion), the gastrointestinal tract (acute reversible lupus gastrointestinal ischemia), and the joints (arthritis,

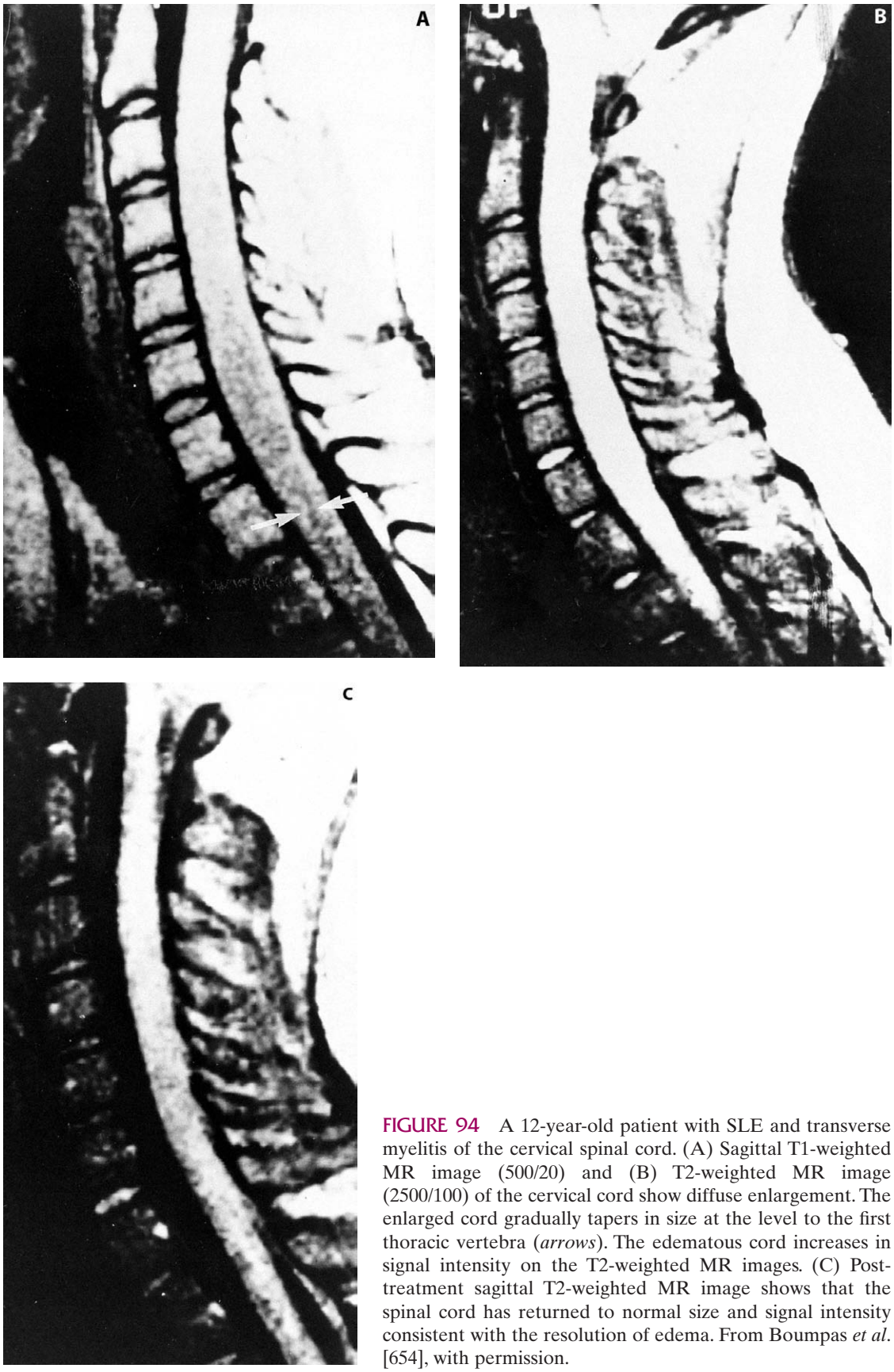


FIGURE 94 A 12-year-old patient with SLE and transverse myelitis of the cervical spinal cord. (A) Sagittal T1-weighted MR image (500/20) and (B) T2-weighted MR image (2500/100) of the cervical cord show diffuse enlargement. The enlarged cord gradually tapers in size at the level to the first thoracic vertebra (*arrows*). The edematous cord increases in signal intensity on the T2-weighted MR images. (C) Post-treatment sagittal T2-weighted MR image shows that the spinal cord has returned to normal size and signal intensity consistent with the resolution of edema. From Boumpas *et al.* [654], with permission.

instability). MRA is the preferred modality for defining lupus vasculitis, arteriosclerosis, and acute thrombosis. The modalities that produce cross-sectional images (ECG power Doppler/duplex sonography, CT, MRI) and the modalities that evaluate metabolism and perfusion (MRS, FDG-PET, SPECT, and MRA/MRI) have advanced dramatically and continue to improve our understanding of patients with SLE by defining their more subtle lesions and radiographically and clinically occult lesions, and their response to therapy and its complications.

References

1. Esdaile, J. M., Danoff, D., Rosenthall, L., *et al*. (1981). Deforming arthritis in systemic lupus erythematosus. *Ann. Rheum. Dis.* **40**, 124.
2. Weissman, B. N., Rappoport, A. S., Sosman, J. L., *et al*. (1978). Radiographic findings in the hands in patients with systemic lupus erythematosus. *Radiology* **126**, 313.
3. Resnick, D. (1988). Systemic lupus erythematosus. *In* "Diagnosis of Bone and Joint Disorders" (D. Resnick and G. Niwayama, eds.), pp. 1165–1187. Saunders, Philadelphia.
4. Braunstein, E. M., Weissman, B. N., Sosman, J. L., Schur, P. H. (1983). Radiologic findings in late-onset systemic lupus erythematosus. *AJR* **140**, 587.
5. Dubois, E. L., Friou, G. J., and Chandor, S. (1972). Rheumatoid nodules and rheumatoid granulomas in systemic lupus erythematosus. *JAMA* **220**, 515.
6. Bywaters, E. G. L. (1975). Jaccoud's syndrome: A sequel to the joint involvement of systemic lupus erythematosus. *Clin. Rheum. Dis.* **1**, 125.
7. De la Sota, M., Garcia-Morteo, O., and Maldonado-Cocco, J. A. (1985). Jaccoud's arthropathy of the knees in systemic lupus erythematosus. *Arthritis Rheum.* **28**, 825.
8. Aptekar, R. G., Lawless, O. J., and Decker, J. L. (1974). Deforming non-erosive arthritis of the hand in systemic lupus erythematosus. *Clin. Orthop.* **100**, 120.
9. Kahn, M. F. (1986). Jaccoud's syndrome in a rheumatology unit. *Clin. Rheumatol. Pract.* Winter, 124.

9a. Franceschini, F., Cretti, L., Quinzanini, M., Rizzini, F. L., and Cattaneo, R. (1993). Deforming arthropathy of the hands in systemic lupus erythematosus is associated with antibodies to SSA/Ro and to SSB/La. *Lupus.* **3**, 419.

10. Manthorpe, R., Bendixen, G., Scholer, H., *et al*. (1980). Jaccoud's syndrome. A nosographic entity associated with systemic lupus erythematosus. *J. Rheumatol.* **7**, 169.
11. Cohen, A. S., Canoso, J. J. (1972). Criteria for the classification of systemic lupus erythematosus-status 1972. *Arthritis Rheum*. **15**, 540.
12. Leskinen, R. H., Skrifvars, B. V., Laasonen, L. S., and Edgren, K. J. (1984). Bone lesions in systemic erythematosus. *Radiology* **153**, 49.
13. Moidel, R. A., and Good, A. E. (1981). Coexistent gout and systemic lupus erythematosus. *Arthritis Rheum*. **24**, 969.
14. Helliwell, M., Crisp, A. J., and Grahame, R. (1982). Coexistent tophaceous gout and systemic lupus erythematosus. *Rheumatol. Rehabil*. **21**, 161.
15. Alarcon, G. S., Ball, G. V., and Goldfarb, P. M. (1981). Systemic lupus erythematosus, gout, and Paget's disease. *Arthritis Rheum.* **24**, 238.
16. Noonan, C. D., Odone, D. T., Engleman, E. P., and Splitter, S. D. (1963). Roentgenographic manifestations of joint disease in systemic lupus erythematosus. *Radiology* **80**, 837.
17. Russell, A. S., Percy, J. S., Rigall, W. M., and Wilson, G. L. (1974). Deforming arthropathy in systemic lupus erythematosus. *Ann. Rheum. Dis*. **33**, 204.

17a. Liebling, M. R., and Gold, R. H. (1981). Erosions of the temporomandibular joint in systemic lupus erythematosus. *Arthritis Rheum.* **29**, 948.

18. Halim, W., Van Der Korst, K., Valkenburg, H. A., *et al*. (1975). Terminal phalangeal osteosclerosis. *Ann. Rheum. Dis*. **34**, 82.
19. McBride, C. S., and Fisher, M. S. (1975). Acrosclerosis in sarcoidosis. *Radiology* **115**, 279.
20. Shapeero, L. G., and Miller, W. T. (1973). The radiology of systemic lupus erythematosus: A new evaluation. *RSNA* 47.
21. Weinberger, A., Kaplan, J. G., and Myers, A. R. (1979). Extensive soft tissue calcification (calcinosis universalis) in systemic lupus erythematosus. *Ann. Rheum. Dis.* **38**, 384.
22. Kohli, M., and Bennett, R. M. (1994). Sacroiliitis in systemic lupus erythematosus. *J. Rheumatol*. **21**, 170.
23. Vivas, J., and Tilakos, N. A. (1985). Sacroiliitis in male systemic lupus erythematosus. *Scand. J. Rheumatol*. **14**, 441. [Abstract]
24. Nassonova, V. S., Alekberova, Z. S., Folomeyev, M. Y., and Mylov, N. (1984). Sacroiliitis in male systemic lupus erythematosus. *Scand. J. Rheumatol. (Suppl.)* **52**, 23.

24a. Vivas, J., and Tilakos, N. A. (1985). Sacroiliitis in male systemic lupus erythematosus. *Bol. Assoc. Med. P. R.* **77**, 271.

25. DeSmet, A. A., Mahmood, T., Robinson, R. G., and Lindsley, H. B. (1984). Elevated sacroiliac joint uptake ratios in systemic lupus erythematosus. *AJR* **143**, 351.
26. Murphey, M. M., Wetzel, L. H., Bramble J. M., *et al*. (1991). Sacroiliitis: MR imaging findings. *Radiology* **180**, 239.
27. Potasman, I., and Bassan, H. M. (1984). Multiple tendon rupture in systemic lupus erythematosus: Case report and review of the literature. *Ann. Rheum. Dis.* **43**, 347.

27a. Babini, S. M., Cocco, J. A., Babini, J. C., *et al*. (1990). Atlantoaxial subluxation in systemic lupus erythematosus: Further evidence of tendinous alteration. *J. Rheumatol.* **17**, 173.

27b. Pritchard, C. H., and Berney, S. (1989). Patellar tendons rupture in systemic lupus erythematosus. *J. Rheumatol.* **16**, 786.

27c. Munk, P. L., Vellet, A. D., Romano, C. C., *et al*. (1994). Emerging role of magnetic resonance imaging in rheumatology. *Can. Assoc. Radiol. J.* **45**, 250.
28. Shapeero, L. G. (1990). MR imaging of the foot and ankle. *In* "Diagnostic Radiology" (A. M. Margulis and C. A. Gouding, eds.), pp. 454–466. University of California, San Francisco.
29. Brandser, E. A., El-Khoury, G. Y., and Saltzman, C. L. (1995). Tendon injuries: Application of magnetic resonance imaging. *J. Can. Assoc. Radiol.* **46**, 9.
30. Kerr, R., and Kingston, S. (1997). Imaging of sports injuries of the wrist and hand. *Semin. Musculoskel. Radiol.* **1**, 5.
30a. Gould, E. S., Taylor, S., Naidich, J. B., Furie, R., and Lane, L. (1987). MR appearance of bilateral spontaneous patellar tendon rupture in systemic lupus erythematosus. *J. Comput. Assist. Tomogr.* **11**, 1096.
31. Nazarian, L. N., Rawool, N. M., Martin, C. E., and Schweitzer, M. E. (1995). Synovial fluid in the hindfoot and ankle: Detection of amount and distribution with US. *Radiology* **197**, 275.
32. Von Holsbeeck, M., and Introcaso, H. H. (1990). Sonography of tendons. *In* "Musculoskeletal Ultrasound," pp. 57–89. Mosby-YearBook, Chicago.
33. Fornage, B., and Rifkin, M. D. (1988). Ultrasound examination of tendons. *Radiol. Clin. North. Am.* **26**, 87.
33a. Rosenthal, S. J., Jones, P. H., and Wetzel, L. H. (2001). Phase inversion tissue harmonic sonographic imaging: A clinical utility study. *Am. J. Roentgen.* **176**, 1393.
33b. Barberie, J. E., Wong, A. D., and Cooperberg, P. L., Carson, B. W. (1998). Extended field-of-view sonography in musculoskeletal disorders. *Am. J. Roentgen.* **171**, 751.
33c. Newman, J. S., Adler, R. S., Bude, R. O., and Rubin, J. M. (1994). Detection of soft-tissue hyperemia: Value of Power Doppler sonography, *Am. J. Roentgen.* **163**, 385.
34. Frankel, V. (1978). The Terry-Thomas sign. *Clin. Orthop. Res.* **135**, 311.
35. Levissohn, M., Palmer, A. K., Coren, A. B., and Zinberg E. (1987). Wrist arthrography: The value of the three compartment injection technique. *Skeletal Radiol.* **16**, 539.
36. Schweitzer, M. E., Brahme, S. K., Hodler, J., *et al*. (1992). Chronic wrist pain: Spin-echo and short tau inversion recovery MR imaging and conventional and MR arthrography. *Radiology* **182**, 205.
37. Shapeero, L. G., Dye, S. F., Lipton, M. D., *et al*. (1988). Functional dynamics of the knee joint by ultrafast cine-CT. *Invest. Radiol.* **23**, 118.
38. Shellock, F. G. (1997). Kinematic MR imaging of the joints. *Semin. Musculoskel. Radiol.* **1**, 143.
39. Dubois, E., and Cozen, L. (1960). Avascular (aseptic) bone necrosis associated with systemic lupus erythematosus. *JAMA* **174**, 966.
40. LaPorte, D. M., Mont, M. A., Mohan, V., Jones, L. C., and Hungerford, D. S. (1968). Multifocal osteonecrosis. *J. Rheumatol.* **1998**, 25.
40a. Ruderman, M., McCarty D. J., Jr. (1964). Aseptic necrosis in systemic lupus erythematosus. Report of a case involving six joints. *Arthritis. Rheum.* **7**, 709.
41. Pietrograde, V., and Mastromarino, R. (1957). Osteopatia da prolungato trattamento cortisonico. *Ortop. Traumat.* **25**, 791.
42. Abeles, M., Urman, J. D., and Rothfield, N. F. (1978). Aseptic necrosis of bone in systemic lupus erythematosus "relationship to corticosteroid therapy". *Arch. Intern. Med.* **138**, 750.
43. Smith, F. E., Sweet, D. E., Brunner, C. M., *et al*. (1976). Avascular necrosis in SLE: An apparent predilection for young patients. *Ann. Rheum. Dis.* **35**, 227.
44. Bergstein, J. M., Wiens, C., and Fish, A. J. (1974). Avascular necrosis of bone in systemic lupus erythematosus. *J. Pediatr.* **85**, 31.
45. Hurley, R. M., Steinberg, R. H., Patriquin, H., *et al*. (1974). Avascular necrosis of the femoral head in childhood systemic lupus erythematosus. *Can. Med. Assoc. J.* **111**, 781.
46. Mitchell, D. G., Rao, V. J., Dalinka, M. K., *et al*. (1987). Femoral head avascular necrosis: Correlation of MR imaging, radiographic staging, radionuclide imaging, and classical findings. *Radiology* **162**, 172.
46a. Abrahim-Zadeh, R., Klein, R. M., Leslie, D., and Norman, A. (1998). Characteristics of calcaneal bone infarction: An MR imaging investigation. *Skeletal Radiol.* **27**, 321.
47. Coleman, B. G., Kressel, H. Y., Dalinka, M. K., *et al*. (1988). Radiographically negative avascular necrosis: Detection with MR imaging. *Radiology* **168**, 525.
48. Lee, M. J., Corrigan, J., Stack, J. P., and Ennis, J. T. (1990). A comparison of modern imaging modalities in osteonecrosis of the femoral head. *Clin. Radiol.* **42**, 427.
49. Tervonen, O., Mueller, D. M., Matteson, E. L., *et al*. (1992). Clinically occult avascular necrosis of the hip: Prevalence in an asymptomatic population at risk. *Radiology* **1822**, 845.
49a. Umans, H., Haramati, N., and Flusser, G. (2000). The diagnostic role of gadolinium-enhanced MRI in distinguishing between acute medullary bone infarct and osteomyelitis. *Magn. Res. Imag.* **18**, 255.
50. Klipper, A. R., Stevens, M. B., Zizic, T. M., *et al*. (1976). Ischemic necrosis of bone in systemic lupus erythematosus. *Medicine* **55**, 251.
51. Griffiths, I. D., Maini, R. N., Scott, J. T. (1979). Clinical and radiological features of osteonecrosis in systemic lupus erythematosus. *Ann. Rheum. Dis.* **38**, 413.
52. Norman, A. (1984). Roentgenologic diagnosis. *In* "Osteoarthritis, Diagnosis and Management" (R. W. Moskowitz, D. S. Howell, K. M. Goldberg, and H. J. Mankin, eds.) p. 155. Saunders, Philadelphia.
53. Norman, A., and Bullough, P. (1963). The radiolucent "crescent line": An early diagnostic sign of avascular necrosis of the femoral head. *Bull. Hosp. Joint. Dis.* **24**, 99.
53a. Aranow, C., and Zelicof, Leslie, D., *et al*. (1997). Clinically occult avascular necrosis of the hip in systemic lupus erythematosus. *J. Rheumatol.* **24**, 2318.

54. Glickstein, M., Neustadter, L., Dalinka, M., and Kricun, M. (1986). Periosteal reaction in systemic lupus erythematosus. *Skeletal Radiol.* **15**, 610.
55. Burson, J. S., Grana, J., Varela, J., Atanew, A. M., and Galdo, F. (1990). Laminar periostitis and multiple osteonecrosis in systemic lupus erythematosus. *Clin. Rheumatol.* **9**, 535.
56. Maldague, B. E., Noel, H. M., and Malghem, J. J. (1978). The intravertebral vacuum cleft: A sign of ischemic vertebral collapse. *Radiology* **129**, 23.
57 Alonso-Bartolomé, P., Martinez-Taboada, V. M., Blanco, R., and Rodrigue-Valverde, V. (1998). Insufficiency fractures of the tibia and fibula. *Semin. Arthritis. Rheum.* **28**, 413.
57a. Stafford, S. A., Rosenthal, D. I., Gebhardt, M. C., Brady, T. J., and Scott, J. A. (1986). MR imaging in stress fracture. *AJR* **147**, 553.
58. Ginzler, E., Diamond, H., Kaplan, K. D., *et al*. (1978). Computer analysis of factors influencing frequency of infection in systemic lupus erythematosus. *Arthritis Rheum*. **21**, 37.
59. Schenfeld, L., Gray, R. G., Poppo, M. J., *et al*. (1981). Bacterial monoarthritis due to *Neisseria meningitidis* in systemic lupus erythematosus. *J. Rheumatol.* **8**, 145.
60. Hunter, T., and Plummer, F. A. (1980). Infectious arthritis complicating systemic lupus erythematosus. *Can. Med. Assoc. J.* **122**, 791.
61. Quismorio, F. P., Lakes, J. T., Zarnow, A. J., *et al*. (1983). Septic arthritis due to *Arizona hinshawii. J Rheumatol.* **10**, 147.
62. Boey, M. L., and Feng, P. H. (1982). Salmonella infection in systemic lupus erythematosus. *Singapore Med. J.* **23**, 147.
63. Tehranzadeh, J., Wang, F., and Mesgarzadeh, M. (1992). Magnetic resonance imaging of osteomyelitis. *Crit. Rev. Diag. Imag.* **33**, 495.
63a. Hopkins, K. L., Li CPK, and Bergman, G. (1995). Gadolinium-DTPA-enhanced magnetic resonance imaging of musculoskeletal infectious processes. *Skeletal Radiol.* **24**, 325.
64. Mirahmadi, K. S., Coburn, J. W., and Bluestone, R. (1973). Calcific periarthritis and hemodialysis. *JAMA* **223**, 548.
65. Isenberg, D. A., and Snaith, M. L. (1981). Muscle disease in systemic lupus erythematosus: A study of its nature, frequency and cause. *J. Rheumatol.* **8**, 917.
66. Claudepiere, P., Saint-Marcoux, B., Larget-Piet, B., *et al*. (1996). Clinical images: Value of magnetic resonance imaging in extensive pyomyositis. *Arthritis Rheum.* **39**, 1760.
67. Baehr, G., Klemperer, P., and Schifrin, A. (1935). A diffuse disease of peripheral circulation (usually associated with lupus erythematosus and endocarditis). *Trans. Acad. Am. Phys.* **50**, 139.
68. Keats, T. E. (1961). The collagen diseases: A demonstration of the nonspecificity of their extrapulmonary manifestations. *Am. J. Roentgen.* **86**, 938.
69. Alarcon-Segovia, D., and Osmundson, P. J. (1965). Peripheral vascular syndromes associated with systemic lupus erythematosus. *Ann. Intern. Med.* **62**, 907.
70. Kabir, D. I., and Malkinson, F. D. (1969). Lupus erythematosus and calcinosis cutis. *Arch. Dermatol.* **100**, 17.
71. Tay, C. R. (1970). Cutaneous manifestations of systemic lupus erythematosus: A clinical study from Singapore. *Aust. J. Dermatol.* **11**, 30.
72. Savin, J. A. (1971). Systemic lupus erythematosus with ectopic calcification. *Br. J. Dermatol.* **84**, 191.
73. Powell, R. J., Bywaters, E. G. L., and Ansell, B. M. (1974). Systemic lupus erythematosus with widespread subcutaneous fat calcification. *Proc. R. Soc. Med.* **67**, 215.
74. Quismorio, F. P., Dubois, E. L., and Chandor, S. B. (1975). Soft-tissue calcification in systemic lupus erythematosus. *Arch. Dermatol.* **111**, 352.
74a. Minami, A., Suda, K., Kaneda, K., Kumakiri, M. (1994). Extensive subcutaneous calcification of the forearm in systemic lupus erythematosus. *J. Hand Surg.* **19B**, 638.
75. Budin, J. A., and Feldman, F. (1975). Soft-tissue calcification in systemic lupus erythematosus. *Am. J. Roentgen.* **124**, 358.
75a. Nomura, M., Ilada, M., Okada, M., and Yoshikawa, K. (1990). Large subcutaneous calcification in systemic lupus erythematosus. *Arch. Dermatol.* **126**, 1057.
76. Mathews, J., Sziklas, J. J., and Spencer, R. P. (1985). Multiple sites extraosseous deposition of bone imaging agent in lupus erythematosus. *Clin. Nucl. Med.* **101**, 730.
77. Wallace, D. J. (1997). The clinical presentation of systemic lupus erythematosus. *In* "Dubois Lupus Erythematosus" (D. J. Wallace and B. H. Hahn, eds.), pp. 627–633. Williams & Wilkins, Baltimore.
78. Garland, L. H., and Sisson, M. A. (1954). Roentgen findings in the "collagen" diseases. *Am. J. Roentgen.* **71**, 581.
79. Gould, D. M., Daves, M. L., and McAfee, J. G. (1958). A review of roentgenographic findings in systemic lupus erythematosus (SLE). *Am. J. Med. Sci.* **235**, 596.
80. Harvey, A. M., Shulman, L. E., Tumulty, P. A., *et al*. (1954). Systemic lupus erythematosus: Review of the literature and critical analysis of 138 cases. *Medicine* **33**, 291.
81. Raine, J. L. (1975). Refractory, massive effusion in system lupus erythematosus treated with talc poudrage. *Ann. Rheum.* **44**, 61.
82. Reda, M. G., and Baigelman, W. (1980). Pleural effusion in systemic lupus erythematosus. *Acta Cytol.* **24**, 553.
83. Carr, D. T., Lillington, G. A., and Mayne, J. G. (1970). Pleural-fluid glucose in systemic lupus erythematosus. *Mayo Clin. Proc.* **45**, 409.
84. Matthay, R. A., Hudson, L. D., and Petty, T. L. (1973). Acute lupus pneumonitis: Response to azathioprine therapy. *Chest* **63**, 117.
85. Myers, S. A., Podczaski, E., and Freese, U. (1980). Acute lupus pneumonitis in the puerperium: A case report and literature review. *J. Reprod. Med.* **25**, 285.
86. Dubois, E. L., and Tuffanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus, computer analysis of 520 cases. *JAMA* **190**, 104.

87. Matthay, R. A., Schwarz, M. I., Petty, T. L., *et al*. (1974). Pulmonary manifestations of systemic lupus erythematosus: Review of twelve cases of acute pneumonitis. *Medicine* **54**, 397.
88. Foldes, I. (1946). Acute systemic lupus erythematosus. *Am. J. Clin. Pathol.* **16**, 160.
89. Rakov, H. L., and Taylor, J. S. (1942). Acute disseminated lupus erythematosus without cutaneous manifestations and with heretofore undescribed pulmonary lesions. *Arch. Int. Med.* **70**, 88.
90. Purnell, D. C., Baggenstoss, A. H., and Olsen, A. M. (1955). Pulmonary lesions in disseminated lupus erythematosus. *Ann. Intern. Med.* **42**, 619.
91. Gross, M., Esterly, J. R., and Earle, R. H. (1972). Pulmonary alteration in systemic lupus erythematosus. *Am. Rev. Respir. Dis.* **105**, 572.
92. Turner-Stokes, L., and Turner-Warwick, M. (1982). Intrathoracic manifestations of systemic lupus erythematosus. *Clin. Rheum. Dis.* **8**, 229.
93. Yeo, P. P. B., and Sinnian, R. (1975). Lupus cor pulmonale with electron microscopy and immunofluorescent antibody structures. *Ann. Rheum. Dis.* **34**, 457.
94. Carette, S., Macher, A. M., Nussbaum, A., *et al*. (1984). Severe acute pulmonary disease in patients with systemic lupus erythematosus: Ten years' experience at the National Institutes of Health. *Semin. Arthritis Rheum.* **14**, 52.
95. Thom, R. T. O. A., Peters, W. G., de Bruine, F. T., and Willemze, R. (1987). Pulmonary complications of cytosine arabinoside therapy: Radiographic findings. *Am. J. Roentgen.* **149**, 23.
96. Gorevic, P. D., Katler, E. L., and Agus, B. (1980). Pulmonary nocardiosis: Occurrence in men with systemic lupus erythematosus. *Arch. Intern. Med.* **140**, 361.
97. Feng, P. H., and Tan, T. H. (1982). Tuberculosis in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **41**, 11.
98. Levin, D. C. (1971). Proper interpretation of pulmonary roentgen changes in systemic lupus erythematosus. *Am. J. Roentgen.* **111**, 510.
99. Webb, W. R., and Gamsu, G. (1981). Cavitary pulmonary nodules with systemic lupus erythematosus: Differential diagnosis. *Am. J. Roentgen.* **136**, 27.
99a. Kim, H. Y., Im, J.-G., Goo, J. M., Lee, J. K., Song, J. W., and Kim, K. S. (1999). Pulmonary tuberculosis in patients with systemic lupus erythematosus. *Am. J. Roentgen.* **173**, 1639.
100. Israel, H. C. (1953). The pulmonary manifestations of disseminated lupus erythematosus. *Am. J. Med. Sci.* **266**, 387.
100a. Cheema, G. S., and Quismorio, F. P. J. R. (2000). Interstitial lung disease in systemic lupus erythematosus. *Curr. Opin. Pulm. Med.* **200**(8), 424.
101. Mace, B. E. W., and Sperryn, R. N. (1971). Systemic lupus erythematosus with fibrosing alveolitis. *Proc. R. Soc. Med.* **64**, 58.
102. Vachtenheim, J., and Grossman, J. (1973). Diffuse interstitial lung fibrosis in systemic lupus erythematosus. *Ann. Intern. Med.* **79**, 913.
103. Holden, M. (1973). Massive pulmonary fibrosis due to systemic lupus erythematosus. *N.Y. State J. Med.* **73**, 462.
104. Eisenberg, H., Dubois, E. L., Sherwin, R. P., and Balchum, O. J. (1973). Diffuse interstitial lung disease in systemic lupus erythematosus. *Ann. Intern. Med.* **79**, 37.
105. Lin, R. Y. (1987). Severe spirometric effects in systemic lupus erythematosus: A possible role for bronchioalveolar lavage and gallium scanning. *Clin. Rheumatol.* **6**, 276.
105a. Witt, C., Dorner, T., Hiepe, F., Borges, A. C., Fietze, I., and Baumann, G. (1996). Diagnosis of alveolitis in interstitial lung manifestation in connective tissue diseases: Importance of late inspiratory crackles, 67 gallium scan and bronchoalveolar lavage. *Lupus* **5**, 606.
106. Sawkar, L. A., and Eason, H. F. (1971). Recurrent spontaneous pneumothoraces in systemic lupus erythematosus. *Chest* **60**, 604.
107. Richards, A. J., Swinson, D. R., Talbot, I. C., and Hamilton, E. B. D. (1975). Diffuse pulmonary fibrosis and bilateral pneumothoraces in systemic lupus erythematosus. *Postgrad. Med. J.* **151**, 581.
108. Masuda, A., Tsushima, T., Shizume, K., *et al*. (1990). Recurrent pneumothoraces and mediastinal emphysema in systemic lupus erythematosus. *J. Rheumatol.* **17**, 544.
109. Paira, S. O., and Rovberano, S. (1992). Case report: Bilateral pneumothorax and mediastinal emphysema in systemic lupus erythematosus. *Clin. Rheumatol.* **11**, 571.
110. Passero, F. C., and Myers, A. R. (1980). Hemopneumothorax in systemic lupus erythematosus. *J. Rheumatol.* **7**, 183.
111. Prakash, U. B., Luthra, H. S., and Divertie, M. B. (1985). Intrathoracic manifestations in mixed connective tissue disease. *Mayo Clin. Proc.* **60**, 813.
112. Holgate, S. T., Glass, D. N., Haslam, P., *et al*. (1976). Respiratory involvement in systemic lupus erythematosus: A clinical and immunological study. *Clin. Exp. Immunol.* **24**, 385.
113. Gibson, G. J., Edmonds, J. P., and Hughes, G. R. V. (1977). Diaphragm function and lung involvement in systemic lupus erythematosus. *Am. J. Med.* **63**, 926.
114. Hoffbrand, B. I., and Beck, E. R. (1965). "Unexplained" dyspnoea and shrinking lungs in systemic lupus erythematosus. *Br. Med. J.* **1**, 1273.
114a. Munoz-Rodriguez, F. J., Font, J., Badia, J. R., *et al*. (1997). Shrinking lungs in systemic lupus erythematosus: Improvement with inhaled beta-agonist therapy. *Lupus* **6**, 412.
115. Myhre, J. R. (1959). Pleuropulmonary manifestations of lupus erythematosus disseminatus. *Acta Med. Scand.* **165**, 55.
116. Thompson, P. J., Dhillon, D. P., Ledingham, J., and Turner-Warwick, M. (1985). Shrinking lungs, diaphragmatic dysfunction, and systemic lupus erythematosus. *Am. Rev. Resp. Dis.* **132**, 926.
117. Laroche, C. M., Mulvey, D. A., Hawkins, P. N., *et al*. (1989). Diaphragm strength in the shrinking lung syndrome of systemic lupus erythematosus. *Q. J. Med.* **71**, 429.

118. Huang, C. T., Hennigar, G. R., and Lyons, H. A. (1965). Pulmonary dysfunction in systemic lupus erythematosus. *N. Engl. J. Med.* **272**, 288.
119. Bankier, A. Z., Kiener, H. P., Wiesmayr, M. N., *et al*. (1995). Discrete lung involvement in systemic lupus erythematosus: CT assessment. *Radiology* **196**, 835.
119a. Fenlon, H. M., Doran, M., Sant, S. M., and Breatnach, E. (1996). High-resolution chest CT in systemic lupus erythematosus. *Am. J. Roentgen.* **166**, 301.
120. Sant, S. M., Doran, M., Fenelon, H. M., and Breatnach, E. S. (1997). Pleuropulmonary abnormalities in patients with systemic lupus erythematosus: Assessment with high resolution computed tomography, chest radiography and pulmonary function test. *Clin. Exp. Rheumatol.* **15**, 507.
120a. Ooi, G. C., Ngan, H., Peh, W. C. G., *et al*. (1997). Systemic lupus erythematosus patients with respiratory symptoms: The value of HRCT. *Clin. Radiol.* **91**, 1081.
121. Wells, A. J., Hansell, D. M., and Corrin, B. (1992). High resolution computed tomography as a predictor of lung histology in systemic sclerosis. *Thorax* **47**, 738.
122. Gladman, D. D., and Urowitz, M. B. (1980). Venous syndromes and pulmonary embolism in systemic lupus erythematosus. *Ann. Rheum. Dis.* **39**, 340.
122a. Tamara-Soler, V., Guillermo-ARbo, O. M., and Neva-Cacers, C. (2000). Tromboembolismo pulmon relacionado a sindrome antifosfolipido y lupus eritematoso sistemico. *Rev. Med. Chile* **128**, 899.
123. Asherson, R. A., Gibson, D. G., Evans, D. W., *et al*. (1988). Diagnostic and therapeutic problems in two patients with antiphospholipid antibodies, heart valve lesions, and transient ischaemic attacks. *Ann. Rheum. Dis.* **47**, 947.
123a. Semeniuk, G. B., Re, R., and Freue, R. D. (2001). Trombosis de grandes vasos pulmonare como causa de hipertension pulmonar severa en el lupus erithematoso sistemico. *Medicina* **61**, 319.
124. Yuen, S. F., Lau, K. F., Steinberg, A. W., Grattan-Smith, P. J., and Hodson, E. M. (2001). Intracranial venous thrombosis and pulmonary embolism with antiphospholipid syndrome in systemic lupus erythematosus. *J. Paedriatr. Child Health* **37**, 405.
124a. Howe, H. S., Boey, M. L., Fong, K. Y., and Feng, P. H. (1988). Pulmonary hemorrhage, pulmonary infarction, and the lupus anticoagulant. *Ann. Rheum. Dis.* **47**, 869.
125. Abud-Mendoza, C., Diaz-Jouanen, E., and Alarcon-Segovia, D. (1985). Fatal pulmonary hemorrhage in SLE: Occurrence without hemoptysis. *J. Rheumatol.* **12**, 558.
126. Casteneda, J., Herrero-Beaumont, G., Valenzuela, A., *et al*. (1985). Massive pulmonary hemorrhage, fatal complication of systemic lupus erythematosus. *J. Rheumatol.* **12**, 186.
127. Eagen, J. W., Memoli, V. A., Roberts, J. L., *et al*. (1978). Pulmonary hemorrhage in systemic lupus erythematosus. *Medicine* **57**, 545.
128. Churg, A., Franklin, W., Chan, K. L., *et al*. (1980). Pulmonary hemorrhage and immune-complex deposition in the lung: Complications in a patient with systemic lupus erythematosus. *Arch. Pathol. Lab. Med.* **104**, 388.
129. Gamsu, G., and Webb, W. R. (1978). Pulmonary hemorrhage in systemic lupus erythematosus. *J. Assoc. Can. Radiol.* **29**, 66.
130. Marino, C. T., and Pertschuk, L. P. (1981). Pulmonary hemorrhage in lupus erythematosus. *Arch. Intern. Med.* **141**, 201.
131. Gould, D. B., and Soriano, R. Z. (1975). Acute alveolar hemorrhage in lupus erythematosus. *Ann. Intern. Med.* **83**, 836.
132. Mintz, G., Galindo, L. F., Fernandez-Diez, J., *et al*. (1978). Acute massive pulmonary hemorrhage in systemic lupus erythematosus. *J. Rheumatol.* **5**, 39.
133. Rajani, K. B., Ashbacher, L. V., and Kinney, T. R. (1978). Pulmonary hemorrhage and systemic lupus erythematosus. *J. Pediatr.* **93**, 810.
134. Millman, R. P., Cohen, T. B., Levinson, A. L., *et al*. (1981). Systemic lupus erythematosus complicated by acute pulmonary hemorrhage: Recovery following plasmapheresis and cytotoxic therapy. *J. Rheumatol.* **8**, 1021.
135. Ramirez, R. E., Glasier, C., Kirks, D., *et al*. (1984). Pulmonary hemorrhage associated with systemic lupus erythematosus in children. *Radiology* **152**, 409.
136. Ewan, P. W., Jones, H. A., Rhodes, C. G., and Hughes, J. M. B. (1976). Detection of intrapulmonary hemorrhage with carbon monoxide uptake: Application in Goodpasture's syndrome. *N. Engl. J. Med.* **295**, 1391.
137. Makine, Y., Ogawa, M., Ueda, S., and Ohto, M. (1993). CT appearance of diffuse alveolar hemorrhage in a patient with systemic lupus erythematosus. *Acta Radiol.* **34**, 634.
138. Brentjzens, J. R., O'Connell, D. W., Pawlowski, I. B., *et al*. (1974). Experimental immune complex disease of lung: The pathogenesis of a laboratory model resembling certain human interstitial lung disease. *J. Exp. Med.* **140**, 150.
139. Ichikawa, Y., Shimizu, H., Kobayashi, I., *et al*. (1989). Recurrent lupus pneumonitis with pulmonary hemorrhage associated with chronic thyroiditis and antithyroid hormone antibodies. *Clin. Exp. Rheumatol.* **7**, 309.
140. Kwong, Y. L., Wong, K. L., Kung, I. T., *et al*. (1988). Concomitant alveolar hemorrhage and cytomegalovirus infection in a patient with systemic lupus erythematosus. *Postgrad. Med. J.* **64**, 56.
141. Byrd, R., and Trunk, G. (1973). Systemic lupus erythematosus presenting as pulmonary hemosiderosis. *Chest* **64**, 128.
142. Fayemi, A. O. (1976). Pulmonary vascular disease in systemic lupus erythematosus. *Am. J. Clin. Pathol.* **65**, 284.
143. Santini, D., Fox, D., Kloner, R. A., *et al*. (1980). Pulmonary hypertension in systemic lupus erythematosus: Hemodynamics and effects of vasodilator therapy. *Clin. Cardiol.* **3**, 406.
144. Sack, K. E., Bekheit, S., Fadem, S. Z., and Bedrossian, C. W. M. (1979). Severe pulmonary vascular disease in systemic lupus erythematosus. *South Med. J.* **72**, 1016.
145. Kanemoto, N., Gonda, N., Katsu, M., and Fukuda, J. (1975). Two cases of pulmonary hypertension with

Raynaud's phenomenon: Primary pulmonary hypertension and systemic lupus erythematosus. *Jpn. Heart J.* **16**, 354.
146. Mack, J. W., Fry, M. B., and McIntosh, D. A. (1973). Pulmonary hypertension in systemic lupus erythematosus. *N. Engl. J. Med.* **289**, 157.
147. Sergent, J. S., and Lockshin, M. D. (1973). Primary pulmonary hypertension and systemic lupus erythematosus. *N. Engl. J. Med.* **288**, 1078.
148. Woh, M. R., and Castleman, B. (1973). Case records of the Massachusetts General Hospital: Case 4. *N. Engl. J. Med.* **288**, 204.
149. Nair, S. S., Askari, A. D., Popelka, C. G., and Kleinerman, I. F. (1980). Pulmonary hypertension and systemic lupus erythematosus. *Arch. Intern. Med.* **140**, 109.
150. Perez, H. D., and Kramer, N. (1981). Pulmonary hypertension in systemic lupus erythematosus: Report of four cases and review of the literature. *Semin. Arthritis Rheum.* **11**, 177.
151. Cummings, P. (1973). Primary pulmonary hypertension in systemic lupus erythematosus. *N. Engl. J. Med.* **288**, 1078.
152. Asherson, R. A., Hackett, D., Gharavi, A. E., *et al*. (1986). Pulmonary hypertension in systemic lupus erythematosus: A report of three cases. *J. Rheumatol.* **13**, 416.
153. Stark, P., Sargent, E. N., Boylen, T., and Jaramillo, D. (1987). Pulmonary arterial hypertension as a manifestation of lupus erythematosus. *Radiology* **27**, 370.
154. Simonson, J. S., Schiller, N. B., Petri, M., and Hellman, D. B. (1989). Pulmonary hypertension in systemic lupus erythematosus. *J. Rheumatol.* **16**, 918.
155. Winslow, T. M., Ossipov, M. A., Faxio, G. P., *et al*. (1995). Five-year follow-up study of the prevalence and progression of pulmonary hypertension in systemic lupus erythematosus. *Am. Heart J.* **129**, 510.
156. Winslow, T. M., Ossipov, M., Redberg, R. F., Fazio, G. P., and Schiller, N. B. (1993). Exercise capacity and hemodynamics in systemic lupus erythematosus: A Doppler echocardiographic exercise study. *Am. Heart J.* **126**, 410.
157. Salerni, R., Rodnan, G. P., Leon, D. R., and Shaver, I. A. (1977). Pulmonary hypertension in the CREST syndrome variant of progressive systemic sclerosis (scleroderma). *Ann. Intern. Med.* **86**, 394.
158. Jones, M. B., Osterholm, R. K., Wilson, R. B., *et al*. (1978). Fatal pulmonary hypertension and resolving immune-complex glomerulonephritis in mixed connective tissue disease: A case report and review of the literature. *Am. J. Med.* **65**, 855.
159. Taryle, D. A., and Ellis, J. H. (1979). Systemic lupus erythematosus: An unusual cause of bilateral hilar lymphadenopathy. *South Med. J.* **72**, 896.
159a. Seyrek, N., Paydas, S., Godkel, Y., Tuncer, I., and Sagliker, Y. (1996). Systemic lupus erythematosus-nephrotic syndrome and mediastinal lymphadenopathy mimicking lymphoma. *Nephron* **72**, 489.
160. Harrison, G. N., Lipham, M., Elguindi, A. S., and Loebl, D. H. (1979). Acute sarcoidosis occurring during the course of systemic lupus erythematosus. *South Med. J.* **72**, 1387.
161. Hunter, T., Arnott, J. E., and McCarthy, D. S. (1980). Features of systemic lupus erythematosus and sarcoid occurring together. *Arthritis Rheum.* **23**, 364.
162. Ansari, A., Larson, P. H., and Bates, H. D. (1985). Cardiovascular manifestations of systemic lupus erythematosus: Current perspective. *Prog. Cardiovasc. Dis.* **27**, 421.
163. Badul, E., Garcia-Rubi, D., Robles, E., *et al*. (1985). Cardiovascular manifestations in systemic lupus erythematosus: Prospective study of 100 patients. *Angiology* **36**, 431.
164. Carette, S. (1988). Cardiopulmonary manifestations of systemic lupus erythematosus. *Rheum. Dis. North Am.* **14**, 137.
165. Doherty, E., and Siegel, R. J. (1985). Cardiovascular manifestations of systemic lupus erythematosus. *Am. Heart* **110**, 1257.
166. Sturfelt, G., Eskilsson, J., Nived, O., Truedsson, L., and Valind, S. (1992). Cardiovascular disease in systemic lupus erythematosus: A study of 75 patients from a defined population. *Medicine* **71**, 216.
167. Cervera, R., Font, J., Pare, C., *et al*. (1992). Cardiac disease in systemic lupus erythematosus: A prospective study of 70 patients. *Ann. Rheum. Dis.* **51**, 156.
168. Winslow, T. M., Ossipov, M. A., Fazio, G. P., *et al*. (1993). The left ventricle in systemic lupus erythematosus: Initial observations and a five-year follow-up in a university medical center population. *Am. Heart J.* **125**, 1117.
169. Cujec, B., Sibley, J., and Haga, M. (1991). Cardiac abnormalities in patients with systemic lupus erythematosus. *Can. J. Cardiol.* **7**, 343.
170. Ramonda, R., Doria, A., Villanova, C., *et al*. (1992). Evaluation of cardiac involvement in systemic lupus erythematosus. *Rev. Rhum. Mal. Osteoartic* **59**, 690.
171. Crozier, I. G., Li, E., Mime, M. J., and Nicholls, M. G. (1990). Cardiac involvement in systemic lupus erythematosus detected by echocardiography. *Am. J. Cardiol.* **65**, 1145.
172. Chang, R. W. (1982). Cardiac manifestations of SLE. *Clin. Rheum. Dis.* **8**, 197.
173. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85.
174. Shearn, M. A. (1959). The heart in systemic lupus erythematosus. *Am. Heart J.* **58**, 452.
175. Marks, A. D. (1972). The cardiovascular manifestations of systemic lupus erythematosus. *Am. J. Med. Sci.* **264**, 254.
176. Hejtmancik, M. R., Wright, J. C., Quint, R., and Jennings, F. L. (1964). The cardiovascular manifestations of systemic lupus erythematosus. *Am. Heart J.* **68**, 119.
177. Bourel, M., Gouffault, J., and Boudesseul, B. (1971). Les manifestations cardiovasculaires du lupus érythémateux disséminateux. *Coeur Med. Interne* **10**, 535.

178. Starkey, R. H., and Hahn, B. H. (1973). Rapid development of constrictive pericarditis in a patient with systemic lupus erythematosus. *Chest* **63**, 448.
178a. Frank, H., Globits, S. (1999). Magnetic resonance imaging evaluation of myocardial and pericardial disease. *J. Magn. Reson. Imag.* **10**, 617.
179. Leung, W. H., Wong, K. L., Lau, C. P., *et al*. (1990). Cardiac abnormalities in systemic lupus erythematosus: A prospective M-mode, cross-sectional and Doppler echocardiographic study. *J. Cardiol.* **27**, 367.
180. Collins, R. L., Turner, R. A., Nomeir, A. M., *et al*. (1978). Cardiopulmonary manifestations of systemic lupus erythematosus. *J. Rheumatol.* **5**, 299.
181. Elkayam, U., Weiss, S., and Laniado, S. (1977). Pericardial effusion and mitral valve involvement in systemic lupus erythematosus: Echocardiographic study. *Ann. Rheum. Dis.* **36**, 349.
182. Jacobson, E. J., and Reza, M. J. (1978). Constrictive pericarditis in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 972.
183. Askari, A. D. (1978). Pericardial tamponade with hemorrhagic fluid in systemic lupus erythematosus. *JAMA* **33**, 111.
184. Chia, B. L., Mah, E. P. K., and Feng, P. H. (1981). Cardiovascular abnormalities in systemic lupus erythematosus. *J. Clin. Ultrasound* **9**, 237.
185. Klinkoff, A. V., Thompson, C. R., Reid, G. D., and Tomlinson, C. W. (1985). M-mode and two-dimensional echocardiographic abnormalities in systemic lupus erythematosus. *JAMA* **253**, 3273.
186. Nagyhegyl, G., Nadas, I., Banyai, F., *et al*. (1989). Cardiac and cardiopulmonary changes in systemic lupus erythematosus. *Orv. Hetil* **29**, 215.
187. Meziane, M. A., Fishman, E. K., and Siegelman, S. S. (1984). CT diagnosis of hemopericardium in acute dissecting aneurysm of the thoracic aorta. *J. Comput. Assist. Tomogr.* **8**, 10.
188. Moncada, R., Baker, M., Salinas, M., *et al*. (1982). Diagnostic role of computed tomography in pericardial heart disease: Congenital defects, thickening, neoplasms, and effusions. *Am. Heart J.* **103**, 263.
189. Higgins, C. B., Lanzer, P., Herfken, S., *et al*. (1983). Cardiovascular system. In "Clinical Magnetic Resonance Imaging" (A. R. Margulis, C. B. Higgins, and L. Kaufman, eds.), p. 159. University of California San Francisco.
190. Ehrenfeld, M., Asman, A., Shpilberg, O., and Samra, Y. (1989). Cardiac tamponade as the presenting manifestation of systemic lupus erythematosus. *Am. J. Med.* **86**, 626.
191. Goldenberg, D. L., Leff, G., and Grayzel, A. L. (1975). Pericardial tamponade in systemic lupus erythematosus. *N.Y. State Med. J.* **75**, 910.
192. Nour-Eddine, M., Bennis, A., Soulami, S., and Chraibi, N. (1996). Tamponnade cardiaque révélatrice d'un lupus érythémateux systémique. *Ann. Cardiol. Angeiol.* **45**, 71.
193. Gulati, S., and Kumar, L. (1992). Cardiac tamponade as an initial manifestation of systemic lupus erythematosus in early childhood. *Ann. Rheum.* **51**, 279.
194. Porcel, J. M., Selva, A., Tornos, M. P., *et al*. (1989). Resolution of cardiac tamponade in systemic lupus erythematosus with indomethacin. *Chest* **96**, 1193.
195. Berbir, N., Allen, J., and Dubois, E. (1977). Le risque de la péricardiocentèse dans le lupus érythémateux disséminateux: Apropos d'un cas et revue de la littérature. *Rev. Rheum.* **44**, 359.
196. Coe, M. D., Hamer, D. H., Levy, C. S., *et al*. (1990). Gonococcal pericarditis with tamponade in a patient with systemic lupus erythematosus. *Arthritis Rheum.* **33**, 1438.
197. Wolkove, N., and Frank, H. (1974). Lupus pericarditis. *Can. Med. Assoc. J.* **111**, 1331.
198. Walley, T. (1976). Cross-sectional echocardiography in the diagnosis of Libman-Sacks endocarditis *Int. J. Cardiol.* **20**, 406.
199. Herzog, C. A., Carson, P., Michaud, L., and Asinger, R. W. (1988). Two dimensional echocardiographic imaging of left ventricular mural vegetations. *Am. Heart J.* **115**, 684.
199a. Barjatiya, M. K., Shah, N. K., Kothari, S. S., *et al*. (1992). Spontaneous left venticular thrombus in a patient of systemic lupus erythematosus. *J. Assoc. Phys. India* **40**, 195.
200. Barzizza, F., Venco, A., Grandi, A. M., and Finardi, G. (1987). Mitral valve prolapse in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **5**, 59.
201. Galve, E., Candell-Riera, J., and Pigrau, C. (1988). Prevalence, morphologic types, and evolution of cardiac valvular disease in systemic lupus erythematosus. *N. Engl. J. Med.* **319**, 817.
202. Doherty, N. E., III, Feldman, G., Maurer, G., and Siegel, R. J. (1988). Echocardiographic findings in systemic lupus erythematosus. *Am. J. Cardiol.* **61**, 1144.
203. Kahan, A., Amor, B., de Vernejoul, F., and Saporta, L. (1985). The diagnostic importance of two-dimensional echocardiography. *Br. J. Rheumatol.* **24**, 187.
204. Enomoto, K., Kaji, Y., and Mayumi, T. (1991). Left ventricular function in patients with stable systemic lupus erythematosus. *Jpn. Heart J.* **32**, 445.
205. Fujimoto, S., Kagoshima, T., Nakajima, T., and Dohi, K. (1994). Doppler echocardiographic assessment of left ventricular diastolic function in patients with systemic lupus erythematosus. *Cardiology* **85**, 267.
206. Leung, W. H., Wong, K. L., Lau, C. P., *et al*. (1990). Doppler echocardiographic evolution of left ventricular diastolic function in patients with systemic lupus erythematosus. *Am. Heart J.* **120**, 82.
207. Roldan, C. A., Shively, B. K., and Crawford, M. H. (1996). An echocardiographic study of valvular heart disease associated with systemic lupus erythematosus. *N. Engl. J. Med.* **335**, 1424.
208. Morelli, S., Perrone, C., and Vasesini, G. (1995). Tricuspid valve steno-insufficiency in systemic lupus erythematosus. *Lupus* **4**, 318.
209. Enomoto, K., Kaji, Y., Mayumi, T., *et al*. (1991). Frequency of valvular regurgitation by color Doppler echocardiography in systemic lupus erythematosus. *Am. J. Cardiol.* **67**, 209.

210. Hospenpud, J. D., Montaro, A., Hart, M. V., *et al*. (1984). Myocardial perfusion abnormalities in asymptomatic patients with systemic lupus erythematosus. *Am. J. Med.* **77**, 286.
210a. Wackers, F. J. Th., Mattera, J., Errison, D., *et al*. (1992). Quantitative dual isotope (T1201/Tc99m sestamibi) rest/exercise imaging: Is defect reversibility the same as with 99mTc sestamibi alone? *J. Nucl. Med.* **33**, 855.
211. Duska, F., Bradna, P., and Popslsil, M. (1987). Pyrophosphate scintigraphy and other noninvasive methods in the detection of cardiac involvement in some systemic connective tissue diseases. *J. Nucl. Med.* **26**, 28.
211a. Heo, J., Cave, V., Wasserleben, V., *et al*. (1994). Planar and tomographic imaging with technetium 99m labeled tetrofosmin: Correlation with thallium 201 and coronary artery disease. *J. Nucl. Med.* **4**, 317.
212. Utz, I. A., Herfkens, R. J., Heinsimer, J. A., *et al*. (1988). Valvular regurgitation: Dynamic MR imaging. *Radiology* **168**, 91.
213. Pflugfelder, P. W., Landzberg, J. S., Cassidy, M. M., *et al*. (1989). Comparison of cine-MR imaging with Doppler echocardiography for the evaluation of aortic regurgitation. *Am. J. Roentgen.* **152**, 729.
214. Been, M., Thomson, B. J., Smith, M. A., *et al*. (1988). Myocardial involvement in systemic lupus erythematosus detected by magnetic resonance imaging. *Eur. Heart J.* **9**, 1250.
215. Caputo, G. R., and Higgins, C. B. (1990). Fast computed tomography, magnetic resonance imaging, and positron emission tomography. *Invest. Radiol.* **25**, 838.
216. Ahmad, M., Johnson, R. F., Fawcett, H. D., and Schreiber, M. H. (1988). Magnetic resonance imaging in patients with unstable angina: Comparison with acute myocardial infarctions and normals. *MRI* **6**, 527.
217. de Roos, A., Doornbos, I., vander Wall, E. E., and van Voorthvisen, A. E. (1988). MR imaging of acute myocardial infarction: Value of Gd-DTPA. *Am. J. Roentgen.* **150**, 531.
217a. Johnston, D. L., Mulvagh, S. L., and Cashion, R. W. (1989). Nuclear magnetic resonance imaging of acute myocardial infarction with 24 hours of chest pain onset. *Am. J. Cardiol.* **64**, 172.
218. Shapeero, L. G., Vanel, D., Verstraete, K. L., and Bloem, J. L. (2002). Fast magnetic resonance imaging with contrast for soft-tissue sarcoma viability. *Clin. Orthop. Rel. Res.* **397**, 212.
218a. Lim, T.-H., and Choi, S. I. (1999). MRI of myocardial infarction: *J. Magn. Reson. Imag.* **10**, 656.
219. Krauss, X. H., Vander Wall, E. E., and Doornbos, J. (1989). Value of magnetic resonance imaging in patients with a myocardial infarction: Comparison with thallium-201 scintigraphy. *Cardiovasc. Intervent. Radiol.* **12**, 119.
220. Laufer, J., Frand, M., and Milo, S. (1982). Valve replacement for severe tricuspid regurgitation caused by Libman-Sacks endocarditis. *Br. Heart J.* **48**, 294.
221. Vaughton, K. C., Walker, D. R., and Sturridge, M. F. (1979). Mitral valve replacement for mitral stenosis caused by Libman-Sacks endocarditis. *Br. Heart J.* **41**, 730.
222. EI-Ghobarey, A., Grennan, D. M., Hadidi, T., and EI-Bodawy, S. (1976). Aortic incompetence in systemic lupus erythematosus. *Br. Med. J.* **12**, 915.
223. Thandroyen, F. T., Matisonn, R. E., and Weir, E. K. (1978). Severe aortic incompetence caused by systemic lupus erythematosus. *S. Afr. Med. J.* **54**, 166.
224. Evans, D. T. P., and Sloman, J. G. (1981). Mitral stenosis and mitral incompetence due to Libman-Sacks endocarditis with mitral valve replacement. *Aust. N.Z. J. Med.* **11**, 526.
225. Murray, F. T., Fuleihan, D. S., Cornwall, C. S., and Pinals, R. S. (1975). Acute mitral regurgitation from ruptured chordae tendineae in systemic lupus erythematosus. *J. Rheumatol.* **2**, 454.
226. Milne, J. R., Doyle, D. V., Banim, S. E., and Huskisson, E. C. (1981). Systemic lupus erythematosus as a cause of severe mixed mitral valve disease. *J. Rheumatol.* **8**, 516.
227. Rawsthorne, L., Ptacin, M. J., Choi, H., *et al*. (1981). Lupus valvulitis necessitating double valve replacement. *Arthritis Rheum.* **24**, 561.
228. Bignold, L. P., Bailey, I. K., and Kronenberg, H. (1980). Myocardial infarction, papillary muscle dysfunction and mitral valvular incompetence in systemic lupus erythematosus. *Aust. N.Z. J. Med.* **10**, 236.
229. Borenstein, D. G., Fye, W. B., Arnett, F. C., and Stevens, M. B. (1978). The myocarditis of systemic lupus erythematosus: Association with myositis. *Ann. Intern. Med.* **89**, 619.
230. del Rio, A., Vasquez, J. J., Sobrino, L. A., *et al*. (1978). Myocardial involvement in systemic lupus erythematosus: A noninvasive study of left ventricular function. *Chest* **74**, 414.
231. Maniscalco, B., Felner, J., McCans, *et al*. (1975). Echocardiographic abnormalities in systemic lupus erythematosus. *Circulation* **525** (Suppl. II), 836.
232. Bulkley, B. H., and Roberts, W. C. (1975). The heart in systemic lupus erythematosus and the changes induced in it by corticosteroid therapy. *Am. J. Med.* **58**, 243.
233. Senningen, R. P., Borer, J. S., Redwood, D. R., *et al*. (1974). Libman-Sacks endocardoma: Diagnosis during life with radiographic, fluoroscopic and angiocardiographic findings. *Radiology* **113**, 597.
234. Hoffman, R., Lethen, H., Zunker, U., *et al*. (1994). Rapid appearance of severe mitral regurgitation under high-dosage corticosteroid therapy in a patient with systemic lupus erythematosus. *Eur. Heart J.* **15**, 138.
235. Gordon, R. J., Weilbaecher, D., Davy, S. M., *et al*. (1996). Valvulitis involving a bioprosthetic valve in a patient with systemic lupus erythematosus. *J. Am. Soc. Echocardiogr*. **9**, 104.
236. Khamashta, M. A., Cervera, A., Asherson, R. A., *et al*. (1990). Association of antibodies against phospholipids with heart valve disease in systemic lupus erythematosus. *Lancet* **1**, 1541.
237. Leung, W. H., Wong, K.-L., Lau, C.-P., Wong, C.-K., and Liu, H.-W. (1990). Association between antiphospholipid antibodies and cardiac abnormalities in patients with systemic lupus erythematosus. *Am. J. Med.* **89**, 414.

237a. Li, E. K., Crozier, I. A., Milne, M. J., Nicholls, M. G., and Cohen, M. G. (1990). Lack of association between anticardiolipin antibodies and heart valve disease in Chinese patients with systemic lupus erythematosus. *Lancet* **336**, 504.
238. Ford, P. M., Ford, S. E., and Lillicrap, D. P. (1988). Association of lupus anticoagulant with severe heart disease in systemic lupus erythematosus. *J. Rheumatol.* **15**, 597.
239. Metz, D., Jolly, J., Graciet-Richard, J., *et al*. (1994). Anomalies cardiaques dans le lupus érythémateux disséminateux diagnostiques par echographie Doppler prévalence et association au syndrome des antiphospholipides. *Presse Med.* **23**, 1797.
240. Appelbe, A. F., Olson, D., Mixon, R., Craver, J. M., and Martin, R. P. (1991). Libman-Sacks endocarditis mimicking intracardiac tumor. *Am. J. Cardiol.* **68**, 817.
241. Atallah, A., Hamousin Metregiste, R., Samuel, J., Petit, G., and Makoy, F. (1995). Thrombus intraventriculaire gauche compliquant une maladie lupique. *Arch. Mal. Coeur. Vaiss.* **88**, 1043.
242. Nihoyannopoulos, P., Gomez, P. M., Joshi, J., *et al*. (1990). Cardiac abnormalities in systemic lupus erythematosus: Association with raised anticardiolipin antibodies. *Circulation* **82**, 369.
243. Gleason, C. B., Stoddard, M. F., Wagner, S. G., *et al*. (1993). A comparison of cardiac valvular involvement in the primary antiphospholipid syndrome versus anticardiolipin-negative systemic lupus erythematosus. *Am. Heart J.* **125**, 1123.
244. Roldan, C. A., Shively, B. K., Lau, C., *et al*. (1992). Systemic lupus erythematosus valve disease by transesophageal echocardiography and the role of antiphospholipid antibodies. *J. Am. Coll. Cardiol.* **20**, 1127.
245. Hojnik, M., George, J., Ziporen, L., and Shoenfeld, Y. (1996). Heart valve involvement (Libman-Sacks endocarditis) in the antiphospholipid syndrome. *Circulation* **93**, 1579.
246. Logar, D., Kveder, T., Rozman, B., and Dobovisek, J. (1990). Possible association between anti-Ro antibodies and myocarditis or cardiac conduction defects in adults with systemic lupus erythematosus. *Ann. Rheum. Dis.* **49**, 627.
247. Isaeva, L. A., Deliagin, V. M., and Bazhenoa, L. K. (1988). Manifestations of carditis in diffuse connective tissue disease in children. *Cor. Vasa.* **30**, 211.
248. Sasson, Z., Rasooly, Y., Chow, C. W., Marshall, S., and Urowitz, M. B. (1992). Impairment of left ventricular diastolic function in systemic lupus erythematosus. *Am. J. Cardiol.* **69**, 1629.
248a. Astorri, E., Fiorina, P., Ridolo, E., Contini, G. A., Albertini, D., and Dall'Aglo, P. (1997). Studio ecocardiografico Doppler della funzione ventricolare sinistra in pazienti con lupus eritematoso sistemico. *Cardiologia* **42**, 1179.
249. Gur, H., Keren, G., Auerbuch, M., and Levo, Y. (1988). Severe congestive lupus cardiomyopathy complicated by an intracavitary thrombus: A clinical and echocardiographic follow-up. *J. Rheumatol.* **15**, 1278.
250. Bahl, V. K., Aradhye, S., Vasan, R. S., *et al*. (1992). Myocardial systolic function in systemic lupus erythematosus: A study based on radionuclide ventriculography. *Clin. Cardiol.* **15**, 433.
251. Urowitz, M. B., Bookman, A. A., Koehler, B. E., *et al*. (1976). The bimodal mortality pattern of systemic lupus erythematosus. *Am. J. Med.* **60**, 221.
252. Decker, J. L. (1979). Mortality in lupus nephritis. *Ann. Rheum.* **22**, 764.
253. Lipton, M. J., Higgins, C. B., Farmer, D., and Boyd, D. P. (1984). Cardiac imaging with a high-speed cine-CT scanner: Preliminary results. *Radiology* **152**, 579.
254. Meller, I., Conde, C. A., and Deppisch, L. M. (1975). Myocardial infarction due to coronary atherosclerosis in three young adults with systemic lupus erythematosus. *Am. J. Cardiol.* **35**, 309.
255. Tsakraklides, V. G., Blieden, L. C., and Edwards, J. E. (1974). Coronary arteriosclerosis and myocardial infarction associated with systemic lupus erythematosus. *Am. Heart J.* **87**, 837.
256. Haider, Y. S., and Robert, W. C. (1981). Coronary arterial disease in systemic lupus erythematosus: Quantification of degrees of narrowing in 20 necropsy patients. *Am. J. Med.* **70**, 775.
257. Benisch, B. M., and Pervez, N. (1974). Coronary artery vasculitis and myocardial infarction with systemic lupus erythematosus. *N.Y. State J. Med.* **74**, 873.
258. Ishikawa, S., Segar, W. E., Gilbert, E. F., *et al*. (1978). Myocardial infarct in a child with systemic lupus erythematosus. *Am. J. Dis. Child* **132**, 696.
259. Rosenthal, T., Neufeld, H., Kishon, Y., *et al*. (1980). Myocardial infarction in a young woman with systemic lupus erythematosus. *Angiology* **31**, 573.
260. Homcy, C. J., Liberthson, R. R., Fallon, J. T., *et al*. (1982). Ischemic heart disease in systemic lupus erythematosus in the young patient: Report of six cases. *Am. J. Cardiol.* **49**, 478.
261. Wilson, V. E., Eck, S. L., and Bates, E. R. (1992). Evaluation and treatment of acute myocardial infarction complicating systemic lupus erythematosus. *Chest* **101**, 420.
262. Heibel, R. H., O'Toole, J. D., Curtiss, E. L., *et al*. (1976). Coronary arteritis in systemic lupus erythematosus. *Chest* **69**, 700.
263. Bonfiglio, T. A., Botti, R. E., and Hagstrom, J. W. C. (1972). Coronary arteritis, occlusion and myocardial infarction due to lupus erythematosus. *Am. Heart J.* **83**, 153.
263a. Agatston, A. S., Janowitz, W. R., Kaplan, G., Gasso, J., Hildner, F., and Viamonte M., Jr. (1994). Ultrafast computed tomography-detected coronary calcium reflects the angiographic extent of coronary arterial atherosclerosis. *Am. J. Cardiol.* **74**, 1424.
263b. Von Pelt, J. M. (1998). Coronary electron beam computed tomography (EBCT) in 13 SLE patients with 2 or more cardiovascular risk factors. *Arthritis Rheum.* **41**, S139. [Abstract]
263c. Manzi, S., Kuller, L. H., Edmundowica, D., and Sutton-Tyrrell, K. (2000). Vascular imaging: Changing the face of cardiovascular research. *Lupus* **9**, 176.

264. Takatsu, Y., Hattori, R., Sakaguchi, K., *et al*. (1985). Acute myocardial infarction associated with systemic lupus erythematosus documented by coronary arteriograms. *Chest* **88**, 147.
264a. Sakuma, H., Kawada, N., Takeda, K., and Higgins, C. (1999). MR measurements of coronary blood flow. *J. Magn. Reson. Imag.* **10**, 728.
265. Sumino, H., Kanda, T., Sasaki, T., Kanazawa, N., and Takeuchi, H. (1995). Myocardial infarction secondary to coronary aneurysm in systemic lupus erythematosus. *Angiology* **46**, 527.
266. Nobrega, T. P., Klodas, E., Breen, J. E., *et al*. (1996). Giant coronary artery aneurysms and myocardial infarction in a patient with systemic lupus erythematosus. *Cath. Cardiovasc. Diagn.* **39**, 75.
266a. Howe, H. S., Wong, J. S. L., Ding, D. P., *et al*. (1997). Mycotic aneurysm of a coronary artery in SLE: A rare complication of salmonella infection. *Lupus* **6**, 404.
267. Hanson, J. A., LLoyd, M. E., and Hughes, G. R. V. (1994). Aortic root thrombus causing stroke in a patient with systemic lupus erythematosus. *Scand. J. Rheumatol.* **23**, 156.
268. Ferrante, F. M., Myerson, G. E., and Johnson, I. A. (1982). Subclavian artery thrombosis mimicking the aortic arch syndrome in systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1501.
269. Gharavi, A. E. (1985). Aortic arch syndrome associated with anticardiolipin antibodies and the lupus anticoagulant: Comment on Ferrante paper. *Arthritis Rheum.* **28**, 594.
270. Soubrier, M., Carrie, D., Urosevic, Z., *et al*. (1995). Aortic occlusion in a patient with antiphospholipid antibody syndrome in systemic lupus erythematosus. *Int. Angiol.* **14**, 233.
271. Menon, J., Karande, S. C., Khambekar, K. P., *et al*. (1996). Systemic lupus erythematosus with aortoarteritis. *Ind. Pediatr.* **33**, 238.
272. Igarashi, T., Nagaoka, I., Matsunaga, K., *et al*. (1989). Aortitis syndrome (Takayasu's arteritis) associated with systemic lupus erythematosus. *J. Rheumatol.* **16**, 1579.
273. Kwong, T., Leondias, J. C., and Ilowite, N. T. (1994). Asymptomatic superior vena cava thrombosis and pulmonary embolism in an adolescent with SLE and antiphospholipid antibodies. *Clin. Exp. Rheumatol.* **12**, 215.
274. Sclair, M., Nassar, H., Bar-Ziv, Y., and Putterman, C. (1995). Case report: Dissecting aortic aneurysm in systemic lupus erythematosus. *Lupus* **4**, 71.
274a. Choi, K. H., Rim, S. J., Lee, S. K., Jang, B. C., and Cho, S. H. (1999). Dissecting aortic aneurysm with aortic-valve insufficiency in systemic lupus erythematosus. *Nephrol. Dialys. Transplant.* **14**, 969.
274b. Kamayama, K., Kuramachi, S., Ueda, T., *et al*. (1999). Takayasu's aortitis with dissection in systemic lupus erythematosus. *Scand J. Rheumatol.* **28**, 187.
275. Chakravarty, K., and Scott, D. G. (1992). Mycotic aneurysm of the aortic arch masquerading as systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 1079.
276. Glueck, H. I., Kant, K. S., Weiss, M. A., *et al*. (1985). Thrombosis in systemic lupus erythematosus: Relation to the presence of circulating anticoagulants. *Arch. Intern. Med.* **145**, 1389.
277. Gladstein, G. S., Rynes, R. I., Parham, N., and Bartholomew, L. E. (1979). Gangrene of a foot secondary to systemic lupus erythematosus with large vessel vasculitis. *J. Rheumatol.* **6**, 549.
278. Kaufman, J. L., Bancilla, E., and Slade, J. (1986). Lupus vasculitis with tibial artery thrombosis and gangrene. *Arthritis Rheum.* **29**, 1291.
279. Jindal, B. K., Martin, M. F. R., and Gayner, A. (1983). Gangrene developing after minor surgery in a patient with undiagnosed systemic lupus erythematosus and lupus anticoagulant. *Ann. Rheum. Dis.* **42**, 347.
280. Bowles, C. A. (1990). Vasculopathy associated with the antiphospholipid antibody syndrome. *Rheum. Dis. Clin. North Am.* **16**, 47.
281. Ter Borges, Vander Meer, J., and De Wolf, J. T. (1988). Arterial thrombotic manifestations in young women with lupus anticoagulant. *Clin. Rheumatol.* **7**, 74.
282. Anderson, C. A., Tsuruda, J. S., Shapeero, L. G., and Lee, R. (1990). Artifacts in maximum intensity projection display of magnetic resonance angiograms. *Am. J. Roentgen.* **54**, 623.
283. Sheppard, S. (1995). Basic concepts in magnetic resonance angiography. *Radiol. Clin North Am.* **33**, 91.
284. Douek, P. C., Revel, D., and Chazel, S. L. (1995). Fast MR angiography of the aortoiliac arteries and arteries of the lower extremity: Value of bolus-enhanced whole-volume subtraction technique. *Am. J. Roentgen.* **165**, 431.
285. Snidow, J. J., Harris, V. J., Trerotoloa, S. O., *et al*. (1995). Interpretations and treatment decisions based on MR angiography versus conventional arteriography in symptomatic lower extremity ischemia. *Cardiovasc. Interven. Radiol.* **6**, 595.
286. Peck, B., Hoffman, G. S., and Franck, W. A. (1978). Thrombophlebitis in systemic lupus erythematosus. *JAMA* **240**, 1728.
287. Scully, R. E. (1990). Case records of the Massachusetts General Hospital: Case 2-1990. *N. Engl. J. Med.* **332**, 754.
288. St. Clair, W., Jones, B., Rogers, J. S., *et al*. (1981). Deep venous thrombosis and a circulating anticoagulant in systemic lupus erythematosus. *Am. J. Dis. Child* **135**, 230.
289. O'Leary, M. J., Limpisvasti, P., Nordyke, R. A., and Kistner, R. L. (1980). Massive thrombosis in SLE. *Hawaii Med. J.* **39**, 81.
290. Williams, H., Laurent, R., and Gibson, T. (1980). The lupus coagulation inhibitor and venous thrombosis: A report of four cases. *Clin. Lab. Haematol.* **2**, 139.
291. Seabold, J. E., Conrad, G. R., Kimball, D. A., *et al*. (1988). Pitfalls in the diagnosis of venous thrombophlebitis by Indium-111 platelet scintigraphy. *J. Nuclear Med.* **29**, 1169.
292. Naidich, I. B., Feinberg, A. W., Karp-Harman, H., *et al*. (1988). Contrast venography: Reassessment of its role. *Radiology* **168**, 97.

293. Appelman, P. T., Dejong, T. E., and Lampmann, L. E. (1987). Deep venous thrombosis of the leg: US findings. *Radiology* **163**, 743.
294. Cronan, J. J., Dorfman, G. S., and Grusmark, I. (1988). Lower extremity deep venous thrombosis: Further experience with refinements of US assessment. *Radiology* **168**, 101.
295. Rose, S. C., Zweibel, W. J., Nelson, B. D., *et al*. (1990). Symptomatic lower extremity venous thrombosis: Accuracy, limitations, and role of color duplex flow imaging on diagnosis. *Radiology* **175**, 639.
296. Osler, W. (1895). On the visceral manifestations of erythema exuditavum multiforme. *Am. J. Med. Sci.* **110**, 629.
297. Haserick, J. R. (1955). Modern concepts of systemic lupus erythematosus: A review of 126 cases. *J. Chronic Dis.* **1**, 317.
298. Hoffman, B. I., and Katz, W. A. (1980). The gastrointestinal manifestations of systemic lupus erythematosus. *Semin. Arthritis Rheum.* **9**, 237.
299. Cook, C. D., Wedgwood, R. J., Craig, J. M., *et al*. (1960). Systemic lupus erythematosis: Description of 37 cases in children and a discussion of endocrine therapy in the 32 cases. *Pediatrics* **26**, 570.
300. Brown, C. H., Shirey, E. K., and Haserick, I. R. (1956). Gastrointestinal manifestations of systemic lupus erythematosus. *Gastroenterology* **31**, 649.
301. Bruce, J., and Sircus, W. (1959). Disseminated lupus erythematosus of the alimentary tract. *Lancet* **1**, 795.
302. Couris, G. D., Block, M. A., and Rupe, C. E. (1964). Gastrointestinal complications of collagen diseases: Surgical implications. *Arch. Surg.* **89**, 695.
303. Shapeero, L. G., Myers, A., Oberkircher, P. E., and Miller, W. T. (1974). Acute reversible lupus vasculitis of the gastrointestinal tract. *Radiology* **112**, 569.
304. Ginzburg, L., and Klein, E. (1974). Late intestinal stenosis following strangulated hernia. *Ann. Surg.* **88**, 204.
305. Wolf, B. S., and Marshak, R. H. (1956). Segmental infarction of the small bowel. *Radiology* **66**, 701.
306. Hamelin, L. (1966). Insuffisance vasculaire mésentérique. *J. Can. Assoc. Radiol.* **17**, 107.
307. Kistin, M. G., Kaplan, M. M., and Harrington, J. T. (1978). Diffuse ischemic colitis associated with systemic lupus erythematosus: Response to subtotal colectomy. *Gastroenterology* **75**, 1147.
308. Tsuchiya, M., Okazaki, I., Asakura, H., and Ohkubo, T. (1975). Radiographic and endoscopic features of colonic ulcers in systemic lupus erythematosus. *Am. J. Gastroenterol.* **64**, 277.
309. Schwartz, S., Boley, S. J., Robinson, K., *et al*. (1964). Roentgenologic features of vascular disorders of the intestines. *Radiol. Clin. North. Am.* **2**, 71.
310. Phillips, J. C., and Howland, W. J. (1968). Mesenteric arteritis in systemic lupus erythematosus. *JAMA* **206**, 1569.
311. Asherson, R. A., Morgan, S. H., Harris, E. N., *et al*. (1986). Arterial occlusion causing large bowel infarction: A reflection of clotting diathesis in SLE. *Clin. Rheum.* **5**, 102.
312. Keating, J. P., King, B. R., Kenwright, D. N., and Brasch, H. D. (1998). Vasculitis-induced colonic stricture: Report of two cases. *Dis. Colon. Rectum.* **4**, 1316.
312a. Finkbiner, R. B., and Decker, J. P. (1963). Ulceration and perforation of the intestine due to necrotizing arteriolitis. *N. Engl. J. Med.* **268**, 14.
313. Hermann, G. R. (1967). Intussusception secondary to mesenteric arteritis: Complication of systemic lupus erythematosus in a 5-year-old child. *JAMA* **200**, 74.
314. Decrop, E., Ponette, E., Baert, A. L., *et al*. (1990). Preoperative radiological diagnosis of acute necrotizing enteritis in systemic lupus erythematosus. *J. Belge Radiol.* **73**, 31.
315. Litwin, M., Kurulcio, L., and Kurylcio, S. (1968). Perforation of the cecum in the course of lupus erythematosus disseminatus. *Pol. Przegl. Chir.* **40**, 1388.
316. Pollak, V. E., Grove, W. J., Kark, R. M., *et al*. (1958). Systemic lupus erythematosus simulating acute surgical condition of the abdomen. *N. Engl. J. Med.* **259**, 258.
317. Vachtenheim, J., and Kabatkova, Z. (1966). Mesenteric arteritis with subsequent infarction of the intestine in systemic lupus erythematosus. *Cesk. Gastroenterol. Vyz.* **20**, 278.
318. Zizic, T. M., Shulman, L. E., and Stevens, M. B. (1975). Colonic perforations in systemic lupus erythematosus. *Medicine* **54**, 411.
319. Kleinman, P., Meyers, M. A., Abbott, G., and Kazam, E. (1976). Necrotizing enterocolitis with pneumatosis intestinalis in systemic lupus erythematosus and polyarteritis. *Radiology* **121**, 595.
320. Stoddard, C. J., Kay, P. H., Simms, J. M., *et al*. (1978). Acute abdominal complications of systemic lupus erythematosus. *Br. J. Surg.* **65**, 625.
320a. Alcocer-Gouyonnet, F., Chan-Nunez, C., Hernadez, J., Guzman, J., and Gamboa-Dominguez, A. (2000). Acute abdomen and lupus enteritis: Thrombocytopenia and pneumatosis intestinalis as indicators for surgery. *Am. Surg.* **66**, 193.
321. Zizic, T. M., Classen, J. N., and Stevens, M. B. (1982). Acute abdominal complications of systemic lupus erythematosus and polyarteritis nodosa. *Am. J. Med.* **73**, 525.
321a. Testini, M., Margari, A., Piccinni, G., D'Abbicco, D., and Amoruso, M. (2000). A catastrophic complication of systemic lupus erythematosus: Massive mesenteric infarction. *Hepato-Gastroenterol.* **47**, 761.
322. Heiberg, E., Wolverson, M. K., Sundaram, M., and Shields, J. B. (1988). Body computed tomography findings in systemic lupus erythematosus. *J. Comput. Assist. Tomogr.* **12**, 68.
323. Boulter, M., Brink, H., Mathia, S. L., *et al*. (1987). Unusual cranial and abdominal computed tomographic CT scan appearances in a case of systemic lupus erythematosus (SLE). *Ann. Rheum. Dis.* **46**, 162.
324. Kirshy, D. M., Gordon, D. H., and Atweh, N. A. (1991). Abdominal computed tomography in lupus mesenteric arteritis. *Comp. Med. Imaging Graph.* **15**, 369.
324a. Wakiyama, S., Yoshimura, K., Shimada, M., and Sugimachi, K. (1996). Lupus peritonitis mimicking

acute surgical abdomen in a patient with systemic lupus erythematosus: Report of a case. *Jpn. J. Surg.* **26**, 715.
325. Freiman, D., Chin, H., and Bilaniuk, L. (1975). Pneumatosis intestinalis in systemic lupus erythematosus. *Radiology* **116**, 563.
326. Derksen, O. S. (1978). Pneumatosis intestinalis in a female patient with systemic lupus erythematosus. *Radiol. Clin. N. Am.* **47**, 334.
327. Binstadt, D. H., and L'Heureux, P. R. (1977). Pneumatosis cystoides intestinalis in childhood systemic lupus erythematosus. *Minn. Med.* **60**, 408.
328. Vas, W. G., Seelig, R., Mahanta, B., *et al*. (1988). Neutropenic colitis evaluated with computed tomography. *J. Comput. Assist. Tomogr.* **12**, 211.
329. O'Neill, P. B. (1961). Gastrointestinal abnormalities in collagen diseases. *Am. J. Dig. Dis.* **6**, 1069.
330. Stevens, M. B., Hookman, P., Siegel, C. I., *et al*. (1964). Aperistalsis of the esophagus in patients with connective-tissue disorders and Raynaud's phenomenon. *N. Engl. J. Med.* **270**, 1218.
331. Gutierrez, F., Valenzuela, J. E., Ehremann, G. R., *et al*. (1982). Esophageal dysfunction in patients with mixed connective-tissue diseases and systemic lupus erythematosus. *Dig. Dis. Sci.* **27**, 592.
332. Tatelman, M., and Keech, M. K. (1966). Esophageal motility in systemic lupus erythematosus rheumatoid arthritis, and scleroderma. *Radiology* **86**, 1041.
333. Ramirez-Mata, M., Reyes, P. A., Alarcon-Segovia, D., and Garza, R. (1974). Esophageal motility in systemic lupus erythematosus. *Dig. Dis.* **19**, 132.
334. Turner, K., Rittenberg, G., Lipshutz, W., *et al*. (1973). Esophageal dysfunction in collagen disease. *Am. J. Med. Sci.* **265**, 191.
335. Morton, R. E., Miller, A. L., and Kaplan, R. (1976). Systemic lupus erythematosus: Unusual presentation with gastric polyps and vasculitis. *South Med. J.* **69**, 507.
336. Bazinet, P., and Mann, G. A. (1971). Malabsorption in systemic lupus erythematosus. *Am. J. Dig. Dis.* **16**, 460.
337. Siurala, M., Lulkunen, H., Toivonen, S., *et al*. (1965). Digestive tract in collagen diseases. *Acta. Med. Scand.* **178**, 13.
337a. Mader, R., Adawi, M., and Schonfeld, S. (1997). Malabsorption in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **15**, 659.
338. Trentham, D. E., and Masi, A. T. (1976). Systemic lupus erythematosus with a protein-losing enteropathy. *JAMA* **236**, 287.
339. Tsukhara, M. J., Matsuo, K., and Kojima, H. (1980). Protein-losing enteropathy in a boy with lupus erythematosus. *J. Pediatr.* **97**, 778.
340. Pachas, W. N., Linscheer, W. G., and Pinals, R. S. (1971). Protein-losing enteropathy in systemic lupus erythematosus. *Am. J. Gastroenterol.* **55**, 162.
340a. Hizawa, K., Ilda, M., Aoyagi, K., *et al*. (1998). Double-contrast radiographic assessment of lupus-associated enteropathy. *Clin. Radiol.* **53**, 825.
341. Weiser, M. M., Andres, G. A., Brentjens, J. R., *et al*. (1981). Systemic lupus erythematosus and intestinal venulitis. *Gastroenterology* **81**, 570.
342. Oddis, C., and McGlynn, T. I. (1984). Abdominal computed tomography scan in acute lupus abdominal serositis. *J. Comput. Assist. Tomogr.* **8**, 337.
343. Neuffer, F. R., Jones, T. B., and Dubulison, R. L. (1983). Systemic lupus erythematosus: Another etiology of the target sign. *J. Ultrasound Med.* **2**, 135.
344. Asherson, R. A., Thompson, R. P. H., MacLachian, N., *et al*. (1989). Budd-Chiari syndrome, visceral arterial occlusions, current fetal loss and the "lupus anticoagulant" in systemic lupus erythematosus. *J. Rheumatol.* **16**, 219.
345. Auerbach, M., and Levo, Y. (1986). Budd-Chiari syndrome as the major thrombotic complication of systemic lupus erythematosus with the lupus anticoagulant. *Ann. Rheum. Dis.* **45**, 435.
346. Yun, Y. Y., Yoh, K. A., Yang, H. I., *et al*. (1996). A case of Budd-Chiari syndrome with high antiphospholipid antibody in a patient with systemic lupus erythematosus. *Korean. J. Int. Med.* **11**, 82.
347. Ducobu, J., Schreiber, S., Schockert, J., *et al*. (1987). Budd-Chiari syndrome in a patient with systemic lupus erythematosus. *Acta. Clin. Belg.* **42**, 450.
348. Scully, R. E. (1990). Case records of the Massachusetts General Hospital: Case 2-1990. *N. Engl. J. Med.* **332**, 754.
349. Miller, V. E., and Berland, L. L. (1985). Pulsed Doppler duplex sonography and CT of portal vein thrombosis. *Am. J. Roentgen.* **145**, 73.
350. Gansbeke, D. V., Auni, E. F., Delacour, C., *et al*. (1985). Sonographic features of portal vein thrombosis. *Am. J. Roentgen.* **144**, 749.
351. Mathieu, D., Vasile, N., and Grenier, P. (1985). Portal thrombosis: Dynamic CT features and course. *Radiology* **154**, 737.
352. Zirinsky, K., Markisz, J. A., Rubenstein, W. A., *et al*. (1988). MR imaging of portal venous thrombosis: Correlation with CT and sonography. *Am. J. Roentgen.* **150**, 283.
353. Young, N., and Wong, K. P. (1991). Antibody to cardiolipin causing hepatic infarction in a postpartum patient with systemic lupus erythematosus. *Australas. Radiol.* **35**, 83.
354. Longstreth, P. L., Korobkin, M., and Palubinskas, A. J. (1974). Renal microaneurysms in a patient of systemic lupus erythematosus. *Radiology* **113**, 65.
355. Trambert, J., Reinitz, E., and Buchbinder, S. (1989). Ruptured hepatic artery aneurysms in a patient with systemic lupus erythematosus: Case report. *Cardiovasc. Intervent. Radiol.* **12**, 32.
356. Sutton, E., Malatjalian, D., and Hayne, O. A., and Hanly, J. F. (1989). Liver lymphoma in systemic lupus erythematosus. *J. Rheumatol.* **16**, 1584.
357. Weill, B. J., Menkes, C. J., Cormier, C., *et al*. (1988). Hepatocellular carcinoma after Danazol therapy. *J. Rheumatol.* **15**, 1447.

358. Short, T. S. (1907). Fatal case of acute lupus erythematosus. *Br. J. Dermatol.* **19**, 271.
359. Ginzler, A. M., and Fox, T. T. (1940). Disseminated lupus erythematosus: A cutaneous manifestation of a systemic disease (Libman-Sacks). *Arch. Intern. Med.* **65**, 26.
360. Gold, S. C., and Gowing, N. F. C. (1953). Systemic lupus erythematosus: A clinical and pathological study. *Q. J. Med.* **22**, 457.
361. Fox, R. A., and Rosahn, P. D. (1943). The lymph nodes in disseminated lupus erythematosus. *Am. J. Pathol.* **19**, 73.
362. Cruickshank, B. (1958). Lesion of lymph nodes in rheumatoid disease and disseminated lupus erythematosus. *Scot. Med. J.* **3**, 110.
363. Castellino, R. A., Marglin, S., and Blank, N. (1980). Hodgkin's disease, the non-Hodgkin's lymphomas, and the leukemias in the retroperitoneum. *Semin. Roentgenol.* **15**, 288.
364. Greco, A., Jelliffe, A. M., Maher, E. J., and Leung, A. W. L. (1988). MR imaging of lymphomas: Impact on therapy. *J. Comput. Assist. Tomogr.* **12**, 785.
365. Wyburn-Mason, R. (1979). SLE and lymphoma. *Lancet* **1**, 156.
366. Agudelo, C. A., Schumacher, H. R., Glick, J. H., and Molina, J. (1981). Non-Hodgkin's lymphoma in systemic lupus erythematosus: Report of 4 cases with ultrastructural studies in 2 patients. *J. Rheumatol.* **8**, 69.
367. Walden, P. A. M., Philalithis, P. E., Joekes, A. M., and Bagshaw, K. D. (1977). Development of a lymphocytic lymphoma during immunosuppressive therapy with azathioprine for systemic lupus erythematosus with renal involvement induced by phenylbutazone. *Clin. Nephrol.* **8**, 317.
368. McCarty, G. A., Rice, J. R., Fetter, B. F., and Pisetsky, D. S. (1982). Lymphocytic lymphoma and systemic lupus erythematosus, their coexistence with antibody to the Sm antigen. *Arch. Pathol. Lab. Med.* **106**, 196.
368a. Tardivon, A., Munck, J.-N., Shapeero, L. G., *et al*. (1995). Impact of MR imaging in malignant lymphoma: Comparison with blind biopsy and clinical data. *Ann. Oncol.* **6**, 795.
368b. Shapeero, L. G. (2003). Dynamic MR imaging: diffusion and whole body imaging of recurrences and metastases. *Proc. Int. Skelet. Soc*.
369. Wilson, W. A., Perez, M. C., Marwah, R., Foreman, J. B., and McGrath, H., Jr. (1989). Scintigraphic quantitation of splenic function in SLE: Correlation with IgM levels in serum. *J. Clin. Exp. Rheumatol.* **7**, 251.
370. Malleson, P., Petty, R. E., Nadel, H., and Dimmick, J. E. (1988). Functional asplenia in childhood onset systemic lupus erythematosus. *J. Rheumatol.* **15**, 1648.
371. Pilier, P., and Furie, R. (1990). Functional asplenia in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **20**, 185.
372. Petterson, T., and Julkunen, H. J. (1992). Asplenia in a patient with systemic lupus erythematosus and antiphospholipid antibodies. *J. Rheumatol.* **19**, 1159.
373. Rosenfield, A. T., and Siegel, N. J. (1981). Renal parenchymal disease: Histopathologic-sonographic correlation. *Am. J. Roentgen.* **137**, 793.
374. Stanley, J. H., Cornella, R., Loevinger, E., *et al*. (1984). Sonography of lupus nephritis. *Am. J. Roentgen.* **142**, 1165.
375. Longmaid, H. E., III, Rider, E., and Tymkin, J. (1987). Lupus nephritis: New sonographic findings. *J. Ultrasound Med.* **6**, 75.
376. Arnold, M. H., and Schrieber, L. (1988). Case report: Splenic and renal infarction in systemic lupus erythematosus: Association with anti-cardiolipin antibodies. *Clin. Rheumatol.* **7**, 406.
377. Silver, T. M., Kass, E. J., Thornbury, J. R., *et al*. (1976). The radiological spectrum of acute pyelonephritis in adults and adolescents. *Radiology* **118**, 65.
378. Corriere, I. N., Jr., and Sander, C. M. (1982). The diagnosis and immediate therapy of acute renal and perirenal infections. *Urol. Clin. North. Am.* **9**, 219.
379. Lee, J. K. T., McClennan, B. L., Melson, G. L., and Stanley, R. J. (1980). Acute focal bacterial nephritis: Emphasis on gray scale sonography and computed tomography. *Am. J. Roentgen.* **135**, 87.
380. Rosenfield, A. T., Glickman, M. G., Taylor, K. J. M., *et al*. (1979). Acute bacterial nephritis (acute lobar nephronia). *Radiology* **132**, 553.
381. Appel, G. B., Williams, G. S., Meltzer, J. I., and Pirani, C. L. (1966). Renal vein thrombosis, nephrotic syndrome, and systemic lupus erythematosus, an association in four cases. *Ann. Intern. Med.* **85**, 310.
382. Strahlman, E. R., Davidson, N. E., Kim, W. S., and Hockberg, M. C. (1982). Clinical conferences at the Johns Hopkins Hospital: Lupus nephritis. *Johns Hopkins Med. J.* **150**, 101.
383. Glazer, G. M., Francis, I. R., Gross, B. H., and Amendala, M. A. (1984). Computed tomography of renal vein thrombosis. *J. Comput. Assist. Tomogr.* **198**, 288.
384. Hamilton, C. R., Jr., and Tumulty, P. A. (1968). Thrombosis of renal veins and inferior vena cava complicating lupus nephritis. *JAMA* **206**, 2315.
385. Bradley, W. G., Jr., Jacobs, R. P., Trew, P. A., *et al*. (1981). Renal vein thrombosis: Occurrence in membranous glomerulonephropathy and lupus nephritis. *Radiology* **139**, 571.
386. Hricak, H., Crooks, L., Sheldon, P., and Kaufman, L. (1983). Nuclear magnetic resonance of imaging of the kidney. *Radiology* **146**, 425.
387. Ekelund, L., and Lindholm, T. (1974). Angiography in collagenous disease of the kidney. *Acta. Radiol.* **15**, 413.
388. Bookstein, J. J., and Clark, R. L. (1980). Renal Microvascular Disease: Angiographic–Microangiographic Correlates. Little Brown, Boston.
389. Weisman, M. H., McDanald, E. C., and Wilson, C. B. (1981). Studies of the pathogenesis of interstitial cystitis, obstructive uropathy, and intestinal malabsorption

in a patient with systemic lupus erythematosus. *Am. J. Med.* **70**, 875.
390. Orth, R. W., Weisman, H. M., Cohen, A. H., *et al*. (1983). Lupus cystitis: Primary bladder manifestations of systemic lupus erythematosus. *Ann. Intern. Med.* **98**, 323.
391. Boye, E., Morse, M., Huttner, L., *et al*. (1979). Immune-complex-mediated interstitial cystitis as a major manifestation of systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **13**, 67.
392. De La Serna, A. R., and Alarcon-Segovia, D. (1981). Chronic interstitial cystitis as an initial major manifestation of systemic lupus erythematosus. *J. Rheumatol.* **8**, 808.
393. Tsushima, Y., Uozumi, Y., and Yano, S. (1996). Reversible thickening of the bowel and urinary bladder wall in systemic lupus erythematosus: A case report. *Radiat. Med.* **14**, 95.
393a. Tanaka, H., Waga, S. H., Tateyama, T., *et al*. (2000). Interstitial cystitis and ileus in pediatric-onset systemic lupus erythematosus. *Pediatr. Nephrol.* **14**, 589.
394. Plotz, P. H., *et al*. (1979). Bladder complications in patients receiving cyclophosphamide for systemic lupus erythematosus or rheumatoid arthritis. *Ann. Intern. Med.* **91**, 221.
395. Elliott, R. W., Essenhigh, D. M., and Morley, A. R. (1982). Cyclophosphamide treatment of systemic lupus erythematosus: Risk of bladder cancer exceeds benefit. *Br. J. Med.* **284**, 1160.
396. Wada, T., Yokoyama, H., Ikeda, K., Tomosugi, N., and Kobayashi, K. (1992). Neurogenic bladder due to peripheral neuropathy and a visual disturbance in an elderly man with systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 547.
397. Shapeero, L. G., Friedland, G. W., and Perkash, I. (1983). The transrectal sonographic voiding cystourethrogram: Studies in neuromuscular bladder dysfunction. *Am. J. Roentgen.* **141**, 83.
398. Shapeero, L. G. (1989). Imaging renal transplants and their complications. *In* "Diagnostic Radiology" (A. R. Margulis and C. A. Gooding, eds.), pp. 527–534. University of California, San Francisco.
399. Coyne, S. S., Walsh, J. W., Tisnado, J., *et al*. (1981). Surgically correctable renal transplant complications: An integrated clinical and radiological approach. *Am. J. Roentgen.* **136**, 1113.
400. Bennett, L. N., Voegeli, D. R., Crummy, A. B., *et al*. (1986). Urologic complications following renal transplantation: Role of interventional radiologic procedures. *Radiology* **160**, 531.
401. Stone, J. H., Amend, W. J. C., and Criswell, L. A. (1997). Outcome of renal transplantation in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **27**, 17.
402. Cattran, D., and Aprile, M. (1991). Renal transplantation in lupus erythematosus. *Ann. Intern. Med.* **114**, 991.
403. Meija, G., Zimmerman, S., Glass, N., *et al*. (1983). Renal transplantation in patients with systemic lupus erythematosus. *Arch. Intern. Med.* **143**, 2089.
404. Nyberg, G., Karlberg, I., Svalander, C., Hedman, L., and Blohme, I. (1990). Renal transplantation in patients with systemic lupus erythematosus: Increased risk in early graft loss. *Scand J. Urol. Nephrol.* **24**, 307.
405. Bumgardner, G., Mauer, S., Payne, W., *et al*. (1988). A single-center 15 year results of renal transplantation in patients with systemic lupus erythematosus. *Transplantation* **46**, 703.
406. Sokumbi, D., Wadhwa, N., Waltzer, W., and Rapaport, F. T. (1993). Renal transplantation in patients with end-stage renal disease secondary to systemic lupus erythematosus. *Transplant. Proc.* **25**, 3328.
407. Bitker, M., Barrou, B., Ourhama, S., *et al*. (1993). Renal transplantation in patients with systemic lupus erythematosus. *Transplant. Proc.* **25**, 2172.
408. Hariharan, S., Schroeder, T., Carey, M., and First, M. R. (1992). Renal transplantation in patients with systemic lupus erythematosus. *Clin. Transplant.* **6**, 345.
409. Goss, J., Cole, B., Jendrisak, M., *et al*. (1991). Renal transplantation for systemic lupus erythematosus and recurrent lupus nephritis. *Transplantation* **52**, 805.
410. Terashita, G., and Cook, D. (1987). Original disease of the recipient. "Clinical Transplants" (P. Teraskai, ed.), pp. 373–379. Los Angeles.
411. Letourneau, I. G., Day, D. L., Ascher, N. L., *et al*. (1988). Imaging of renal transplants. *Am. J. Roentgen.* **150**, 833.
412. Curry, N. S., Cochran, M., and Barbaric, Z. L. (1984). Interventional radiologic procedures in the renal transplant. *Radiology* **152**, 647.
413. Grossman, R. A., Dafoe, D. C., Schoenfeld, R. B., *et al*. (1982). Percutaneous transluminal angioplasty treatment of renal transplant artery stenosis. *Transplantation* **34**, 33.
414. Hattner, R. S., Engelstad, B. I., and Dae, M. W. (1984). Radionuclide evaluation of renal transplant. *In* "Nuclear Medicine Annual" (L. M. Freeman and H. S. Weissman, eds.), p. 319. Raven Press, New York.
415. Kirchner, P. T., and Rosenthall, L. (1982). Renal transplant evaluation. *Semin. Nuclear Med.* **12**, 370.
416. Kim, E. E., Pjura, G., Lowry, P., *et al*. (1986). Cyclosporin-A nephrotoxicity and acute cellular rejection in renal transplant recipients: Correlation between radionuclide and histologic findings. *Radiology* **159**, 443.
417. Townsend, R. R., Tomlanovich, S. J., Goldstein, R. B., and Filly, R. A. (1980). Combined Doppler and morphologic sonographic evaluation of renal transplant rejection. *J. Ultrasound. Med.* **9**, 199.
418. Rifkin, M. D., Needleman, L., Pasto, M. E., *et al*. (1987). Evaluation of renal transplant rejection by duplex Doppler examination: Value of the resistive index. *Am. J. Roentgen.* **148**, 759.
419. Don, S., Kopecky, K. K., Filo, R. S., *et al*. (1989). Duplex Doppler US of renal allografts: Causes of elevated resistive index. *Radiology* **171**, 709.
420. Allen, K. S., Jorkasky, K. D., Arger, P. H., *et al*. (1988). Renal allografts: Prospective analysis of Doppler sonography. *Radiology* **169**, 371.
421. Linkowski, G. D., Warvariv, V., Filly, R. A., *et al*. (1987). Sonography in the diagnosis of acute renal allograft rejection and cyclosporine nephrotoxicity. *Am. J. Roentgen.* **148**, 291.

422. Hoddick, W., Filly, R. A., Backman, U., *et al*. (1986). Renal allograft rejection: US evaluation. *Radiology* **161**, 469.
423. Nicolet, V., Carignan, L., Dubuc, G., *et al*. (1988). Thickening of the renal collecting system: A nonspecific finding at US. *Radiology* **168**, 411.
424. Warshauer, D. M., Taylor, K. J. W., Bia, M. J., *et al*. (1988). Unusual causes of increased vascular impedance in renal transplants: Duplex Doppler evaluation. *Radiology* **169**, 367.
425. Pozniak, M., Kelcz, F., Stratta, R. J., *et al*. (1988). Extraneous factors affecting resistive index. *Invest. Radiol.* **23**, 899.
426. Shapeero, L. G., and Vordermark, J. S. (1989). Papillary necrosis in the renal transplant. *J. Ultrasound Med.* **8**, 579.
427. Hoffman, J. C., Schnur, M. I., and Koenigsberg, M. (1982). Demonstration of renal papillary necrosis by sonography. *Radiology* **145**, 785.
428. Amend, W. J. C., *et al*. (1981). Recurrent systemic lupus erythematosus involving renal allografts. *Intern. Med.* **94**, 444.
429. Yakub, Y. N., Freeman, R. B., and Pabico, R. C. (1981). Renal transplantation in systemic lupus erythematosus. *Nephron* **27**, 197.
430. Nyberg, D. (1987). Rejection and recurrence of SLE nephritis in cyclosporine treated recipients. *Transplantation* **19**, 163A.
431. Becker, J. A., Butt, K., and Lipkowitz, G. (1989). Segmental infarction of the renal allograft: Ultrasound/MRI observations. *Urol. Radiol.* **11**, 109.
432. Harris, K. M., Schwartz, M. L., Slasky, B. S., *et al*. (1987). Post transplantation cyclosporine-induced lymphoproliferative disorders: Clinical and radiologic manifestations. *Radiology* **162**, 697.
433. Penn, I. (1990). Cancers complicating organ transplantation. *N. Engl. J. Med.* **323**, 1767.
433a. Cibere, J., Sibley, J., and Haga, M. (2001). Systemic lupus erythematosus and the risk of malignancy. *Lupus* **10**, 384.
433b. Sultan, S. M., Ioannou, Y., and Isenberg, D. A. (2000). Is there an association of malignancy with sytemic lupus erythematosus? An analysis of 276 patients with long-term review. *Rheumatology* **29**, 1147.
433c. Sweeney, D. M., Manzi, S., Jonsky, J., *et al*. (1995). Risk of malignancy in women with systemic lupus erythematosus. *J. Rheumatol.* **22**, 1478.
434. Smith, T. P., Hunter, D. W., Letourneau, J. G., *et al*. (1988). Urinary obstruction in renal transplants: Diagnosis by antegrade pyelography and results of percutaneous treatment. *Am. J. Roentgen.* **15**, 507.
435. Thomalla, J. V., Steidle, C. P., Leapman, S. B., *et al*. (1988). Ureteral obstruction of a renal allograft secondary to *C. krusei*. *Transplant. Proc.* **20**, 551.
436. Jaskowski, Jones, R. M., and Murie, J. A. (1987). Urologic complications in 600 consecutive renal transplants. *Br. J. Surg.* **74**, 922.
437. Letourneau, J. G., Day, D. L., and Feinberg, S. B. (1982). Ultrasound-computed tomographic evaluation of renal transplants. *Radiol. Clin. North Am.* **25**, 267.
438. Hunter, D. W., Castaneda-Zuniga, W. R., and Coleman, C. C. (1983). Percutaneous techniques in the management of urological complications in renal transplant patient. *Radiology* **148**, 407.
439. Voegeli, D. R., Crummy, A. B., McDermott, J. C., *et al*. (1988). Percutaneous dilation of ureteral strictures in renal plant patients. *Radiology* **69**, 185.
440. Hanto, D. W., and Simmons, R. L. (1987). Renal transplantation: Practical considerations. *Radiol. Clin. North Am.* **25**, 239.
441. Silver, T. M., Campbell, D., Wicks, J. D., *et al*. (1981). Peritransplant fluid collections: Ultrasound evaluation and clinical significance. *Radiology* **138**, 145.
442. Spigos, D. G., Tan, W., Pave, D. G., *et al*. (1977). Diagnosis of extravasation after renal transplantation. *Am J Roentgen.* **129**, 409.
443. Rabinovici, R., Rivkind, A., Lebensart, P. D., *et al*. (1986). Ultrasonographic diagnosis of vesical leakage in a renal transplant recipient. *Urol. Radiol.* **8**, 112.
444. Greenberg, B. M., Perloff, L. J., Grossman, R. A., *et al*. (1985). Treatment of lymphocele in renal allograft recipients. *Arch. Surg.* **120**, 501.
445. Niemattalh, M. A., Dong, Q., Schoenberg, S. O., Cho, K. J., and Prince, M. R. (1999). Magnetic resonance imaging in renal transplantation. *J. Magn. Res. Imag.* **10**, 357.
445a. Gedroyc, W. M. W., Reidy, J. F., and Saxton, H. M. (1987). Arteriography of renal transplantation. *Clin. Radiol.* **38**, 239.
446. Stanley, P., Malekzadeh, M., and Diament, M. J. (1987). Post-transplant renal artery stenosis: Angiographic study in 32 children. *Am. J. Roentgen.* **148**, 487.
447. Picus, D., Neeley, J. P., McClennan, B. J., *et al*. (1985). Intraarterial digital subtraction angiography of renal transplants. *Am. J. Roentgen.* **145**, 93.
448. Raynaud, A., Bedrossian, J., Remy, P., *et al*. (1986). Percutaneous transluminal angioplasty of renal transplant arterial stenoses. *Am. J. Roentgen.* **146**, 853.
449. Taylor, K. J. W., Morse, S. S., Rigsby, C. M., *et al*. (1987). Vascular complications in renal allografts: Detection with duplex Doppler US. *Radiology* **162**, 31.
450. Delbeke, D., Sacks, G. A., and Sandler, M. P. (1989). Diagnosis of allograft renal vein thrombosis. *Clin. Nuclear Med.* **14**, 415.
451. Reuther, G., Wanjura, D., and Bauer, H. (1989). Acute renal vein thrombosis in renal allografts: Detection with duplex Doppler US. *Radiology* **170**, 557.
452. Middleton, W. D., Kellman, G. M., Melson, G. L., *et al*. (1989). Post-biopsy renal transplant arteriovenous fistulas: Color Doppler US characteristics. *Radiology* **171**, 253.
453. Morton, M. J., and Charboneau, J. W. (1989). Arteriovenous fistula after biopsy of renal transplant: Detection and monitoring with color flow and duplex ultrasonography. *Mayo. Clin. Proc.* **64**, 531.
454. Barker, C. F., Freeman, D. B., Ring, E. J., *et al*. (1982). Percutaneous transluminal angioplasty and fibrinolytic therapy for renal transplant arterial stenosis and thrombosis. *Transplantation* **33**, 447.

455. Schmidt, K. G., Ulmer, H. E., Silverman, N. H., Kleinman, C. S., and Copel, J. A. (1991). Perinatal outcome of fetal complete atrio-ventricular heart block: A multicenter experience. *J. Am. Coll. Cardiol.* **17**, 1360.
456. Michaelson, M., and Engle, M. A. (1972). Congenital complete heart block: An international study of the natural history. *Cardiovasc. Clin.* **4**, 85.
457. McCune, C. M., Mantakas, M. E., Tingelstad, L. B., and Ruddy, S. (1977). Congenital heart block in newborns of mothers with connective tissue disease. *Circulation* **56**, 82.
458. Truccone, M. J., and Mariona, F. G. (1986). Prenatal diagnosis and outcome of congenital complete heart block: The role of fetal echocardiography. *Fetal Ther.* **1**, 210.
459. Chameides, L., Truex, R. C., Vetter, V., *et al*. (1977). Association of maternal systemic lupus erythematosus with congenital complete heart block. *N. Engl. J. Med.* **297**, 1204.
460. Friedman, D. M. (1992). Fetal echocardiography in the assessment of lupus pregnancies. *Am. J. Reprod. Immunol.* **28**, 164.
460a. Buyon, J. P., Walluck, J., Kleinman, C., and Copel, J. (1996). Neonatal lupus. *Curr. Opin. Rheumatol.* **8**, 485.
461. Silverman, E. D. (1993). Congenital heart block and neonatal lupus erythematosus: Prevention is the goal. *J. Rheumatol.* **20**, 7.
462. Scott, J. S., Maddison, P. J., Taylor, P. V., *et al*. (1983). Connective-tissue disease, antibodies to ribonucleoprotein, and congenital heart block. *N. Engl. J. Med.* **309**, 209.
463. Singsen, B. H., Akhter, J. E., Weinstein, M. M., and Sharp, G. C. (1985). Congenital complete heart block and SSA antibodies: Obstetric implications. *Am. J. Obstet. Gynecol.* **152**, 655.
464. Reed, B. R., Lee, L. A., Harmon, C., *et al*. (1983). Autoantibodies to SS-A/Ro in infants with congenital heart block. *J. Pediatr.* **103**, 889.
465. Buyon, J. P., Ben-Chetrit, E., Karp, S., *et al*. (1989). Acquired congenital heart block: Pattern of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La system in neonatal lupus. *J. Clin. Invest.* **84**, 627.
466. Litsey, S. E., Noonan, J. A., O'Connor, W. N., Cottrill, C. M., and Mitchell, B. (1985). Maternal connective tissue disease and congenital heart block: Demonstration of immunoglobulin in cardiac tissue. *N. Engl. J. Med.* **312**, 98.
467. Taylor, P. V., Scott, J. S., Gerlis, L. M., *et al*. (1986). Maternal antibodies against fetal cardiac antigens in congenital complete heart block. *N. Engl. J. Med*. **315**, 667.
468. Lee, L. A., Coulter, S., Erner, S., and Chu, H. (1989). Cardiac immunoglobulin deposition in congenital heart block associated with maternal anti-Ro autoantibodies. *Am. J. Med*. **83**, 793.
469. Lockshin, M. D., Bonfa, E., Elkon, K., and Druzin, M. L. (1988). Neonatal lupus risk to newborns of mothers with systemic lupus erythematosus. *Arthritis Rheum*. **31**, 697.
470. Ramsey-Goldman, R., Horn, D., Deng, J. S., *et al*. (1986). Anti-SS-A antibodies and fetal outcome in maternal systemic lupus erythematosus. *Arthritis Rheum*. **29**, 1269.
471. Ferrazzini, G., Fasnacht, M., and Arbenz, U. (1996). Neonatal lupus erythematosus with congenital heart block and severe heart failure due to myocarditis and endocarditis of the mitral valve. *Neonatal Ped Intensive Care* **22**, 464.
472. Hanly, J. G., Gladman, D. D., Rose, T. H., *et al*. (1988). Lupus pregnancy, a prospective study of placental changes. *Arthritis Rheum*. **31**, 359.
473. Harris, E. N., Chan, J. K., Asherson, R. A., *et al*. (1986). Thrombosis, recurrent fetal loss, and thrombocytopenia: Predictive value of the anticardiolipin antibody test. *Arch. Intern. Med*. **146**, 2153.
474. Lockshin, M. D., Druzin, M. L., Goei, S., *et al*. (1985). Antibody to cardiolipin as a predictor of fetal distress on death in pregnant patients with systemic lupus erythematosus. *N. Engl. J. Med*. **313**, 152.
475. Lubbe, W. F., Butler, W. S., Palmer, S. J., and Liggins, G. C. (1983). Fetal survival after prednisone suppression of maternal lupus anticoagulant. *Lancet* **18**, 1361.
476. Derksen, R. H. W. M., Boumas, B. N., and Kater, L. (1986). The association between the lupus anticoagulant and cerebral infarction in systemic lupus erythematosus. *Semin. J. Rheumatol*. **15**, 179.
477. Abramowsky, C. R., Vegas, M. E., Swinehart, G., and Gyues, M. T. (1980). Decidual vasculopathy in the placenta in lupus erythematosus. *N. Engl. J. Med*. **303**, 668.
478. De Wolf, F., Carreras, L. O., and Merman, P. (1982). Decidual vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accidents, recurrent fetal loss and a lupus anticoagulant. *Am. J. Obstet. Gynecol*. **142**, 829.
479. Carroll, B. A. (1990). Obstetric duplex sonography in patients with lupus anticoagulant syndrome. *J. Ultrasound Med*. **9**, 17.
480. Meizner, I., Katz, M., and Press, F. (1988). Assessment of utero-placental blood flow velocity wave forms in a pregnant patient with systemic lupus erythematosus. *J. Clin. Ultrasound* **16**, 55.
481. Trudinger, B. J., Giles, W. B., Cook, C. M., *et al*. (1985). Fetal umbilical artery flow velocity waveforms and placental resistance: Clinical significance. *Br. J. Obstet. Gynaecol*. **92**, 605.
482. Erskine, R. L. A., and Ritchie, J. W. K. (1985). Umbilical artery flow characteristics in normal and growth retarded fetuses. *Br. J. Obstet. Gynaecol*. **92**, 605.
483. McCune, M. J., and Golbus, J. (1988). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am*. **14**, 149.
484. Kushner, M. J., Tobin, M., Fazekas, F., *et al*. (1990). Cerebral blood flow variations in CNS lupus. *Neurology* **40**, 99.
485. Ellis, S. G., and Verity, M. A. (1979). The central nervous system involvement in systemic lupus erythematosus: A review of neuropsychiatric findings in 57 cases. *Semin. Arthritis Rheum*. **8**, 212.

486. Boumpas, D. T., Scott, D. E., and Balow, J. E. (1993). Neuropsychiatric lupus: A case of guarded optimism. *J. Rheumatol*. **20**, 1641.
487. Johnson, R. T., and Richardson, E. P. (1968). The neurological manifestations of systemic lupus erythematosus. *Medicine* **47**, 337.
488. Breshnihan, B. (1982). CNS lupus. *Clin. Rheum. Dis*. **8**, 183.
489. Asherson, R. A., Khamashta, M. A., Gil, A., *et al*. (1989). Cerebrovascular disease and antiphospholipid antibodies in systemic lupus erythematosus, lupus-like disease, and the primary antiphospholipid syndrome. *Am. J. Med*. **86**, 391.
490. Bluestein, H. G. (1987). Neuropsychiatric manifestations of systemic lupus erythematosus. *N. Engl. J. Med*. **317**, 309.
491. Robbins, M. L., Kornguth, S. E., Bell, C. L., *et al.* (1988). Anti-neurofilament antibody evaluation in neuropsychiatric systemic lupus erythematosus: Combination with anticardiolipin antibody assay and magnetic resonance imaging. *Arthritis Rheum*. **31**, 623.
492. Hughes, G. R. V., Harris, E. N., and Gharavi, A. E. (1986). The anticardiolipin syndrome. *J. Rheumatol*. **13**, 486.
493. Derksen, R. H., Bourman, V. N., and Kater, L. (1986). The association between the lupus anticoagulant and cerebral infarction in systemic lupus erythematosus. *J. Rheumatol*. **15**, 79.
494. Levine, S. R., Kim, S., Deegan, M. J., and Welch, K. M. (1987). Ischemic stroke associated with anticardiolipin antibodies. *Stroke* **18**, 1101.
495. Boey, M. L., Golaco, C. B., Gharavi, A. E., *et al.* (1983). Thrombosis in systemic lupus erythematosus: Striking association with the presence of circulating lupus anticoagulant. *Br. Med. J*. **287**, 1021.
496. Kushner, M., and Simonian, N. (1989). Lupus anticoagulants, anti-cardiolipin antibodies, and cerebral ischemia. *Stroke* **20**, 225.
497. Kelley, R. E., and Berger, J. R. (1987). Ischemic stroke in a girl with lupus anticoagulant. *Pediatr. Neurol*. **31**, 58.
498. Drake, M. E., Jr. (1988). Lupus anticoagulant and lacunar infarctions. *Eur. Neurol*. **28**, 174.
499. Toubi, E., Khamashta, M. A., Panarra, A., and Hugher, G. R. V. (1995). Association of antiphospholipid antibodies with central nervous system disease in systemic lupus erythematosus. *Am. J. Med*. **99**, 397.
500. Sibbitt, W. L., Jr., Sibbitt, R. R., Griffey, R. H., Eckel, C., and Bankhurst, A. D. (1989). Magnetic resonance and computed tomographic imaging in evaluation of acute neuropsychiatric disease in systemic lupus erythematosus. *Ann. Rheum. Dis*. **48**, 1014.
501. West, S. G., Emlen, W., Wener, M. H., and Kotzin, B. L. (1995). Neuropsychiatric lupus erythematosus: A 10-year prospective study on the value of diagnostic tests. *Am. J. Med*. **99**, 153.
502. Sewell, K. L., Livneh, A., Aranow, C. B., and Grayzel, A. I. (1989). Magnetic resonance imaging versus computed tomographic scanning in neuropsychiatric systemic lupus erythematosus. *Am. J. Med*. **86**, 625.
503. Nomura, K., Yanano, S., Ikeda, Y., *et al.* (1999). Asymptomatic cerebrovascular lesions detected by magnetic resonance imaging in patients with systemic lupus erythematosus. *Intern. Med*. **38**, 785.
504. Kozora, E., West, S. G., Kotzin, B. L., *et al.* (1998). Magnetic resonance imaging abnormalities and cognitive deficits in systemic lupus erythematosus patients without overt central nervous system disease. *Arthritis Rheum*. **41**, 42.
505. Rovaris, M., Ciboddo, G., Gerevini, S., *et al.* (2000). Brain involvement in systemic immune mediated disease: Magnetic resonance and magnetisation transfer imaging study. *J. Neurol. Neurosurg. Psychiat.* **68**, 170.
506. Bosma, G. P., Rood, M. J., Zwinderman, A. H., Huizinga, T. W. J., and Van Buchem, M. A. (2000). Evidence of central nervous system damage in patients with neuropsychiatric systemic lupus erythematosus demonstrated by magnetization transfer imaging. *Arthritis Rheum*. **43**, 49.
507. Colamussi, P., Giganti, M., Cittanti, C., *et al.* (1995). Brain single-photon emission tomography with 99mTC-HMPAO in neuropsychiatric systemic lupus erythematosus: Relations with EEG and MRI findings and clinical manifestations. *Eur. J. Nuclear Med*. **22**, 17.
508. Kao, C.-H., Lan, J.-L., Chang Lai, S.-P., *et al.* (1999). The role of FDG-PET, HMPAO-PET and MRI in the detection of brain involvement patients with systemic lupus erythematosus. *Eur. J. Nucl. Med*. **25**, 129.
509. Kovacs, J. A., Urowitz, M. B., Gladman, D. D., and Zeman, R. (1995). The use of single photon emission computerized tomography in neuropsychiatric SLE: A pilot study. *J. Rheumatol*. **22**, 1247.
510. Falcini, F., De Cristofaro, M. T. R., and Ermini, M. (1998). Regional cerebral blood flow in juvenile systemic lupus erythematosus: A prospective SPECT study. *J. Rheumatol*. **35**, 583.
511. Lin, W. Y., Wang, S. J., Yen, T. C., and Lan, J. L. (1997). Technetium-99m-HMPAO brain SPECT in sytemic lupus erythematosus with CANS involvement. *J. Nuclear Med*. **38**, 1125.
512. Szer, I. S., Miller, J. H., Rawlings, D., Shaham, B., and Bernstein, B. (1993). Cerebral perfusion abnormalities in children with central nervous system manifestation of lupus detected by single photon emission computed tomography. *J. Rheumatol*. **20**, 2143.
513. Hanly, J. G. (1998). Single photon emission computed tomography scanning in neuropsychiatric systemic lupus erythematosus. *J. Rheumatol*. **25**, 401.
514. Russo, R., Gilday, D., Laxer, R. M., Eddy, A., and Silverman, E. D. (1998). Single photon emission tomography scanning in childhood systemic lupus erythematosus. *J. Rheumatol*. **25**, 576.
515. Pinching, A. J., Travers, R. L., Hughes, G. R. V., *et al.* (1978). Oxygen 15 brain scanning for detection of cerebral involvement in systemic lupus erythematosus. *Lancet* **1**, 898.

516. Awada, H. H., Marno, H. L., Luft, A. G., *et al.* (1987). Cerebral blood flow in systemic lupus erythematosus with and without central nervous system involvement. *J. Neurol. Neurosurg. Psychiatry* **50**, 1597.
517. Kao, C.-H., Ho, Y.-J., Lan, J.-L., *et al.* (1999). Discrepancy between regional cerebral blood flow and glucose metabolism of the brain in systemic lupus erythematosus patients with normal brain magnetic resonance imaging findings. *Arthritis Rheum*. **42**, 61.
518. Stoppe, G., Wiidhagen, K., Seidel, J. W., *et al.* (1990). Positron emission tomography in neuropsychiatric lupus erythematosus. *Neurology* **40**, 304.
519. Otte, A., Weiner, S. M., Peter, H. H., *et al.* (1997). Brain glucose utilization in systemic lupus erythematosus with neuropsychiatric symptoms: A controlled positron emission tomography study. *Eur. J. Nucl. Med*. **24**, 787.
520. Meyer, G. I. J., Schober, O., Stoppe, G., *et al.* (1989). Cerebral involvement in systemic lupus erythematosus (SLE): Comparison of positron emission tomography (PET) with other imaging methods. *Psychiatry Res*. **29**, 367.
521. Pawlik, G., and Heiss, W. D. (1989). Positron emission tomography and neuropsychological function. *In* "Neuropsychological Function and Brain Imaging". (E. D. Bigler, R. A. Yeo, E. and Turkheimer, eds.), pp. 65–138. Plenum Press, New York.
522. Weiner, S. M., Otte, A., Schumacher, M., *et al.* (2000). Alterations of cerebral glucose metabolism indicate progress to severe morphogical brain lesion in neuropsychiatric systemic lupus erythematosus. *Lupus* **9**, 386.
523. Weiner, S. M., Otte, A., Hoegerle, S., *et al.* (1998). Neuropsychiatric systemic lupus erythematosus before and after immunosuppressive treatment: A FDG PET study. *Lupus* **7**, 57.
524. Weiner, S. M., Otte, A., Schumacher, M., *et al.* (2000). Diagnosis and monitoring of central nervous system involvement in systemic lupus erythematosus: Value of F-18 fluorodeoxygluscose PET. *Ann. Rheum. Dis*. **59**, 377.
525. Griffey, R. H., Brown, M. S., Bankhurst, A. D., *et al.* (1990). Depletion of high energy phosphates in the central nervous system of patients with systemic lupus erythematosus, as determined by phosphorus 31 nuclear magnetic resonance spectroscopy. *Arthritis Rheum*. **33**, 829.
526. Davie, C. A., Feinstein, A., Kartsounis, L. D., *et al.* (1995). Proton magnetic resonance spectroscopy of systemic lupus erythematosus involving the central nervous system. *J. Neurol.* **242**, 522.
527. Keshavan, M. S., Kapur, S., and Pettegrew, J. W. (1991). Magnetic resonance spectroscopy in psychiatry: Potential, pitfalls, and promise. *Am. J. Psychiat*. **148**, 976.
528. Sibbitt, W. L., Jr., and Sibbitt, R. R. (1993). Magnetic resonance spectroscopy and positron emission tomography scanning in neuropsychiatric systemic lupus erythematosus. *Rheum. Dis. Clin. North. Am*. **19**, 851.
529. Bennahum, D. A., Messner, R. P., and Shoop, J. D. (1974). Brain scan findings in central nervous system involvement by systemic lupus erythematosus. *Ann. Intern. Med*. **81**, 763.
530. Tan, R. F., Gladman, D. D., Urowitz, M. B., and Milne, N. (1979). Brain scan diagnosis of central nervous system involvement in systemic lupus erythematosus. *Ann. Rheum. Dis*. **37**, 357.
531. Grigor, R., Edmonds, J., Lewkonia, R., *et al.* (1978). Systemic lupus erythematosus: A prospective analysis. *Ann. Rheum. Dis*. **37**, 121.
532. Omdal, R., Sleseth, B., Klow, N. E., *et al.* (1989). Clinical neurological, electrophysiological and cerebral CT scans findings in systemic lupus erythematosus. *Scand. J. Rheumatol*. **18**, 283.
533. Golloub, C. A., Marcelo, N. C., and Leahy, W. (1989). Reversible prolonged neurologic deficit associated with transient cranial abnormalities and systemic lupus erythematosus. *South Med. J*. **82**, 650.
534. Weisberg, L. A. (1986). The cranial computed tomographic findings in patients with neurological manifestations of systemic lupus erythematosus. *Comput. Radiol*. **10**, 63.
535. Carette, S., Urowitz, M. B., and Grossman, H. (1982). Cranial computerized tomography in systemic lupus erythematosus. *J. Rheumatol*. **9**, 855.
536. Gaylis, N. B., Altman, R. D., Ostrov, S., and Quencer, R. (1982). The selective value of computed tomography of the brain cerebritis due to systemic lupus erythematosus. *J. Rheumatol*. **9**, 850.
537. Shah, Z. R., Belion, E. M., Toor, A. H., and Shields, R. (1988). CT demonstration of brain infarcts due to deep venous thrombosis in systemic lupus erythematosus. *Comput. Med. Imag. Graph*. **12**, 241.
538. Parnass, S. M., Goodwin, J. A., Patel, D. V., *et al.* (1987). Dural sinus thrombosis: A mechanism for pseudotumor cerebri in systemic lupus erythematosus. *J. Rheumatol*. **14**, 152.
539. Gordon, T., Maddison, P. J., and Isenberg, D. A. (1986). Rapid development of cerebral atrophy in systemic lupus erythematosus. *Br. J. Rheumatol*. **25**, 296.
540. Ivanova, M. M., Blizniuk, O. I., Tadua, F. J., and Tumanova, A. A. (1989). Lesions of the central nervous system in systemic lupus erythematosus in the computerized-tomographic image. *Klin. Med. Wochenschr*. **93**, 429.
541. Bovim, G., Jorstad, S., and Schrader, H. (1990). Subdural hematoma presenting as headache in systemic lupus erythematosus. *Cephalalgia* **10**, 25.
542. Nascimento, O. J., De Freitas, M. R., Cavalcanti, J. L., and Netto, M. B. (1985). Cerebral hemorrhage as the initial manifestation of systemic lupus erythematosus: Clinical and histopathological study of 2 patients. *Arq. Neuropsiquiatr*. **43**, 187.
543. Savitz, M. H., Katz, S. S., Letsch, S. D., *et al.* (1987). Mirror-image intracerebral hemorrhages in a patient with systemic lupus erythematosus. *Mt. Sinai. J. Med*. **54**, 522.

544. Ishige, N., Sunami, K., Sato, A., *et al.* (1985). A case of systemic lupus erythematosus associated with spontaneous bilateral epidural hematomas. *No Shinkel Geka* **13**, 345.
545. Nagayasu, S., Hanakita, I., Miyake, H., *et al.* (1986). A case of systemic lupus erythematosus associated with multiple intracranial aneurysms. *No Shinkei Geka*. **14**, 1251.
546. Futran, J., Shore, A., Urowitz, M. B., and Grossman, H. (1987). Subdural hematoma in systemic lupus erythematosus: Report and review of the literature. *J. Rheumatol*. **14**, 378.
547. Haas, L. F. (1982). Stroke as an early manifestation of systemic lupus erythematosus. *J. Neurol. Neurosurg. Psychiatry* **45**, 544.
548. Vern, B. A., and Butler, M. (1983). Transient thalamic hypodensity in lupus erythematosus with generalized seizures. *Neurology* **33**, 1081.
549. Isshi, K., Hirohata, S., Hashimoto, T., and Miyashita, H. (1994). Systemic lupus erythematosus presenting with diffuse low density lesions in the cerebral white matter on computed axial tomography scans: Its implication in the pathogenesis of diffuse central nervous system lupus. *J. Rheumatol*. **21**, 1758.
550. Borenstein, D. G., and Jacobs, R. P. (1982). Aqueductal stenosis: A possible late sequela of central nervous system inflammation in systemic lupus. *South. Med. J*. **75**, 475.
551. Mazer, S., Araujo, J. C., Ribeiro, R. C., and Kasting, G. (1983). Unusual computed appearance of cerebral toxoplasmosis. *AJNR* **4**, 458.
552. Lehman, T. J. A., Bernstein, B., Hanson, V., *et al.* (1981). Meningococcal infection complicating systemic lupus erythematosus. *J. Pediatr*. **99**, 94.
553. Khan, M. A., and Sbar, S. (1975). Cryptococcal meningitis in corticosteroid-treated systemic lupus erythematosus. *Postgrad. Med. J*. **51**, 660.
554. Speller, D. C. E., Fakunle, F., Cairns, S. A., and Stephens, M. (1977). Cryptococcal meningitis complicating lupus erythematosus: Two patients treated with flucytosine and amphotericin. *Br. J. Clin. Pathol*. **30**, 254.
555. Lammens, M., Robberecht, W., Waer, M., Carton, H., and Dome, R. (1990). Purulent meningitis due to aspergillosis in a patients with systemic lupus erythematosus. *Neurol. Neurosurg*. **94**, 39.
556. Malas, D., and Weiss, S. (1977). Progressive multifocal leukoencephalopathy and cryptococcal meningitis with systemic lupus erythematosus and thymoma. *Ann. Neurol*. **1**, 188.
557. Goldman, J. A., Fleischer, A. S., Leifer, W., *et al.* (1979). *Candida albicans* mycotic aneurysm associated with systemic lupus erythematosus. *Neurosurgery* **4**, 325.
558. Kim, W.-U., Lee, S.-H., and Shim, B.-Y. (2000). Intramedullary tuberculosis manifested as Brown-Sequard syndrome in a patient with systemic lupus erythematosus. *Lupus* **9**, 147.
559. Brant-Zawadzki, M., Mills, C. M., and Norman, D. (1983). Central nervous system. *In* "Clinical Magnetic Resonance Imaging" (A. R. Margulis, C. B. Higgins, and L. Kaufman, eds.), p. 91. Radiology Research and Education Foundation, San Francisco.
560. Gonzalez-Scarano, F., Lisak, R. P., Bilaniuk, L. T., *et al.* (1979). Cranial computed tomography in diagnosis of systemic lupus erythematosus. *Ann. Neurol.* **5**, 158.
561. Miguel, E. C., Pereira, R. M. R., Pereira C. A. D. B., *et al.* (1994). Psychiatric manifestations of systemic lupus erythematosus: Clinical features, symptoms, and signs of central nervous system activity in 43 patients. *Medicine* **73**, 224.
562. Kaell, A. T., Sheety, M., Lee, B. C. P., and Lockshin, M. D. (1986). The diversity of neurologic events in systemic lupus erythematosus: Prospective clinical and computed tomographic classification of 82 events in 71 patients. *Arch. Neurol*. **43**, 273.
563. McCune, W. J., MacGuire, A., Aisen, A., and Gebarski, S. (1988). Identification of brain lesions in neuropsychiatric systemic lupus erythematosus by magnetic resonance scanning. *Arthritis Rheum*. **31**, 159.
564. Ostrov, S. G., Quencer, R. M., Gaylis, M. B., and Altman, R. D. (1982). Cerebral atrophy in systemic lupus erythematosus: Corticosteroid or disease-induced phenomenon? *AJNR* **3**, 21.
565. Sibbitt, W. L., Jr., Haseler, L. J., Griffey, R. H., *et al.* (1994). Analysis of cerebral structural changes in systemic lupus erythematosus by proton MR spectroscopy. *Am. J. Neurorad*. **15**, 923.
566. Chinn, R. J., Wilkinson, J. D., Hall-Craggs, M. A., *et al.* (1997). Magnetic resonance imaging of the brain and cerebral proton spectroscopy in patients with systemic lupus erythematosus. *Arthritis Rheum*. **40**, 36.
567. Yokota, S., Morki, T., Kosuga, K., *et al.* (1985). Basal ganglia calcification in two children with systemic lupus erythematosus. *Ryumachi* **25**, 115.
568. Daud, A. B., and Nuruddin, R. N. (1988). Solitary paraventricular calcification in cerebral lupus erythematosus: A report of two cases. *Neuroradiology* **30**, 84.
569. Nordstrom, D. M., West, S. G., and Andersen, P. A. (1985). Basal ganglia calcifications in central nervous system lupus erythematosus. *Arthritis Rheum*. **28**, 1412.
570. Yamamoto, K., Nogaki, H., Takase, Y., and Morimatsu, M. (1992). Systemic lupus erythematosus associated with marked intracranial calcification. *Am. J. Neurorad*. **13**, 1340.
571. Raya, P. G., Aguado, A. G., Merlo, M. J. S., *et al.* (1994). Massive cerebral calcification in systemic lupus erythematosus: Report of an unusual case. *Lupus* **3**, 133.
572. Raymond, A. A., Zariah, A. A., Samad, S. A., Chin, C. N., and Kong, N. C. T. (1996). Brain calcification in patient with cerebral lupus. *Lupus* **5**, 123.
573. Aisen, A. M., Gabrielsen, T. O., and McCune, M. J. (1985). MR imaging of systemic lupus erythematosus involving the brain. *Am. J. Roentgen*. **144**, 1027.
574. Lim, L., Ron, M. A., Ormerod, J. E., *et al.* (1988). Psychiatric and neurological manifestations in systemic lupus erythematosus. *Q. J. Med*. **66**, 27.

575. Suzuki, K. J., Hara, M., Nakajima, S., *et al.* (1989). Analysis of systemic lupus erythematosus (SLE) involving the central nervous system by magnetic resonance imaging (MRI). *Ryumachi* **29**, 88.
576. Vermess, M., Bernstein, R. M., Bydder, G. M., *et al.* (1983). Nuclear magnetic resonance (NMR) of the brain in systemic lupus erythematosus. *J. Comput. Assist. Tomogr*. **7**, 461.
577. Cauli, A., and Montaldo, Peltz M. T. (1994). Abnormalities of magnetic resonance imaging of the central nervous system in patients with systemic lupus erythematosus. Correlate with disease severity. *Clin. Rheumatol.* **13**, 615.
578. McAbee, G. N., and Barasch, E. S. (1990). Resolving MRI lesions in lupus erythematosus selectively involving the brainstem. *Pediatr. Neurol*. **6**, 186.
579. Sewell, K. L., Livneh, A., Aranow, C. B., and Grayzel, A. I. (1986). Magnetic resonance imaging versus computed tomographic scanning in neuropsychiatric systemic lupus erythematosus. *Am. J. Med.* **86**, 625.
580. Jacobs, L., Kinkel, P. R., Costello, P. B., *et al.* (1988). Central nervous systemic lupus erythematosus: The value of magnetic resonance imaging. *J. Rheumatol*. **15**, 601.
581. Gonzalez-Crespo, M. R., Blanco, F. J., and Ramos, A. (1995). Magnetic resonance imaging of the brain in systemic lupus erythematosus. *Br. J. Rheumatol*. **34**, 1055.
582. Sibbitt, W. L., Jr., Brooks, W. M., Haseler, L. J., *et al.* (1995). Spin-spin relaxation of brain tissues in systemic lupus erythematosus. *Arthritis Rheum*. **38**, 810.
583. Bell, C. L., Partington, C., Robbins, M., *et al.* (1991). Magnetic resonance imaging of central nervous system lesions in patients with lupus erythematosus. *Arthritis Rheum*. **34**, 843.
584. Miller, D. H., Ormerod, J. E., Gibson, A., *et al.* (1987). MR brain scanning in patients with vasculitis: Differentiation from multiple sclerosis. *Neuroradiology* **2**, 229.
585. Hughes, G. R. V. (1980). Central nervous system lupus diagnosis and treatment. *J. Rheumatol*. **7**, 405.
586. Hiraiwa, M., Nonaka, C., Abe, T., and Tio, M. (1983). Positron emission tomography in systemic lupus erythematosus. *Am. J. Neurorad*. **4**, 541.
587. Kushner, M. J., Tobin, M., Fazekas, F., *et al.* (1990). Cerebral blood flow variations in CNS lupus. *Neurology* **40**, 99.
588. Kodama, K., Okada, S., Hino, T., *et al.* (1995). Single photon emission computed tomography in systemic lupus erythematosus with psychiatric symptoms. *J. Neurol. Neurosurg. Psychiatry* **58**, 307.
589. Emmi, L., Bramati, M., De Cristofaro, M. T. R., *et al.* (1993). MRI and SPECT investigation of the CNS in SLE patients. *Clin. Exp. Rheumatol*. **11**, 13.
590. Stoppe, G., Wildhagen, K., Meyer, G. J., and Schober, O. (1989). Use of fluorodeoxyglucose PET in the diagnosis of central nervous system lupus erythematosus and a comparison with CT and MRI. *Nuklearmedizin* **28**, 187.
591. Nossent, J. C., Hovestadt, A., Schonfeld, D. H. W., and Swaak, A. J. G. (1991). Single-photon-emission computed tomography of the brain in the evaluation of cerebral lupus. *Arthritis Rheum*. **34**, 1397.
592. Rubbert, A., Marienhagen, J., Pirner, K., *et al.* (1993). Single-photon-emission computed tomography: Analysis of cerebral blood flow in the evaluation of central nervous system involvement in patients with systemic lupus erythematosus. *Arthritis Rheum*. **36**, 1253.
593. Grunwald, F., Schomburg, A., Badali, A., *et al.* (1995). 18FDG and acetazolamide-enhanced 99mTc-HMPAO SPECT in systemic lupus erythematosus. *Eur. J. Nuclear Med*. **2**, 1073.
594. Volkow, N. D., Warner, N., McIntyre, R., *et al.* (1988). Cerebral involvement in systemic lupus erythematosus. *Am. J. Physiol. Imag*. **3**, 91.
595. Carbotte, R. M., Denburg, S. D., Denburg, J. A., Nahmias, C., and Garnett, E. S. (1992). Fluctuating cognitive abnormalities and cerebral glucose metabolism in neuropsychiatric systemic lupus erythematosus. *J. Neurol. Neurosurg. Psychiat*. **55**, 1054.
596. Sibbit, W. L., Jr., Griffey, R. H., Haseler, L. J., *et al.* (1993). Analysis of cerebral structural changes in systemic lupus erythematosus by proton magnetic resonance spectroscopy. *Clin. Res*. **41**, 42A,
597. Ferris, E. J. (1974). Collagen disorders: Lupus erythematosus. *In* "Radiology of the Skull and Brain: Angiography" (T. H. Newton, and D. G. Potts, eds.), p. 2582. CV Mosby, St. Louis.
598. Kelley, R. E., Stokes, N., Reyes, P., and Hank, S. J. (1980). Cerebral transmural angiitis and ruptured aneurysm, a complication of systemic lupus erythematosus. *Arch. Neurol*. **37**, 526.
599. Atanes Sandoval, A., Sanchez Burson, J. M., Grana, G. J., *et al.* (1989). Multiple aneurysms and cerebral vasculitis in systemic lupus erythematosus. *Med. Clin*. **92**, 577.
600. Yoshimoto, T., Ueno, K., Ihara, T., *et al.* A case of systemic lupus erythematosus with neurological manifestations as initial symptoms. *No Shinkei Geka* **17**, 489.
601. Weiner, D. K., and Allen, N. B. (1991). Large vessel vasculitis of the central nervous system in systemic lupus erythematosus: Report and review of the literature. *J. Rheumatol*. **18**, 748.
602. Liem, M. D., Gzesh, D. J., and Flanders, A. E. (1996). MRI and angiographic diagnosis of lupus cerebral vasculitis. *Neuroradiology* **38**, 134.
603. Nagayama, Y., Kusudo, K., and Imura, H. (1989). A case of central nervous systemic lupus associated with ruptured cerebral berry aneurysm. *Jpn. J. Med*. **28**, 530.
604. Asai, A., Matsutani, M., Kohno, T., *et al.* (1989). Multiple saccular cerebral aneurysms associated with systemic lupus erythematosus. *Neurol. Med. Chir*. **29**, 245.
605. Hashimoto, N., Handa, H., and Taki, W. (1986). Ruptured cerebral aneurysms in patients with systemic lupus erythematosus. *Surg. Neurol*. **26**, 512.
606. Nagayasu, S., Hanakita, I., Miyake, H., *et al.* (1986). A case of systemic lupus erythematosus associated with

multiple intracranial aneurysms. *No Shinkei Geka* **14**, 1251.
607. Dungan, D. D., and Jay, M. S. (1992). Stroke in an early adolescent with systemic lupus erythematosus and coexistent antiphospholipid antibodies. *Pediatrics* **90**, 96.
608. Trevor, R. P., Sonheimer, F. K., Fessel, W. J., and Wolpert, S. M. (1972). Angiographic demonstration of major cerebral vessel occlusion in systemic lupus erythematosus. *Neuroradiology* **4**, 202.
609. Kwon, S. U., Koh, J. Y., and Kim, J. S. (1999). Vertebrobasilar artery territory infarction as an initial manifestation of systemic lupus erythematosus. *Clin. Neurol. Neurosurg*. **101**, 62.
610. Molle, D., Guillevin, L., Herrman, G., and Godeau, P. (1982). Thrombophlebitis of the superior sagittal sinus in a patient with systemic lupus erythematosus. *Semin. Hop. Paris* **58**, 1215.
611. Uziel, Y., Laxer, R. M., Blaser, S., *et al.* (1995). Cerebral vein thrombosis in childhood systemic lupus erythematosus. *J. Pediatr*. **126**, 722.
612. Vidailhet, M., Piette, J.-C., Wechsler, B., Bousser, M.-G., and Brunet, P. (1990). Cerebral venous thrombosis in systemic lupus erythematosus. *Stroke* **21**, 1226.
613. Li, E. K., Kay, R. L., and Cohen, M. G. (1990). Cerebral venous thrombosis in systemic lupus erythematosus. *Stroke* **21**, 1226.
614. Li, E. K., and Chan, M. S. (1990). Is pseudotumor cerebri in SLE a thrombotic event? *J. Rheumatol*. **17**, 983.
615. Kaplan, R. E., Springate, J. E., Feld, L. G., and Choen, M. E. (1985). Pseudotumor cerebri associated with cerebral venous sinus thrombosis, internal jugular vein thrombosis and systemic lupus erythematosus. *J. Pediatr*. **107**, 226.
616. Parnass, S. M., Goodwin, J. A., Patel, D. V., Levinson, D., and Reinhard, J. D. (1987). Dural sinus thrombosis: A mechanism for pseudotumor cerebri in systemic lupus erythematosus. *J. Rheumatol.* **14**, 152.
617. Lee, M. K., Kim, J. H., Kang, H. R., *et al*. (2001). Systemic lupus erythematosus complicated with cerebral venous sinus thrombosis: A report of two cases. *J. Korean Med. Sci*. **16**, 361.
618. Shiozawa, Z., Yoshida, M., Kobayashi, K., Tsunoda, S., and Mano, T. (1986). Superior sagittal sinus thrombosis and systemic lupus erythematosus. *Ann. Neurol*. **20**, 272.
619. Flusser, D., Abu-Shakra, M., Baumgarten-Kleiner, A., Flusser, G., and Sukenik, S. (1996). Superior sagittal sinus thrombosis in a patient with systemic lupus erythematosus. *Lupus* **5**, 334.
620. Tan, E. K., Chan, L. L., and Auchus, A. P. (2001). Reversible parkinsonism in systemic lupus erythematosus. *J. Neurol. Sci*. **193**, 53.
621. Kwong, K. L., Chu, R., and Wong, S. N. (2000). Parkinsonism unusual neurologic complication in childhood systemic lupus erythematosus. *Lupus* **9**, 474.
622. Lim, S. M., Oldfield, M., and Abbott, R. J. (1998). Systemic lupus erythematosus presenting with steroid-responsive parkinsonism and post-hemiplegic dystonia. *Eur. J. Neurol*. **5**, 308.
623. Krupp, L. B., Lipton, R. B., Swerdow, M. L., *et al.* (1985). Progressive multifocal encephalopathy: Clinical and radiologic features. *Ann. Neurol*. **17**, 344.
624. Newton, P., Aldridge, R. D., Lessells, A. M., and Best, P. V. (1986). Progressive multifocal leukoencephalopathy complicating systemic lupus erythematosus. *Arthritis Rheum*. **29**, 337.
625. Tomura, N., Watanabe, M., Kato, T., Nishino, K., and Kowada, M. (1994). Case report: Progressive multifocal leukoencephalopathy with prominent medullary veins on angiogram. *Clin. Radiol*. **49**, 66.
626. Marsteller, L. P., Marsteller, H. B., Braun, A., *et al.* (1987). An unusual CT appearance of lupus cerebritis. *Am. J. Neurorad*. **8**, 737.
627. Kaye, B. R., Neuwelt, C. M., London, S. S., and De Armond, S. J. (1992). Central nervous system systemic lupus erythematosus mimicking progressive multifocal leukoencephalopathy. *Ann. Rheum. Dis*. **51**, 1152.
628. Cogen, M. S., Kline, L. B., and Duvall, E. R. (1987). Bilateral internuclear ophthalmoplegia in systemic lupus erythematosus. *J. Clin. Neuroophthalmol*. **7**, 69.
629. Cogan, D., Kubick, C., and Smith, W. (1980). Unilateral internuclear ophthalmoplegia. *Arch. Ophthalmol*. **44**, 783.
630. Dutta, I., Golar, S., Balakrishman, C., *et al.* (2000). Unilateral inter-nuclear ophthalmoplegia in systemic lupus erythematosus. *J. Ass. Phys. India* **48**, 1210.
631. Yigit, A., Bingol, A., Mutluer, N., and Tascilar, N. (1986). The one-and-a-half syndrome in systemic lupus erythematosus. *J. Neuroophthalmol*. **16**, 274.
632. Bailey, A., Sayre, G., and Clark, E. (1956). Neuritis associated with systemic lupus erythematosus. *Arch. Neurol. Psych*. **75**, 251.
633. Sklar, E. M., Schatz, N. J., Glaser, J. S., Post, M. J., and Ten Hove, M. (1996). MR of vasculitis-induced optic neuropathy. *Am. J. Neurorad*. **17**, 121.
634. Jordan, D. R., McDonald, H., Olberg, B., McKim, D., and McKendry, R. (1993). Orbital panniculitis as the initial manifestation of systemic lupus erythematosus. *Ophthal. Plast. Reconst. Surg*. **9**, 71.
635. Gray, R. E., Jenkins, E. A., Hall, M. A., Kanski, J. J., and Ansell, B. M. (1989). Recurrent acute proptosis in atypical systemic lupus erythematosus. *Clin. Rheumatol*. 528.
636. Lipsmeyer, E. A. (1972). Development of cerebral lymphoma in a patient with systemic lupus erythematosus treated with immunosuppression. *Arthritis Rheum*. **15**, 183.
637. Cras, P., Franck, C., and Martin, J. J. (1989). Primary intracerebral lymphoma in systemic lupus erythematosus treated with immunosuppression. *Clin. Neuropathol*. **8**, 200.
638. Poon, T., Matoso, L., Tchertkoff, V., *et al.* (1989). CT features of primary cerebral lymphoma in AIDS and non-AIDS patients. *J. Compt. Assist. Tomogr*. **13**, 6.
639. Lee, Y.-Y., Bruner, J. M., van Tassel, P., and Libschitz, H. (1986). Primary central nervous system lymphoma: CT and pathological correlation. *Am. J. Roentgen*. **147**, 747.

640. Schwaighofer, B. W., Hesselink, J. R., Press, G. A., *et al.* (1989). Primary intracranial lymphoma: MR manifestations. *AJNR* **10**, 725.
641. Enzmann, D. E., Brant-Zawadzki, M., and Britt, R. H. (1980). CT of central nervous system infections in immunocompromised patients. *Am. J. Roentgen*. **135**, 263.
642. Widener, H. L., and Littman, B. H. (1978). Ibuprofen-induced meningitis in systemic lupus erythematosus. *JAMA* **239**, 1062,
643. Sergent, J. S., and Lockshin, M. (1978). Azathioprine-induced meningitis in systemic lupus erythematous. *JAMA* **240**, 529.
644. Lancman, M. E., Mesroplan, H., and Granillo, R. J. (1989). Chronic aseptic meningitis in a patient with systemic lupus erythematosus. *Can. J. Neurol. Sci*. **16**, 354.
645. Shaskey, D. J., Mijer, J. F., Williams, H. J., and Sawitzke, A. D. (1995). Subdural fluid collections: An unusual manifestation of CNS disease in a connective tissue disorder. *Clin. Rheumatol*. **14**, 108.
646. Salmaggi, A., Lamperti, E., Eoli, M., *et al.* (1994). Spinal cord involvement and systemic lupus erythematosus: Clinical and magnetic resonance findings in 5 patients. *Clin. Exp. Rheumatol*. **12**, 389.
647. Thakara, P., and Greenspan, B. (1979). Transverse myelopathy in systemic lupus erythematosus. *Arch. Phys. Med. Rehab*. **60**, 323.
648. Lavalle, C., Pixarro, S., Dreukard, C., Sanchez-Guerrero, J., and Alarcon-Segovia, D. (1990). Transverse myelitis: A manifestation of systemic lupus erythematosus strongly associated with antiphospholipid antibodies. *J. Rheumatol*. **17**, 34.
649. Kewalramani, L. A., Orth, M. S., Saleem, S., and Bertrand, D. (1978/1979). Myelopathy associated with systemic lupus erythematosus (erythema nodosum). *Paraplegia* **16**, 282.
650. Andrianakos, A. A., Duffy, J., Suzuki, M., and Sharp, J. T. (1975). Transverse myelopathy in systemic lupus erythematosus, report of three cases and review of the literature. *Ann. Int. Med*. **83**, 616.
651. Scharf, I., Nahir, M., and Hemli, J. (1977). Transverse myelitis with systemic lupus erythematosus. *J. Neurol*. **215**, 231.
652. Hachen, H. J., and Chantraine, A. (1979/1980). Spinal cord involvement in systemic lupus erythematosus. *Paraplegia* **17**, 337.
653. Sinkovics, J. G., Gyorkey, F., and Thoma, G. W. (1969). A rapidly fatal case of systemic lupus erythematosus: Structures resembling viral nucleoprotein strands in the kidney and activities of lymphocytes in culture. *Texas Rep. Biol. Med*. **27**, 887.
654. Boumpas, D. T., Patrona, S. M. J., Dalakas, M. C., *et al.* (1990). Acute transverse myelitis in systemic lupus erythematosus: Magnetic resonance imaging and review of the literature. *Rheumatology* **17**, 89.
655. Marabani, M., Zoma, A., Hadley, D., and Sturrock, R. D. (1989). Transverse myelitis occurring during pregnancy in a patient with systemic lupus erythematosus. *Ann. Rheum. Dis*. **48**, 160.
656. Kenik, J. G., Krohn, K., Kelly, R. B., *et al.* (1987). Transverse myelitis and optic neuritis in systemic lupus erythematosus: A case report with magnetic resonance imaging finding. *Arthritis Rheum*. **30**, 947.
657. Klaiman, M. D., and Miller, S. D. Transverse myelitis complicating systemic lupus erythematosus: Treatment including hydroxychloroquine. *Am. J. Phys. Med. Rehab*. **72**, 158.
658. Provenzale, J. M., Barboriak, D. P., Gaensler, E. H. L., Robertson, R. L., and Mercer, B. (1994). Lupus-related myelitis: Serial MR findings. *Am. J. Neurorad*. **15**, 1911.
659. Simeon-Aznar, C. P., Tolosa-Vilella, C., Cuenca-Luque, R., Jordana-Comanjuncosa, R., and Ordi-Ros, J. (1992). Transverse myelitis in systemic lupus erythematosus: Two cases with magnetic resonance imaging. *Br. J. Rheumatol*. **31**, 555.
660. Stahl, H. D., Ettlin, T. H., Pleshmann, A., *et al.* (1994). Central nervous system lupus: Concomitant occurrence of myelopathy and cognitive dysfunction. *Clin. Rheumatol*. **13**, 273.
661. Yazawa, S., Kawasaki, S., and Ohi, T. (2001). Development of severe longitudinal atrophy of thoracic spinal cord following lupus-related myelitis. *Int. Med*. **50**, 353.
662. Schanktz, V., Loestergaard, L. L., and Junker, P. (1998). Shrinking spinal cord following transverse myelopathy in a patient with systemic lupus erythematosus and the phospholipid antibody syndrome. *J. Rheumat*. **25**, 1425.
663. Miller, D. H., Buchanan, N., and Barker, G. (1992). Gadolinium-enhanced magnetic resonance imaging of the central nervous system in systemic lupus erythematosus. *J. Neurol*. **239**, 460.
664. Baca, V., Sanchez-Vaca, G., Martinez-Muniz, I., Ramirez-Lacayo, M., and Lavalle, C. (1996). Successful treatment of transverse myelitis in a child with systemic lupus erythematosus. *Neuropediatrics* **27**, 42.
665. Zenone, T., Steineur, M. P., Sibille, M., Durieru, I., and Vital Durand, D. (2000). Myelopathie révélatrice d'un lupus: Deux observations et révue de la littérature. *Rev. Méd. Interne* **21**, 1114.
666. Neumann-Andersen, G., and Lindgren, S. (2000). Involvement of the entire spinal cord and medulla oblongata in acute catastrophic-onset transverse myelitis in SLE. *Clin. Rhematol*. **19**, 156.
667. Liu, G. T., Greene, J. M., and Charness, M. E. (1990). Brown–Sequard syndrome in a patient with systemic lupus erythematosus. *Neurology* **40**, 1474.
668. Markusse, H. M., Haan, J., Tan, W. D., and Breedveld, F. C. (1989). Anterior spinal artery syndrome in systemic lupus erythematosus. *Br. J. Rheumatol*. **28**, 344.
669. Dell'Isola, B., Vidailhet, M., Gatfosse, M., *et al.* (1991). Recovery of anterior spinal artery syndrome in a patient with systemic lupus erythematosus and antiphospholipid antibodies. *Br. J. Rheumatol*. **30**, 314.
670. Maravilla, K. R., Weinreb, J. C., Suss, R., and Nunnally, R. L. (1984). Magnetic resonance demonstration of multiple sclerosis plaques in the cervical cord. *AJNR* **5**, 685.
671. Uldry, P.-A., Regli, F., and Uske, A. (1993). Magnetic resonance imaging in patients with multiple sclerosis

and spinal cord involvement: 28 cases. *J. Neurol.* **240**, 41.
672. Appenzeller, S., Kobayashi, E., Costallat, L. T. L., *et al.* (2000). Magnetic resonance imaging in the evaluation of patients with aseptic meningoencephalitis and connective tissue disorders. *Arq. Neuropsiquiat.* **58**, 45.
673. Arroyo, I. L., Barron, K. I. S., and Brewer, E. J., Jr. (1988). Spinal cord compression by epidural lipomatosis in juvenile rheumatoid arthritis. *Arthritis Rheum.* **31**, 447.
674. Crayton, H. E., Partington, C. R., and Bell, C. L. (1992). Spinal cord compression by epidural lipomatosis in a patient with systemic lupus erythematosus. **35**, 482.
675. Satoh, S., Hirahakata, M., Kameda, H., *et al.* (1998). A case of systemic lupus erythematosus associated with spinal epidural hematoma. *Jpn. J. Clin. Immun.* **21**, 166.
676. Mohazab, H. R., Langer, B., and Spigos, D. (1993). Spinal epidural hematoma in a patient with lupus coagulopathy: MR findings. *Am. J. Roentgen.* **160**, 853.

20

MONITORING PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Lisa A. Mandl
Matthew H. Liang

INTRODUCTION

First described over a thousand years ago [1], systemic lupus erythematosus (SLE) frequently manifested as an acute and fatal disease of the young. However, advances in treatment and management since the early 1970s have been so successful that for a large percentage of patients, lupus is now a chronic disease. This has necessitated changes in the approach to monitoring disease. Preventing cumulative organ damage, treating flares early, and minimizing exposure to potentially toxic drugs are now essential tasks in longitudinal management.

Important types of disease assessment include patient reports of signs and symptoms, immunologic markers, organ-specific indices of physiologic function, nonspecific tests of inflammation, and other composite clinical and laboratory indices. None are perfectly reliable, sensitive, or meaningful by themselves. They should be viewed as complementary methods of determining a patient's clinical status in a sometimes confusing clinical presentation.

Each lupus patient has a unique pattern of disease manifestation and laboratory parameters that are useful in assessing disease activity. Ultimately, the clinical relevance of any parameter must be determined by its correlation with physiologic function and with the patient's symptoms. This chapter reviews methods for monitoring patients with lupus.

Once the diagnosis of SLE has been established, the clinician must work out a plan with the patient for ongoing care. The patient must understand that follow-up is required because lupus can be an unpredictable disease. During follow-up, current disease activity, cumulative end organ damage, and ongoing treatment can be assessed. Regular visits also keep the physician up to date with the patient's life circumstances and help maintain a constructive patient–physician relationship. This is particularly important, as recommendations will not be followed if they are not compatible with the patient's lifestyle. The most effective programs occur when patients are active participants in their own care.

The frequency of follow-up for lupus patients can vary. Once a week to once a fortnight may be necessary for an unstable patient who needs close monitoring. Patients who are stable, with minimal or little disease activity on no medications might be seen twice a year. For those on medication, the frequency of visits is also determined by the need to monitor drug toxicity. Studies defining the epidemiology of disease flares are emerging and suggest that on average, patients have a flare every 1 to 2 years [2]. This underscores the need to equip the patient with information so that they can monitor their disease and alert their physician at the earliest sign of a flare. It also shows the need to follow patients regularly and not simply on an as needed basis.

CLINICAL ASSESSMENT

The objective of clinical assessment is to evaluate the various organ systems that can be affected by lupus. Once it is determined that there might be involvement,

other causes of the symptoms must be ruled out and then, most importantly, the physician must determine whether the symptoms represent reversible or irreversible organ damage. The following discussion is organized by organ systems and includes a suggested history, a description of the most common manifestations, and an approach for assessing disease activity and damage.

Constitutional Symptoms

- "Has there been any unintentional change in your weight over the last month?"
- "How is your energy? How does your fatigue affect you?"
- "Have you had any fever in the last month? Did you take your temperature, or have sweats, chills, or shakes?"

Using a variety of definitions, the prevalence of weight loss in case series ranges from 9 to 71% [2, 3]. Significant weight loss (greater than 10% of the body weight, not due to dieting) is probably rare even in patients with active disease, perhaps because these patients often receive steroids, which increase appetite and weight. Therefore, significant weight loss should not be attributed primarily to SLE, but instead alert one to look for other causes, such as hyperthyroidism, malignancy, chronic infection, malabsorption, or poor intake.

Fatigue or decreased stamina is nearly universal in patients with active lupus. It is the limiting problem for one-third to one-half of patients [4]. Fatigue can be the first manifestation of an impending flare and is frequently the first thing that improves as a lupus flare is controlled. Lupus patients often say that their fatigue is like having the flu all the time or that they hit the wall during the day. One can assess fatigue severity by getting the patient to talk about the activities that are made difficult because of the fatigue. Fatigue that prevents one from doing essential or recreational activities is severe fatigue. The precise pathophysiology is unknown but deconditioning likely plays a role, as aerobic training improves the symptoms [5].

When fatigue is an isolated problem (i.e., not in the setting of a flare), the physician should determine whether it might be due to some other disorder. Depression, deconditioning, steroid myopathy, thyroid disease, and sleep disturbance can cause fatigue that is modifiable by addressing the underlying cause. Fatigue out of proportion to disease activity is often due to fibromyalgia [7]. This "dual diagnosis" is frequently ignored and can result in inappropriate therapy for "unresponsive" SLE. In this situation, directed fibromyalgia treatment might be more effective.

In the febrile lupus patient, infection and other causes of fever must be always be excluded. There is no typical fever pattern of lupus. Fever without chills or rigors or positive cultures in patients with leukopenia, a normal C reactive protein, and serologic evidence of lupus activity, such as elevated double-stranded DNA, points to a lupus flare rather than infection, although there is overlap [8].

Mucocutaneous Manifestations

- "Do you have problems with sunlight? Does it make your lupus worse or bring out a rash?"
- "Do your fingers or toes turn dead white?"
- "Have you noticed more hair on your pillow than usual? Do you have bald spots?"
- "Have you had a rash in the last month?"
- "Have you had any sores in your nose or mouth?"

The cutaneous manifestations of lupus are protean, and can involve the epidermal, dermal, and subdermal layers of the skin.

The American College of Rheumatology (ACR) definition of photosensitivity is "an unusual reaction to sunlight by a patient's history or physician observation" [9]. "Unusual reaction" is a vague term and is not explained more specifically. Photosensitivity rashes are usually mild and transient and therefore are only occasionally observed by the physician. Thus, the history is often the only practical way to ascertain the phenomenon. When various objective measures of photosensitivity are compared to the patient's history, there is considerable disagreement [10]. Our criterion for photosensitivity is that sunlight makes the person's lupus symptoms worse or brings out a rash in both the sun-exposed and the unexposed areas within 24 h after sun exposure. Photosensitivity is not well correlated with disease activity [11] and may be associated with decreased mortality [12]. The severity of a photosensitive rash is judged by four features: the extent, the amount of erythema, the thickness, and the scarring or scarring potential (see Table 1).

Raynaud's phenomenon is seen in roughly one-fourth of patients. Clinically, the phenomenon varies during a patient's course and is usually triggered by clear precipitants such as cold or emotional stress. The classic triphasic response is white, blue, and red corresponding to vasospasm, cyanosis, and reperfusion, but this is relatively uncommon. Most studies label patients as having Raynaud's if their fingers are unusually sensitive to cold and turn at least two colors [13].

Raynaud's phenomenon does not necessarily parallel lupus activity or inflammation, but it may herald a flare in otherwise quiescent disease. However, new

TABLE 1 Mucocutaneous Manifestations as Indicators[a] of Disease Activity

Alopecia	±	Photosensitivity	±
Maculopapular rash	+	Raynaud's	±
Malar erythema	+	Skin vasculitis	+
Oral/nasal ulcers	+	Discoid lesions	+
		Panniculitis	+

[a] +, useful indicator of disease activity; ±, sometimes useful.

TABLE 2 Eye Manifestations as Indicators[a] of Disease Activity

Conjunctivitis sicca	–	Retinal vasculitis	+
Episcleritis	+	Retinal microangiopathy	?
Uveitis/iritis	+	Optic neuritis	?

[a] +, useful indicator of disease activity; –, not useful as indicator of disease activity; ?, unclear usefulness.

onset Raynaud's in a patient over 50 years old may be associated with an occult malignancy and should trigger an age- and sex-appropriate workup [14].

Alopecia in SLE can occur as abnormal patchy or diffuse hair loss with or without scarring. Alopecia may be related to disease activity, but can also be caused by steroid therapy and cytotoxic agents [15]. Alopecia results from disturbed growth of the hair follicles that are actively maturing, not the hair follicles in their quiescent phase. We ask patients if they have observed unusual hair loss on their pillow or if they have any bald spots. When alopecia is apparent by inspection, approximately 50% of the hair is gone and should be rated as severe hair loss. Broken and frayed hairs at the front hairline ("lupus hair") is a pattern of nonscarring alopecia that occurs in SLE and is sometimes apparent on inspection even if the patient denies hair loss.

Oral, nasal, and rarely vaginal mucosa ulcers can be manifestations of active disease. They manifest most commonly as shallow ulcers on the buccal mucosa, hard palate, or in the nose and can be painless or painful. They are usually about 1–2 cm in size, but can be larger and extend into the posterior pharynx [16].

Discoid rash, subacute cutaneous lupus, and acute cutaneous lupus are specific subsets of lupus, and skin inflammation should be used to assess disease activity. However, a myriad of other nonspecific inflammatory skin lesions can occur in SLE, including cutaneous vasculitis, urticaria, livedo reticularis, and hypo/hyperpigmented lesions. Interestingly, the total number of skin lesions, both LE specific and LE nonspecific, appears to correlate with disease activity [17]. Multiple cutaneous lesions may indicate the need for more aggressive systemic therapy.

Ocular Manifestations

- "Have you had problems with your vision?"
- "Have you had any eye pain?"

All structures of the eye can be involved in lupus. Inflammatory lesions of the eye include episcleritis, iritis, retinal vasculitis, cotton wool spots (or cytoid bodies), cranial neuropathies, and optic neuritis (Table 2). Patients with secondary Sjogren's syndrome have keratoconjuctivitis, an inflammatory lesion, secondary to dryness. Classic retinopathy manifests as cotton wool spots and is due either to vasculitis or to accelerated atherosclerosis. Retinopathy can also result from thrombosis due to an associated antiphospholipid syndrome [18]. A baseline fundoscopic examination should be performed on all patients. Retinopathy is related to disease activity, and some believe that it is also correlated with cerebral involvement [19, 20]. Patients without active ocular complaints are unlikely to have an occult sight-threatening disease process [21].

Lymphoreticular Manifestations

- "Have you had swollen glands?"

Lymphadenopathy is a manifestation of immune hyperactivity and can mirror disease activity. Nodes are typically nontender, soft, and range from 0.5 to a few centimeters. Nodes are usually either cervical or axillary, although hilar adenopathy has been described. Generalized lymphadenopathy is rare and usually correlates with disease activity [22]. Splenomegaly is not uncommon.

Cardiopulmonary Manifestations

- "Have you had any problems with breathing or shortness of breath?"
- "Have you had any or chest discomfort or pressure?"

The most common cardiopulmonary manifestations of SLE are caused by inflammation of the serosal surfaces, resulting in pleuritis or pericarditis. Clinical manifestations are classically pain with inspiration and expiration. With a significant effusion, dyspnea may result. As opposed to pleuritis, pericarditis improves with sitting forward and worsens when leaning back. Finding a pleuritic or pericardial rub is not essential, as many patients have symptoms without clear physical findings. Relatively small asymptomatic pleural and

TABLE 3 Cardiopulmonary Manifestations as Indicators[a] of Disease Activity

Pericarditis[b]	+	Pleuritis[b]	+
Myocarditis[b]	+	Pneumonitis[b]	+
Libman–Sacks endocarditis	±	Interstitial lung disease	±
Myocardial infarction	±	Reversible hypoxemia	+

[a] +, useful indicator of disease activity; ± sometimes useful.
[b] After exclusion of infection.

TABLE 4 Musculoskeletal Manifestations as Indicators[a] of Disease Activity

Arthralgia	±
Synovitis	+
Myalgia	+
Myositis	+

[a] +, useful indicator of disease activity; ±, sometimes useful.

pericardial effusions can be demonstrated by a chest X-ray or by echocardiography. Pericardial effusions can cause tamponade but this is fortunately rare [23]. Inflammation of the myocardium leads to myocarditis (Table 3). Myocarditis is rare and is manifested by decreased ejection fraction or arrhythmia. If the outer surface of the endocardium is involved, then pericardial symptoms may also be present.

Pneumonitis and interstitial lung disease are other manifestations of the lung [24]. Pneumonitis is an inflammatory lesion, and interstitial lung disease can be inflammatory and/or fibrotic. Both are manifested by dyspnea. Acute pneumonitis is usually accompanied by fever, cough, and pleuritic pain and occurs primarily in patients with active multisystem involvement from SLE [25]. Diffuse interstitial lung disease may develop slowly at any time during the disease course and does not necessarily reflect disease activity. Most cases develop in patients with long-standing disease and a history of recurrent pleuritic chest pain. Interstitial lung disease may also follow an episode of acute pneumonitis. In the dyspneic patient, it is important to separate active pneumonitis from chronic interstitial changes, as the treatments are very different. A high-resolution computed tomography scan of the chest, performed prone, is the preferred imaging study to distinguish between these two pathologies. Bronchoalveolar lavage can then be performed to identify active alveolar inflammation. Pulmonary hemorrhage, sometimes massive, occurs in the setting of acute hemorrhagic pneumonitis/capillaritis and the mortality is quite high [26]. The pathology suggests acute immune complex deposition with inflammation. The "shrinking lung syndrome" is characterized by unexplained dyspnea, small lung volumes, and an elevated diaphragm. There is a restrictive pattern on pulmonary function testing. It is unclear if the primary defect is diaphragmatic weakness or a restriction in chest wall expansion; whether it is a manifestation of active disease is also unclear [27].

A rare syndrome of acute reversible hypoxemia is characterized by otherwise unexplained hypoxemia without parenchymal lung disease and responds to corticosterold therapy. It is hypothesized to be related to leukocyte aggregation [28, 29].

Gastrointestinal Manifestations

- "Have you had any belly or stomach pain over the last month?"

Gastrointestinal complaints may be due to the involvement of abdominal structures by SLE or concurrent processes such as infection or side effects of drugs. The abdominal manifestations of lupus are principally vasculitis of the small and large bowel, the peritoneal membrane, and the pancreas. The most common is a sterile peritonitis, the presentation of which can range from painless ascites to an acute abdomen. A painful large abdominal accumulation of fluid must always be evaluated for the possibility of infection or tumor. Mesenteric vasculitis is mostly associated with active multisystem disease and can result in ischemia, infarction, and perforation. Patients usually present with cramping or constant abdominal pain, vomiting, and fever [30]. Pancreatitis is another potentially life-threatening gastrointestinal manifestation of SLE. It can be the first presenting symptom, but is not necessarily associated with active systemic disease [31, 32]. Although controversial, small studies do not suggest that pancreatitis is related to treatment with systemic corticosteroids [32]. However, there has been at least one fatal case of cytomegalovirus-associated pancreatitis, which occurred while the patient was being treated with corticosteroids [33]. Chronic intestinal pseudo-obstruction (CIPO) is an uncommon but well-recognized gastrointestinal manifestation of SLE. It is due to inflammation of smooth muscle in the bowel wall and responds to immunosuppressive treatment [34].

Musculoskeletal Manifestations

- "Have you had any pain or swelling of your joints?"
- "Have you had any unusually sore muscles?"

Joint symptoms (either arthritis or arthralgias) are the most common feature of active disease (Table 4).

Muscle involvement can occur either as a symptomatic myopathy or as an asymptomatic elevation of muscle enzymes. Both joint and muscle symptoms respond to steroid therapy. To be sure that the symptoms are related to lupus activity, one must exclude other causes, such as fibromyalgia, steroid-induced myopathy, or plaquenil-induced myopathy [35].

Neuropsychiatric Manifestations

- "Have you had any problems with coordination, weakness, or numbness?"
- "Have you had loss of consciousness or seizures?
- "Any difficulties with thinking, concentration, or memory?"
- "Any problems with your mood?"

The ACR has developed standardized nomenclature to define neuropsychiatric lupus syndromes [36]. Up to 90% of patients have some type of central nervous system (CNS) disease [37]. Seizures, psychosis, and aseptic meningitis are the most classic associations. Other neurologic and psychiatric conditions also seen in patients with SLE include headaches, demyelinating disorders, chorea, mood disorders, neuropathies cerebrovascular disease, cognitive dysfunction, and transverse myelopathies, among others [37–39] (Table 5). Whenever any of these manifestations occur, one must always look for other causes, such as tumor, infection, metabolic derangements, and medications, as well as looking for evidence of other organ involvement. However, these manifestations do not *a priori* reflect inflammatory disease activity, as they can also be associated with vascular occlusions, especially in the presence of antiphospholipid antibodies. It is vital to evaluate antiphospholipid antibody status in all SLE patients with neurologic signs [40–43].

TABLE 5 Neuropsychiatric Manifestations[a] as Indicators[b] of Disease Activity

Aseptic meningitis	+	Mononeuritis	±
Cerebrovascular disease	±	Neuropathies (autonomic, cranial, peripheral	±
Cognitive deficits	±	Movement disorder	±
Demyelinating syndrome	±	Plexopathy	±
Encephalopathy/delirium	±	Psychosis	+
Guillain Barré	±	Seizures	+
Headache	±	Transverse myelopathy	±
Mood disorder	±		

[a] Infection, malignancy, and drug toxicity must be excluded.
[b] +, useful indicator of disease activity; ±, sometimes useful.

To assess whether the neuropsychiatric symptom is due to active CNS inflammation, a cerebrospinal fluid sample should be obtained and tested for complete blood cell count, protein, glucose, gram stain, IGG index, and oligoclonal banding. Imaging techniques may be useful to define site and extent of the lesions, and magnetic resonance imaging may be able to distinguish damage due to active inflammation from damage due to previous disease [44]. The evaluation of cerebrovascular disease may include standard arteriograms or subtraction angiography or magnetic resonance angiography. Standard electroencephalography (EEG) should be performed in the diagnostic workup of seizures and encephalopathics. SPECT, PET, and related imaging technologies, as well as quantitative EEG, are considered research tools only [45]. Immunochemical tests for CNS lupus such as antibodies to various neuronal constituents such as glial, sphingomyclin, neurofilament, ribosomal P, neuronal, ganglioside, and galactocerebroside and antilymphocytotoxic antibodies are also investigational [46]. Subsets of anti-DNA antibodies may mediate neuronal death via an apoptotic pathway [47].

LABORATORY MONITORING

Hematologic

Leukopenia, lymphocytopenia, and thrombocytopenia are features of active SLE (although one must always ensure other causes, such as drugs or sepsis, have been excluded) (Table 6). Thus, a complete blood count, including a differential, should be performed regularly.

Hemolytic anemia, which can be Coombs' positive, indicates active disease. The Coombs' test, however, is frequently positive without evidence of active hemolysis and is not a good test of disease activity by itself [48]. Neither iron deficiency anemia nor anemia of chronic disease in isolation is indicative of active SLE. Lymphopenia may also predict flares [49]. Marked thrombocytopenia associated with active SLE may need treatment. A reduction in the platelet count has been observed in more than 20% of all SLE patients [4],

TABLE 6 Hematologic Manifestations as Indicators[a] of Disease Activity

Hemolytic anemia	+
Leucocytopenia	+
Thrombocytopenia	+

[a] +, useful indicator of disease activity.

TABLE 7 Renal Parameters as Indicators[a] of Disease Activity

Hematuria[b]	+
Pyuria[b]	±
Casts (WBC, RBC)	+
Proteinuria[c]	±
Creatinine clearance	±

[a] +, useful indicator of disease activity; ±, sometimes useful. WBC, white blood cells; RBC, red blood cells.
[b] In the absence of infection.
[c] In the absence of renal failure.

usually as a consequence of antiplatelet antibodies, and can be used as a parameter to assess the response to therapy [50]. The combination of thrombocytopenia and hemolytic anemia should always raise the possibility of thrombotic–thrombocytopenic purpura, which can occur in the setting of active and inactive SLE [51].

Renal

Lupus nephritis is often asymptomatic. Signs of inflammatory renal disease and functional impairment should be looked for on a regular basis (Table 7). The urinary sediment is the best tool for assessing active inflammatory renal disease [52].

Semiquantative measurements of hematuria and cellular casts are important tools for monitoring lupus nephritis, especially when done at a dedicated laboratory. Outpatient urine samples should be the second void of the day, collected while the patient is fasting [53]. In patients who are receiving cyclophosphamide, urine microscopy is important in following patients to differentiate hematuria due to glomerular disease from dysmorphic urinary red cells due to cyclophosphamide-induced cystitis or urothelial malignancies.

Proteinuria is an independent risk factor for progressive renal injury in patients with lupus nephritis [54]. However, during flares of lupus nephritis, worsening proteinuria does not predict worse renal outcomes [55]. Quantitative urinary protein may increase with physical activity or decrease because of declining glomerular filtration or treatment with drugs that alter renal blood. Twenty-four-hour urine collections remain the gold standard measurement of urinary protein excretion and should be performed on at least a yearly basis.

Pyuria without hematuria or proteinuria requires that infection be excluded, although it can be a sign of active renal or systemic SLE [56]. Impaired renal function is associated with substantial renal tissue damage from inflammation over time, and therefore spot checks of creatinine and creatinine clearance are not necessarily good gauges of renal disease activity. However, lupus flares associated with a rapid >30% increase in creatinine and an active nephritic urinary sediment have worse outcomes than nephritic flares without changes in creatinine [53]. A sustained doubling of the serum creatinine is a well-accepted outcome measure that reflects at least a 50% fall in the glomerular filtration rate and predicts end stage renal disease in lupus nephritis [53]. In the future, following proinflammatory cytokines such as tumor necrosis factor [57] or urinary excretion of vascular endothelial growth factor [58] may provide more accurate ways of monitoring renal disease over time.

Autoantibodies

The variety of autoantibodies observed in lupus are described elsewhere in this book. Their relationship with disease activity is often difficult to assess: the majority of studies have been retrospective and cross-sectional. Prospective, longitudinal studies, in which immunochemistry is done independent of patient complaints, are limited [59].

Autoantibody testing is often poorly standardized, despite ongoing efforts to establish guidelines and to calibrate measurements by using reference sera [60–63]. These considerations might explain inconsistent findings.

A positive antinuclear antibody is the sine qua non for the diagnosis of SLE. "ANA-negative lupus," a syndrome of clinical lupus without typical autoantibodies, was thought previously to comprise up to 5% of all SLE cases. However, this is now no longer considered a true entity. The phenomenon of "ANA-negative lupus" was based on less sensitive assay techniques and on cross-sectional data, where ANA titers can vary over time and become negative with treatment. Using assays based on human epithelial cells, virtually all patients with clinical SLE have positive extractable nuclear antigens, even if their ANA is negative, and almost all ANA-negative patients will have had a previously positive ANA [64]. However, once documented, following the titers of antinuclear antibody is not helpful in assessing disease activity. Similarly, specific antibodies, such as anti-Ro (anti SSA), anti-La (anti SSB), anti-SM, anti-RMP, and anticardiolipin antibodies, are also useful for diagnosis, but do not help in assessing whether the disease is active [65–69].

The exception to this rule is that antibodies against double-stranded DNA can be useful in assessing disease activity, especially lupus nephritis [70–71]. Several prospective studies demonstrate increasing anti-dsDNA titers preceding clinical flares [71–74]. In a randomized

controlled trial, increasing the prednisone dose in patients with rising anti-dsDNA titers reduced the rate of clinical flares significantly [75]. For quantitative measurements of dsDNA, the Farr assay is superior to the *Crithidia luciliae* test [74].

However, for some patients, the rise and fall of dsDNA do not correlate with clinical events, and persistently high titers are not predictive of flares [60, 76, 77]. Given this degree of individual variability, one should correlate each patient's antibody titers before and after a clinical flare, as that is probably the best way to evaluate whether it can be used legitimately in therapeutic decision making in a given patient.

Immune Complexes

Immune complexes are present in SLE and can be measured using a variety of techniques [78–80]. These tests are not well standardized, and the relationship between immune complexes and disease activity is variable. Immune complex measurement is not part of standard lupus monitoring.

Complement Components

In several studies, clinical lupus exacerbations were preceded or accompanied by falls in complement levels [70, 73, 77]. In some studies, only renal and hematologic flares are associated with decreased complement levels [81]. The total hemolytic complement or CH50 is a functional assay that incorporates the entire complement cascade, but can be normal even when one or more of its components are low. C3 and C4 are not measured by functional assays. CH50 and C4 tend to decrease before clinical signs of disease activity occur, whereas C3 often continues to decline during the height of clinical flare [82]. In patients with active nephritis or vasculitis, C4 levels are often more depressed than C3 levels. However, C3 levels are more predictive of inactive renal disease [77, 82]. Some authors feel that complement levels are more useful in monitoring SLE activity than anti-dsDNA [70, 72], whereas others preferred the latter [74]. As with anti-dsDNA, the association of complement levels and disease activity varies from patient to patient and has to be assessed on an individual basis.

If a patient has a markedly depressed total hemolytic complement, it may be due to an inherited complement deficiency, and therefore following complement is not useful in assessing activity.

Acute-Phase Reactants

The erythrocyte sedimentation rate (ESR) is a sensitive but nonspecific measure of disease activity. It is frequently elevated during flares [59], but does not always mirror the degree of activity and can be elevated in a myriad of other conditions, including infections, malignancies, and paraproteinemias. The role of C-reactive protein (CRP) in monitoring lupus activity has been subject to controversy. In some studies, CRP did not increase during clinical exacerbations [83, 84]. Persistent, mildly elevated levels have been reported in lupus patients with Jaccoud's arthropathy [85]. High CRP levels (>60 mg/liter) are observed in SLE patients with intercurrent infections [86, 87] and in lupus-associated serositis [88]. Mean CRP levels were found to be significantly higher in lupus patients with infections than in those without infections [89], but the range of values overlapped considerably, which means its discriminative value is poor [90]. In newly diagnosed SLE patients, proinflammatory cytokines such as serum interleulan (IL)-1β, IL-6, and tumor necrosis factor-α levels do not correlate with CRP levels, although levels of IL-1ra, another proinflammatory cytokines, do correlate [91]. These data underscore the fact that CRP is not a simple marker of inflammation in lupus. In general, CRP appears to be neither sufficiently sensitive nor specific to be useful in monitoring SLE activity.

DISEASE ACTIVITY INDICES

By the 1980s, there were over 60 different scoring systems for SLE disease activity [59]. A few had been examined for their measurement properties but none of them were widely used. Since then a number of semiquantitative standardized indices, including both clinical and laboratory parameters, have been validated and are used in clinical research [92–95] (see Table 8).

The role of these indices in clinical practice has yet to be determined. Some of these instruments require immunologic testing or specific physical examinations, such as eye examination for cytoid bodies, which are not routine. Nevertheless, they provide an important mnemonic function, may better reflect patients' concerns, and perhaps are more appropriate end points than immunochemical abnormalities [96]. Indeed, studies comparing immunochemical testing to these measures are a way of validating the clinical importance of any immunochemical test. This standardization process should improve the reporting of clinical trials, the ability to compare studies, the conduct of multicenter studies, and the ability to do qualitative and quantitative syntheses of reported trials.

To some degree, these new measures are interchangeable [97]. In addition, with the advent of new biologic agents and the promise of more specific immunologic treatment of lupus, the American Food

TABLE 8 Disease Activity Indices in SLE

Index	Length	Scale	Immunologic testing required[a]	Sensitivity	Notes
BILAG	++++	Categorical	–	+++	Computerized scoring
SLAM	++	Interval	–	++++	Optically scannable
SLEDAI	+	Interval	+	++	
ECLAM	+	Interval	+	?	

[a] Anti-dsDNA and/or Complement.

and Drug Administration and the American College of Rheumatology are developing a priori response criteria, of which one will be an index of disease activity. The measures are discussed in the order of their development.

The **BILAG** instrument was the first of the modem instruments to assess lupus disease activity [98]. The scale, as conceived by its developers, is categorical and reflects an intention to treat eight organ systems separately. It does not easily permit the use of a single numeric value to summarize the overall disease activity. The ratings can be transformed into a continuous scale [99]. The scoring system is complex, varying across organ systems, although a special computer program is available to facilitate scoring. Some of the items, such as lung biopsy, are collected infrequently, leading to relatively high rates of missing data. However, it is a comprehensive instrument, including most signs and symptom that can be seen in lupus. In some studies, BILAG is the most sensitive instrument [95].

The **SLAM** [92] and the current version SLAM-R is being used in the United States, several European countries, Latin America, and Asia. SLAM-R has a more reliable rating of the cardiopulmonary categories and has made laboratory reporting more consistent. SLAM falls between the BILAG and shorter instruments such as SLEDAI and ECLAM in length and is optically scannable with a thorough instruction booklet to guide raters. It can be scored on the spot and does not include expensive immunochemical tests. Each item is graded for severity to quantify changes over time, to better reflect the wide clinical range of SLE. Some investigators believe that the inclusion of milder constitutional symptoms might give undue weight to symptoms that are not specific to lupus. However, like all other instruments, SLAM is administered by expert clinicians who are asked to exclude manifestations not due to lupus. Including constitutional symptoms is useful in covering the entire spectrum of lupus activity, avoiding floor effects, and improving sensitivity to change and to symptoms that matter to patients.

The **SLEDAI** is a short instrument based on multivariate modeling of case scenarios and expert ratings [94]. All variables are assigned organ-specific weights. The instrument includes immunologic tests, and the severity of clinical manifestations is not considered. The advantage of SLEDAI is the comparatively small number of items and the ease of scoring. It may be the most widely used. The lack of severity grading reduces its sensitivity and its usefulness in assessing disease activity over time [92, 95, 100, 101]. The exclusion of mild and moderate clinical symptoms results in a floor effect, in that patients with mild disease cannot be distinguished from patients with an active, organ-threatening disease. In a trial evaluating the safety of hormone replacement therapy and oral contraceptives on SLE, the SLEDAI was insensitive to flares and, as a consequence, was modified to the SELENA-SLEDAI, but it has not yet been extensively validated [2].

ECLAM is a short instrument developed by a multinational group of European rheumatologists [95]. Manifestations are grouped by organ systems and are assigned different weight factors. Disease severity is recorded, but evolving or worsening manifestations are given extra points. The instrument requires complement tests.

ASSESSMENT OF ORGAN DAMAGE

The cumulative inflammatory process of SLE can result in irreversible organ damage, which can be rated with the ACR/SLICC damage index. It has been used primarily for research. The instrument has been shown to be reliable and prognostically important [102]. Early damage as reflected by the initial SLICC/ACR damage index is associated with a higher rate of mortality [103, 104].

MONITORING DRUG TOXICITY

An important task when following a patient with SLE is to monitor the drugs used in treatment for potential toxicity and long-term adverse events. In general, this includes the effects of medications on the

TABLE 9 Recommended Monitoring Strategy for Drugs Used Commonly in Systemic Lupus Erythematosus[a]

Drug	Toxicities requiring monitoring	Baseline evaluations[b]	Monitoring	
			System review	Laboratory
Salicylates, nonsteroidal anti-inflammatory drugs	Gastrointestinal bleeding, hepatic toxicity, renal toxicity, hypertension	CBC, creatinine, urinalysis AST, ALT	Dark/black stool, dyspepsia, nausea/vomiting, abdominal pain, shortness of breath, edema	CBC yearly, creatinine yearly
Glucocorticoids	Hypertension, hyperglycemia, hyperlipidemia, hypokalemia, osteoporosis, avascular necrosis, cataract, weight gain, infections, fluid retention	BP, bone densitometry, glucose, potassium, cholesterol, triglycerides (HDL, LDL)	Polyuria, polydipsia, edema, shortness of breath, BP at each visit, visual changes, bone pain	Urinary dipstick for glucose every 3–6 months, total cholesterol yearly, bone densitometry yearly to assess osteoporosis
Hydroxychloroquine	Macular damage	None unless patient is over 40 years of age or has previous eye disease	Visual changes	Funduscopic and visual fields every 6–12 months
Azathioprine	Myelosuppression, hepatotoxicity, lymphoproliferative disorders	CBC, platelet count, creatinine, AST or ALT	Symptoms of myelosuppression	CBC and platelet count every 1–2 weeks with changes in dose (every 1–3 months thereafter), AST yearly, Pap test at regular intervals
Cyclophosphamide	Myelosuppression, myeloproliferative disorders, malignancy, immunosuppression, hemorrhagic cystitis, secondary infertility	CBC and differential and platelet count, urinalysis	Symptoms of myelosuppression, hematuria, infertility	CBC and urinalysis monthly, urine cytology and Pap test yearly for life
Methotrexate	Myelosuppression, hepatic fibrosis, cirrhosis, pulmonary infiltrates, fibrosis	CBC, chest radiograph within past year, hepatitis B, C serology in high-risk patients, AST, albumin, bilirubin, creatinine	Symptoms of myelosuppression, shortness of breath, nausea/vomiting, oral ulcer	CBC and platelet count every 4–8 weeks, AST or ALT every 4–8 weeks, albumin every 4–8 weeks, serum creatinine, urinalysis

[a] From Rahman *et al*. [104].
[b] CBC, complete blood cell count; AST, aspartate transaminase; ALT, alanine transaminase; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

hematopoetic system, renal function, liver function, and surveillance for malignancies. Establishing the optimal frequency for monitoring patients and selecting the most sensitive tests to use are difficult because many side effects are rare or idiosyncratic. Developing rational monitoring guidelines based completely on evidence is not possible as only a few studies address these issues. In an effort to develop practice guidelines, the ACR has reviewed the pertinent literature and made recommendation for monitoring strategies for drugs used commonly in lupus [105]. One should note that the recommendations reproduced in Table 9 are only guidelines, which must be individualized for specific patients.

Acknowledgments

L. Mandl is supported by a Clinical Investigator Fellowship Award from the American College of Rheumatology, and M. Liang is supported by the Kirkland Scholar Award and NIH Grant P60 AR47782

References

1. Wallace, D. J., and Lyon, I. (1999). Pierre Cazenave and the first detailed modern description of lupus erythematosus. *Semin. Arthritis Rheum*. **28**, 305–313.
2. Petri, M., Buyon, J., and Kim, M. (1999). Classification and definition of major flares in SLE clinical trials. *Lupus* **8**, 685–691.

3. McGehee Harvey, A., Shulman, L. E., Tumulty, A. P., Lockard Conley, C., and Schocnrich, E. H. (1954). Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine* **33**, 291–437.
4. Vitali, C., Bencivelli, W., Idenberg, D. A., Smolen, J. S., Snaith, M. L., Scluto, M., D'Ascanio, A., Bombardlerl, S., European Consensus Study Group for Disease Activity in SLE (1992). Disease activity in systemic lupus erythematosus: Report of the Consensus Study Group of the European Workshop for Rheumatology Research. *Clin. Exp. Rheumatol.* **10**, 527–539.
5. Krupp, L. B., LaRocca, N. G., Muir, J., and Steinberg, A. D. (1990). A study on fatigue in systemic lupus erythematosus. *J. Rheumatol*. **17**, 1450–1452.
6. Robb-Nichsolson, N., Daltroy, L., Eaton, H., Gall, V., Wright, E., Hartley, L. H., Schur, P., and Liang, M. H. (1989). Effects of aerobic conditioning in lupus fatigue. *Br. J. Rheumatol.* **28**, 500–505.
7. Wang, B., Gladman, D. D., and Urowitz, M. B. (1998). Fatigue in lupus is not correlated with disease activity. *J. Rheumatol.* **25**, 892–895.
8. Stahl, N. I., Klippel, J. H., and Decker, J. L. (1979). Fever in systemic lupus erythematosus. *Am. J. Med.* **67**, 935–940.
9. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N., and Winchester, R. J. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. **25**, 1271–1277.
10. Doria, A., Biasimutto, C., Glurardello, A., Satorl, E., Rondinone, R., Piccoli, A., Fomassa, C. V., and Gambari, P. F. (1996). Photosensitiviy in systemic lupus erythematosus: Laboratory testing of ARA/ACR definition. *Lupus* **5**, 263–268.
11. Wysenbeek, A. J., Block, D. A., and Fries, J. F. (1989). Prevalence of expression of photosensitivity in systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**, 461–463.
12. Jacobsen, S., Petersen, J., Ullman, S., *et al.* (1998). A multicentre study of 513 Danish patients with systemic lupus erythematosus. II. Disease mortality and clinical factors of prognostic value. *Clin. Rheumatol*. **17**, 478–484.
13. Brennan, P., Silman, A., Black, C., *et al.* (1993). Validity and reliability of three methods used in the diagnosis of Raynaud's phenomenon: The UK Scleroderma Study Group. *Br. J. Rheumatol.* **32**, 357–361.
14. Naschitz, J. E., Rosner, I., Rozenbaum, M., Zuckerman, E., and Yeshurun, D. (1999). Rheumatic syndromes: Clues to occult neoplasia. *Semin. Arthritis Rheum.* **29**, 43–55.
15. Wysenbeek, A. J., Ecibovici, L., Amit, M., and Weinberger, A. (1991). Alopecia in systemic lupus: Relation to disease manifestations. *J. Rheumatol.* **18**, 1185–1186.
16. Andreason, J. O. (1964). Oral manifestations in discoid and systemic lupus erythematosus. *Acta Odontol. Scand*. **22**, 295–310.
17. Zecevic, R. D., Vojvodic, D., Ristic, B., Pavlovic, M. D., Stefanovic, D., and Karadaglic, D. (2001). Skin lesions: An indicator of disease activity in systemic lupus erythematosus? *Lupus* **10**, 364–367.
18. Giorgi, D., Pace, F., Giorgi, A., Bonomo, L., and Gabrieli, C. B. (1999). Retinopathy in systemic lupus erythematosus: Pathogenesis and approach to therapy. *Hum. Immunol.* **60**, 688–696.
19. Klinkhoff, A. F. V., Beattie, C. W., and Chalmers, A. (1986). Retinopathy in SLE: Relationship to disease activity. *Arthritis Rheum*. **27**, 1152–1156.
20. Jabs, D. A., Fine, S. L., Hochberg, M. C., Newman, S. A., Heiner, G. G., and Stevens, M. B. (1986). Severe retinal vaso-occlusive disease in systemic lupus erythematosus. *Arch. Ophthalmol*. **104**, 558–563.
21. Soo, M. P., Chow, S. K., Tan, C. T., Nadior, N., Yeap, S. S., and Hoh, H. B. (2000). The spectrum of ocular involvement in patients with systemic lupus erythematosus without ocular symptoms. *Lupus* **9**, 511–514.
22. Kitsanou, M., Andreopoulou, E., Bai, M. K., Elisaf, M., and Drosos, A. A. (2000). Extensive lymphadenopathy as the first clinical manifestation in systemic lupus erythematosus. *Lupus* **9**, 140–143.
23. Moder, K. G., Miller, T. D., and Tazelaar, H. D. (1999). Cardiac involvement in systemic lupus erythematosus. *Mayo. Clin. Proc.* **74**, 275–284.
24. Cheema, G. S., and Quismorio, F. P., Jr. (2000). Interstitial lung disease in systemic lupus erythematosus. *Curr. Opin. Pulm. Med.* **6**, 424–429.
25. Ban'le, L. A., Jara, L. J., Medina-Rodriguez, F., Garvia-Figueroa, J. L., and Miranda-Limoll, J. M. (1997). Pulmonary hemorrhage in systemic lupus erythematosus. *Lupus* **6**, 445–448.
26. Rubin, L. A., and Urowitz, M. B. (1983). Shrinking lung syndrome in SLE: A clinical pathologic study. *J. Rheumatol.* **10**, 973–976.
27. Warrington, K. J., Moder, K. G., and Brutinel, W. M. (2000). The shrinking lungs syndrome in systemic lupus erythematosus. *Mayo. Clin. Proc.* **75**, 467–472.
28. Abramson, S. B., Dobro, J., Ebark, M. A., Benton, M., Reibman, J., Epstein, H., Rapoport, D. M., Beirnotit, H. M., and Goldring, R. M. (1991). Acute reversible hyperemia in systemic lupus erythematosus. *Ann. Intern. Med.* **114**, 941–947.
29. Martinez-Taboada, V. M., Blanco, R., Annona, J., Femandez-Suelro, J. L., and Rodriguez-Valluerde, V. (1995). Acute reversible hyperemia in systemic lupus erythematosus: A new syndrome or an index of disease activity? *Lupus* **4**, 259–262.
30. Zizlc, T. M., Classen, J. N., and Stevens, M. B. (1982). Acute abdominal complications of systemic lupus erythematosus and polyarteritis nodosa. *Am. J. Med.* **73**, 525–531.
31. Singh, M., Wani, S., Murtaza, M., Joglekar, S., and Kasubhai, M. (2001). Systemic lupus erythematosus presenting with acute fatal pancreatitis as an initial manifestation. *Am. J. Gastroenterol.* **96**, 2280–2281.
32. Saab, S., Corr, M. P., and Weisman, M. H. (1998). Corticosteroids and systemic lupus erythematosus pancreatitis: A case series. *J. Rheumatol.* **25**, 801–806.
33. Ikura, Y., Matsuo, T., Ogami, M., *et al.* (2000). Cytomegalovirus associated pancreatitis in a patient

with systemic lupus erythematosus. *J. Rheumatol.* **27**, 2715–2717.
34. Hill, P. A., Dwyer, K. M., and Power, D. A. (2000). Chronic intestinal pseudo-obstruction in systemic lupus erythematosus due to intestinal smooth muscle myopathy. *Lupus* **9**, 458–463.
35. Richards, A. J. (1998). Hydroxychloroquine myopathy. *J. Rheumatol.* **25**, 1642–1643.
36. The American College of Rheumatology (1999). Nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum.* **42**, 599–608.
37. Ainiala, H., Loukkola, J., Peltola, J., Korpela, M., and Hietaharju, A. (2001). The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. *Neurology* **57**, 496–500.
38. Feinglass, E. J., Arnett, F. C., Dorsch, C. A., Zizic, T. M., and Stevens, M. B. (1976). Neuropsychiatric manifestations of systemic lupus erythematosus: Diagnosis, clinical spectrum, and relationship to other features of the disease. *Medicine* **55**, 323–339.
39. Abel, T., Gladman, D. D., and Urowitz, M. B. (1980). Neuropsychiatric lupus. *J. Rheumatol.* **7**, 325–332.
40. Boumpas, D. T., Austin, H. A., Fessler, B. J., Balow, J. E., Klippel, J. H., and Lockshin, M. D. (1995). Systemic lupus erythematosus: Emerging concepts. 1. Renal, neuropsychiatric, cardiovascular, pulmonary and hematologic disease. *Ann. Intern. Med.* **122**, 940–950.
41. Mitsia, P., and Levine, S. R. (1994). Large cerebral vessel occlusive disease in systemic lupus erythematosus. *Neurology* **44**, 385–393.
42. Brey, R. L., Gharavi, A. E., and Lockshin, M. D. (1993). Neurologic complications of antiphospholipid antibodies. *Rheum. Dis. Clin. North Am.* **19**, 833–850.
43. Toubi, E., Khamashta, M. A., Panarra, A., and Hughes, G. R. V. (1995). Association of antiphospholipid antibodies with central nervous system disease in systemic lupus erythematosus. *Am. J. Med.* **99**, 397–401.
44. Huizinga, T. W., Steens, S. C., and van Buchem, M. A. (2001). Imaging modalities in central nervous system systemic lupus erythematosus. *Curr. Opin. Rheumatol.* **13**, 383–388.
45. Axford, J. S., Howe, F. A., Heron, C., and Griffiths, J. R. (2001). Sensitivity of quantitative (1)H magnetic resonance spectroscopy of the brain in detecting early neuronal damage in systemic lupus erythematosus. *Ann. Rheum. Dis.* **60**, 106–111.
46. Sanna, G., Piga, M., Terryberry, J. W., *et al.* (2000). Central nervous system involvement in systemic lupus erythematosus: Cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. *Lupus* **9**, 573–583.
47. DeGiorgio, L. A., Konstantinov, K. N., Lee, S. C., Hardin, J. A., Volpe, B. T., and Diamond, B. (2001). A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nature Med.* **7**, 1189–1193.
48. Heddle, N. M., Kelton, J. G., Turchyn, K. L., and Ali, M. A. M. (1988). Hypergammaglobutineamia can be associated with a positive direct antiglobulin test, a non-reactive cluate and no evidence of hemolysis. *Transfusion* **28**, 29–33.
49. Mirzayan, M. J., Schmidt, R. E., and Witte, T. (2000). Prognostic parameters for flare in systemic lupus erythematosus. *Rheumatology (Oxford)* **39**, 1316–1319.
50. Clark, W. F., Linton, A. L., Cordy, P. E., Keown, P. E., Lohmann, R. C., and Lindsay, R. M. (1980). Immunologic findings, thrombocytopenia and disease activity in systemic lupus erythematosus. *Can. Med. J.* **8**, 1191–1195.
51. Stricker, R. B., Davis, J. A., Gershow, J., Yamamoto, K. S., and Kiprov, D. D. (1992). Thrombotic thrombocytopenic purpura complicating systemic lupus erythematosus: Case report and literature review from the plasmapheresis *cra. J. Rheumatol.* **19**, 1469–1473.
52. Herbert, L. A., Dillon, J. J., Middendorf, D. F., Lewis, E. J., and Peter, J. B. (1995). Relationship between appearance of urinary red blood cell/white blood cell casts and the onset of renal relapse in systemic lupus erythematosus. *Am. J. Kidney Dis.* **26**, 432–438.
53. Boumpas, D. T., and Balow, J. E. (1998). Outcome criteria for lupus nephritis trials: A critical overview. *Lupus* **7**, 622–629.
54. Clark, W. F., and Moist, L. M. (1998). Management of chronic renal insufficiency in lupus nephritis: Role of proteinuria, hypertension and dyslipidemia in the progression of renal disease. *Lupus* **7**, 649–653.
55. Moroni, G., Quaglini, S., Maccario, M., Banfi, G., and Ponticelli, C. (1996). "Nephritic flares" are predictors of bad long-term renal outcome in lupus nephritis. *Kidney Int.* **50**, 2047–2053.
56. Rahman, P., Gladman, D. D., Ibanez, D., and Urowitz, M. B. (2001). Significance of isolated hematuria and isolated pyuria in systemic lupus erythematosus. *Lupus* **10**, 418–423.
57. Honkanen, E., von Willebrand, E., Teppo, A. M., Tornroth, T., and Gronhagen-Riska, C. (1998). Adhesion molecules and urinary tumor necrosis factor-alpha in idiopathic membranous glomerulonephritis. *Kidney Int.* **53**, 909–917.
58. Honkanen, E. O., Teppo, A. M., and Gronhagen-Riska, C. (2000). Decreased urinary excretion of vascular endothelial growth factor in idiopathic membranous glomerulonephritis. *Kidney Int.* **57**, 2343–2349.
59. Liang, M. H., Socher, S. A., Roberts, W. N., and Esdaile, J. M. (1988). Measurement of systemic lupus erythematosus activity in clinical research. *Arthritis Rheum.* **31**, 817–825.
60. Esdaile, J. M., Abrahamowicz, M., Joseph, L., MacKenzie, T., Li, Y., and Danoff, D. (1996). Laboratory tests as predictors of disease exacerbations in systemic lupus erythematosus: Why some tests fail. *Arthritis Rheum.* **39**, 370–378.
61. Smolen, J. S., Steiner, G., and Tang, E. M. (1997), Standards of care: The value and importance of standardization. *Arthritis Rheum.* **40**, 410–412.
62. Smolen, J. S., Butcher, B., Fritzler, M. J., Gordon, T., Hardin, J., Kalden, J. R., Lahita, R., Maini, R. N., Reeves, W., Reichlin, M., Rothfield, N., Takasaki, J., VanVenrooi'

W. J., and Tan, E. M. (1997). Reference sera for antinuclear antibodies. II. Further definition of antibody specificities in international antinuclear antibody reference sera by immunofluorescence and Western blot. *Arthritis Rheum.* **40**, 413–418.
63. Egner, W. (2000). The use of laboratory tests in the diagnosis of SLE. *J. Clin. Pathol.* **53**, 424–432.
64. Thomson, K. F., Murphy, A., Goodfield, M. J., and Misbah, S. A. (2001). Is it useful to test for antibodies to extractable nuclear antigens in the presence of a negative antinuclear antibody on Hep-2 cells? *J. Clin. Pathol.* **54**, 413.
65. Spronk, P. E., Limburg, P. C., and Kallenberg, C. G. M. (1995). Serological markers of disease activity in systemic lupus erythematosus. *Lupus* **4**, 86–94.
66. Gompertz, N. R., Isenberg, D. A., and Turner, B. M. (1990). Correlation between clinical features of systemic lupus erythematosus and levels of anti-histone antibodies of the IgG, IgA, and IgM isotypes. *Ann. Rheum. Dis.* **49**, 524–557.
67. Derksen, R. H. W. M., and Mcilof, J. F. (1992). Anti-Ro/SSA and anti-La/SSB autoantibody levels in relation to systemic lupus erythematosus disease activity and congenital heart block: A longitudinal study comprising two consecutive pregnancies in a patient with systemic lupus erythematosus. *Arthritis Rheum.* **35**, 953–959.
68. Buyon, J. P. (1993). Congenital complete heart block. *Lupus* **2**, 291–295.
69. Out, H. J., van Vliet, M., degroot, P. G., and Derksen, R. H. (1992). Prospective study of fluctuations of lupus anticoagulant activity and anticardiolipin antibody titre in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 353–357.
70. Lloyd, W., and Schur, P. H. (1981). Immune complexes, complement, and anti-DNA in exacerbations of systemic lupus erythematosus (SLE). *Medicine* **60**, 201–217.
71. Ho, A., Magder, L. S., Barr, S. G., and Petri, M. (2001). Decreases in anti-double-stranded DNA levels are associated with concurrent flares in patients with systemic lupus erythematosus. *Arthritis Rheum.* **44**, 2342–2349.
72. Miniter, M. F., Stollar, B. D., and Agnello, V. (1979). Reassessment of the clinical significance of native DNA antibodies in systemic lupus crythematosus. *Arthritis Rheum.* 22. 959–968.
73. Swaak, A. J. G., Groenwold, J., and Bronsveld, W. (1986). Predictive value of complement profiles and anti-dsDNA in systemic lupus erythematosus. *Ann. Rheum. Dis.* **45**, 359–366.
74. TerBorg, E. J., Horst, G., Hummel, E. J., Limburg, P. C., and Kallenberg, C. G. M. (1990). Measurement of increases of anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus eryhtematosus: A long-term, prospective study.
75. Bootsma, H., Spronk, P., Derksen, R., deboer, G., Wolters-Dicke, H., Hen-nans, J., Limburg, P., Gmell g-Meyling, F., Kater, L., and Kallenberg, C. (1995). Prevention of relapses in systemic lupus crythematosus. *Lancet* **345**, 1595–1599.
76. LeBlanc, B. A., Gladman, D. D., and Urowitz, M. B. (1994). Serologically active clinically quiescent systemic lupus erythematosus: Predictors of clinical flares. *J. Rheum.* **21**, 2239–2241.
77. Swaak, A. J. G., Aarden, L. A., Statius van Eps, L. W., and Feltkamp, T. E. W. (1979). AntidsDNA and complement profiles as prognostic guide in systemic lupus crythematosus. *Arthritis Rheum.* **22**, 226–235.
78. Nezlin, R. (2000). A quantitative approach to the determination of antigen in immune complexes. *J. Immunol. Methods* **237**, 1–17.
79. George, J., Gilburd, B., Langevitz, P., *et al.* (1999). Beta2 glycoprotein I containing immune-complexes in lupus patients: Association with thrombocytopenia and lipoprotein(a) levels. *Lupus* **8**, 116–120.
80. Nezlin, R., Alarcon-Segovia, D., and Shoenfeld, Y. (1998). Immunochemical determination of DNA in immune complexes present in the circulation of patients with systemic lupus erythematosus. *J. Autoimmun.* **11**, 489–493.
81. Ho, A., Barr, S. G., Magder, L. S., and Petri, M. (2001). A decrease in complement is associated with increased renal and hematologic activity in patients with systemic lupus erythematosus. *Arthritis Rheum.* **44**, 2350–2357.
82. Schur, P. (1997). Complement and systemic lupus crythematosus. *In* "Dubois' Lupus Erythematosus" (D. J. Wallace and B. Hannahs Hahn, eds.), 5th Ed., pp. 245–261. Williams & Wilkins, Baltimore.
83. Zein, N., Ganuza, C., and Kushner, I. (1979). Significance of C-reactive protein elevation in patients with systemic lupus erythematosus. *Arthritis Rheum.* **22**, 7–12.
84. Lauter, S. A., Espinoza, L. R., and Osterland, C. K. (1979). The relationship between c-reactive protein and systemic lupus erythematosus. *Arthritis Rheum.* **22**, 1421–1424.
85. Spronk, P. E., TerBorg, E. J., and Kallenberg, C. G. (1992). Patients with systemic lupus erythematosus and Jaccoud's arthropathy: A clinical subset with increased C-reactive protein response? *Ann. Rheum. Dis.* **51**, 358–361.
86. Bccker, G., Waldburger, M., and Hughes, G. R. V. (1980). Value of serum C-reactive protein measurement in the investigation of fever in SLE. *Ann. Rheum. Dis.* **39**, 50.
87. Hind, C. R. K., Ng, S. C., Feng, P. H., and Papys, M. B. (1985). Serum C-reactive protein measurement in the detection of intercurrent infection in Oriental patients with systemic lupus erythematosus. *Am. Rheum. Dis.* **44**, 260–261.
88. TerBorg, E. J., Horst, G., Limburg, P. C., van Rijswijk, M. H., and Kallenberg, C. G. M. (1990). C-reactive protein levels during disease exacerbations and infections in systemic lupus erythematosus: A prospective, longitudinal study. *J. Rheumatol.* **17**, 1642–1648.
89. Papys, M. B., Lanham, J. G., and debeer, F. C. (1982). C-reactive protein in SLE. *Clin. Rheum. Dis.* **44**, 260–261.
90. Middleton, G. D., McFarlin, J. E., Sipe, J. D., and Lipsky, P. E. (1994). C-reactive protein and systemic lupus crythematosus: elevation does not predict infection. *Arthritis Rheum.* **37**(Suppl.), S321. [Abstract]

91. Liou, L. B. (2001). Serum and *in vitro* production of IL-1 receptor antagonist correlate with C-reactive protein levels in newly diagnosed, untreated lupus patients. *Clin. Exp. Rheumatol.* **19**, 515–523.
92. Liang, M. H., Socher, S. A., Larson, M. G., and Schur, P. H. (1989). Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus eryhtematosus. *Arthritis Rheum.* **32**, 1107–1118.
93. Petri, M., Hellmann, D., and Hochberg, M. (1992). Validity and reliability of lupus activity measures in the routine clinic setting. *J. Rheumatol.* **9**, 53–59.
94. Hawker, G., Gabriel, S. E., Bombardier, C., Goldsmith, C., Caron, D., and Gladman, D. (1993). A reliability study of SLEDAI: A disease activity index for systemic lupus erythematosus. *J. Rheumatol.* **20**, 657–660.
95. Benclvclil, W., Vitali, C., Isenberg, D. A., Smolen, J. S., Snaith, M. L., Scluto, M., Bombardien', S., and the European Consensus Study Group for Disease Activity in SLE (1992). Disease activity in systemic lupus erythematosus: Report of the Consensus Study Group of the European Workshop for Rheumatology Research. *Clin. Exp. Rheumatol.* **10**, 549–554.
96. Fortin, P. R., Abrahamowicz, M., Clarke, A. E., *et al.* (2000). Do lupus disease activity measures detect clinically important change? *J. Rheumatol.* **27**, 1421–1428.
97. Strand, V., Gladman, D., Isenberg, D., Petri, M., Smolen, J., and Tugwell, P. (1999). Outcome measures to be used in clinical trials in systemic lupus erythematosus. *J. Rheumatol.* **26**, 490–497.
98. Symmons, D. P. M., Coppock, J. S., Bacon, P. A., Bresnihan, B., Isenberg, D. A., Maddison, P., McHugh, H., Sbaith, M. L., and Zoma, A. S. (1988). Development and assessment of a computerized index of clinical disease activity in systemic lupus crythematosus. *Q. J. Med.* **69**, 927–937.
99. Hay, E. M., Bacon, P. A., Gordon, C., Isenberg, D. A., Maddison, P., Snaith, M. L., Symmons, D. P. M., Viner, N., and Zoma, A. (1993). The BILAG index: A reliable and valid instrument for measuring clinical disease activity in systemic lupus erythematosus. *Q. J. Med.* **86**, 447–458.
100. Corzillius, M., Schroeder, J. O., Larson, M., Euler, H. H., and Liang, M. (1993). Comparative responsiveness of three disease activity indices in systemic lupus erythematosus. *Arthritis Rheum.* **36**(Suppl.), SI 84.
101. Ward, M. M., Marx, A. S., and Barry, N. N. (2000). Comparison of the validity and sensitivity to change of 5 activity indices in systemic lupus erythematosus. *J. Rheumatol.* **27**, 664–670.
102. Gladman, D. D., Goldsmith, C. H., Urowitz, M. B., *et al.* (2000). The Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index for Systemic Lupus Erythematosus International Comparison. *J. Rheumatol.* **27**, 373–376.
103. Stoll, T., Stucki, G., Gordon, C., and Isenberg, D. A. (1997). A damage index for lupus patients: Where are we now? *Lupus* **6**, 219–222.
104. Rahman, P., Gladman, D. D., Urowitz, M. B., Hallett, D., and Tam, L. S. (2001). Early damage as measured by the SLICC/ACR damage index is a predictor of mortality in systemic lupus erythematosus. *Lupus* **10**, 93–96.
105. American College of Rheumatology Ad Hoc Committee on Systemic Lupus Erythematosus Guidelines (1999). Guidelines for referral and management of systemic lupus erythematosus in adults. *Arthritis Rheum*. **42**, 1785–1796.

21

LABORATORY EVALUATION OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Peter H. Schur

INTRODUCTION

The use of laboratory tests has greatly enhanced our ability to diagnosis systemic lupus erythematosus (SLE) and related disorders. Thus tests such as the antinuclear antibody (ANA) test and tests for antiphospholipid antibodies have in fact been incorporated into the diagnosis of SLE. However, it is important to remember when using these tests, as with any other tests, in a clinical setting to consider their sensitivity, specificity, and predictive value. In addition, laboratory tests are often used to assess the activity of SLE. In this respect, serum complement levels, anti-double-stranded (ds)DNA, and acute-phase reactants have often proved to be useful. Laboratory tests are also used as in any disease to assess organ involvement and function (e.g., renal function). Finally, laboratory tests are often used to evaluate the patient's response to therapy—and again many tests have been useful in this respect (e.g., complement, anti-dsDNA, CBC, renal function).

ANTINUCLEAR ANTIBODIES

Antinuclear antibodies were initially discovered in the 1940s while investigating the immunology of the LE cell test. LE cell tests are rarely performed and have been replaced by the immunofluorescence assay to detect ANAs.

Performance of the ANA Assay

The titer and specificity of antinuclear antibodies vary depending on the antigen substrate used for the assay. Most laboratories currently use HEp2 cells (human epithelial cell tumor line), which provide certain advantages over the frozen sections of murine livers or kidneys [1]. HEp2 cells offer a standardized substrate with larger nucleoli. They also provide better sensitivity for antibodies to nuclear antigens present during cell division, such as the centromere antigens. In addition, ANA titers are almost always higher when measured on HEp2 cells than on frozen sections of murine tissue.

The assay is performed by first incubating acetone-fixed HEp2 cells with patient's serum and then overlaying this combination with fluorescein-tagged antihuman γ globulin [1]. When viewed through a fluorescent microscope, antibodies bound to nuclear antigens produce an apple green nuclear pattern. The pattern of fluorescence and the dilution at which nuclear fluorescence disappears (titer) are subsequently noted. Differences in titer of one tube dilution commonly occur, but are without clinical significance. Certain antibody specificities, such as an anticentromere, can be read directly from the ANA.

In the past, the tremendous interlaboratory variability of ANA results led to a great deal of confusion. However, it is the author's opinion that the ANA is presently reliable and reproducible, as most

laboratories use commercially available tissue culture cell substrates (e.g., HEp2 cells) and participate in proficiency testing.

Enzyme-Linked Immunosorbent Assay

There are ongoing attempts to substitute the enzyme-linked immunosorbent assay (ELISA) for the immunofluorescence ANA assay, the current gold standard [1]. The advantage of an ELISA over the immunofluorescence ANA assay is the ability to automate the procedure. Antigenic substrates for the ELISA have consisted of extracts of nuclei, recombinant antigens, and/or purified antigens.

There are a number of commercial sources of ELISA-ANA testing. Each kit relies on a different method for preparing and coating the nuclear antigens on the microtiter wells, which may account for the variations noted when several kits are compared [2].

Because the ELISA method can be automated and is much less labor-intensive, many commercial laboratories are now screening antinuclear antibody tests by ELISA and then titering only the positive specimens. The reliability of the ELISA assay that employs recombinant nuclear antigens may not be as reliable as other methods, at least when detecting antinuclear antibodies in children [3].

Diseases Associated with a Positive ANA

A positive ANA can be seen with systemic autoimmune diseases, organ-specific autoimmune diseases, and a variety of infections. Their presence does not mandate the presence of illness, as they can also be found in otherwise normal individuals.

Systemic Autoimmune Disease

A positive ANA is an essential component of the definition of some systemic autoimmune disorders, such as systemic lupus erythematosus, but can also be found in association with many autoimmune disorders that are not defined by these antibodies. As a result, the sensitivity of a positive ANA for a particular autoimmune disease can vary widely (see Table 1) [14]:

- SLE: sensitivity, 93%
- Scleroderma: 85%
- Mixed connective tissue disease: 93%
- Polymyositis/dermatomyositis: 61%
- Rheumatoid arthritis (RA): 41%
- Rheumatoid vasculitis: 33%
- Sjogren's syndrome: 48%
- Drug-induced lupus: 100%
- Discoid lupus: 15%
- Pauciarticular juvenile chronic arthritis: 71% [5]
- Raynaud's phenomenon: 36%
- Antiphospholipid syndrome: 50%

Specific Organ Autoimmune Disease

Positive ANAs are occasionally seen in patients with autoimmune diseases that are limited to a specific organ, such as the thyroid gland, liver, or lung. The following sensitivities have been reported in these disorders:

- Hashimoto's thyroiditis: 46% [6]
- Graves' disease: 50% [6]
- Autoimmune hepatitis: 71% [7]
- Primary autoimmune cholangitis: 100% [8]
- Primary pulmonary hypertension: 40% [9]

TABLE 1 Summary of Test Performance Characteristics of the ANA for Major Rheumatic Diseases

		Specificity				Likelihood Ratio	
Disease	Sensitivity Overall	Other CTD	Non-CTD Rheumatic	Healthy	Overall	Positive	Negative
SLE	93%	49%	75%	78%	57%	2.2	0.11
SSc	85%	44%	75%	71%	54%	1.86	0.27
PM/DM	61%	52%	91%	82%	63%	1.67	0.61
Sjogren's	48%	44%	91%	71%	52%	0.99	1.01
Raynaud's	36%	52%	92%	85%	59%	0.88	1.08
JCA	57%	na	na	na	39%	0.95	1.08
With uvetis	80%	na	na	na	53%	1.68	0.39
RA	41%	38%	85%	82%	56%	0.93	1.06

Others

Other well-recognized disorders associated with a positive ANA titer include chronic infectious diseases, such as mononucleosis [10], subacute bacterial endocarditis, and tuberculosis, and some lymphoproliferative diseases [11, 12]. ANAs have also been identified in up to 50% of patients taking certain drugs; however, most of these patients do not develop drug-induced lupus.

False Positives

False-positive ANAs (i.e., ANAs in the absence of autoimmune disease or known antigenic stimuli) are seen more commonly in women and in elderly patients. They are invariably in low titer. Positive ANAs are found commonly in the normal population. When HEp2 cells are used as a substrate, one study of 125 normal individuals found an ANA titer above 1:40 in 32%, above 1:80 in 13%, and above 1:320 in 3% [13]. No patient had anti-dsDNA antibodies. Antibody titers in healthy individuals usually remain relatively constant over time, a finding that can also be seen in patients with known disease.

It is felt that the very low specificity of a positive ANA in the absence of clinical findings of an autoimmune disorder precludes its use as a screening test for disease in the general healthy population.

False Negatives

Certain antinuclear antibodies can be detected at high titer using certain methods and techniques, but are only found at low titer or may even be absent when assayed using other techniques. These confounding findings result from a number of technical and physical nuances, including the method of substrate fixation, the solubility of the antigen (e.g., Ro, La, PCNA, and Ku), and the localization of the antigen outside the nucleus (i.e., Jo-1 and single-stranded DNA). Due to this limitation, a patient with a negative ANA and strong clinical evidence of a systemic autoimmune disorder may require specific antibody assays to accurately diagnosis a rheumatic disease.

In a review of the literature on ANA, including 65 articles that dealt with ANAs and SLE, the sensitivity of a positive ANA was 93%, the specificity vs other connective tissue diseases (CTD) was 49% vs non-CTD rheumatic was 75% vs healthy was 78% (the overall specificity was 57%) [14]. A positive test had a 2.2 likelihood ratio (LR) for the diagnosis of SLE, whereas a negative test had a 0.11 likelihood ratio [14] (see Table 1).

Serial testing of ANAs in patients with SLE has an unknown value [14].

Usefulness of ANA Titer

Unlike nuclear pattern recognition, determination of the titer of antinuclear antibodies still provides clinically relevant information. (1) The presence of very high concentrations of antibody (titer > 1:640) should arouse suspicion of an autoimmune disorder. However, its presence alone is not diagnostic of disease. If no initial diagnosis can be made, it is our practice to watch the patient carefully over time and to exclude ANA-associated diseases. (2) The combination of very low titers of antibody (<1:80) and no signs or symptoms of disease portend a much less ominous prognosis. As a result, these patients need to be reevaluated far less frequently than those with extremely high antibody titers. (3) Little if any data suggest a correlation between ANA titer and the activity of SLE [14].

Types and Usefulness of Staining Pattern

Antinuclear antibodies produce a wide range of different staining patterns, reflecting the presence of antibodies to one or a combination of nuclear antigens. The nuclear staining pattern has been recognized to have a relatively low sensitivity and specificity for different autoimmune disorders, although it was commonly used in the past to detect specific antibody and antigen specificity. At present, specific tests have largely supplanted the use of patterns [15, 16]

- The homogeneous or diffuse pattern represents antibodies to the DNA–histone complex, also called deoxyribonucleoprotein or nucleosome. It is believed that these antibodies are responsible for the LE phenomenon.
- The peripheral or rim pattern is produced by antibodies to DNA.
- Antibodies to Sm, RNP, Ro/SSA, La/SSB, Scl-70, centromere, PCNA, and other antigens produce the speckled pattern.
- The nucleolar pattern is produced by antibodies to RNA polymerase I, fibrillarin, and NOR-90.
- Antibodies to centromeres produce the centromeric pattern.

Despite these general observations, it is increasingly clear that accurate interpretation of different nuclear patterns is confounded by the following difficulties.

- The recognition of specific patterns is operator dependent and does not produce a permanent record. Because the fluorescence fades in 1 to 2 days, one cannot compare a result with other samples without photographing each test result.
- Different serum dilutions can produce varying nuclear patterns.

- One nuclear pattern may obscure and prevent the recognition of another pattern if several antibodies are present simultaneously.
- Certain specificities are visible only on specific substrates. Anti-Ro antibodies and anticentromere antibodies, for example, are not detected with murine organs, but can be found with HEp2 cells.
- Nuclear patterns are neither sensitive nor specific. As a result, no single pattern denotes a single disease and, conversely, several diseases may produce a particular ANA pattern.

Due to these difficulties, specific nuclear pattern recognition is not as useful as previously thought. Pattern recognition has been increasingly supplanted by assays, which detect specific antibodies to an ever-increasing array of nuclear antigens and to antigens not normally found in the nucleus.

Types of Antinuclear Antibodies

Their target antigen defines the different types of ANAs, including single- and double-stranded DNA, nuclear histones, nonhistone nuclear proteins, and RNA–protein complexes. As will be seen, some of these antibodies are relatively specific for a particular disease or for specific clinical manifestations in patients with lupus.

Reflex Testing

Some laboratories have a routine that when an ANA test is ordered they will routinely perform a screening ANA test. Most laboratories, if the screen is positive, will titer the ANA and report patterns. Some laboratories perform what is called "reflex testing." This entails performing tests for anti-dsDNA, Sm, RNP, Ro/SSA, and La/SSB whenever the ANA is positive. This reflexology has the advantage of helping discriminate ANAs associated with SLE (e.g., positive in one of these assays) from nonspecific ANAs immediately instead of waiting for the patient and physician to resort to a second blood specimen and an additional visit to the office/laboratory, making reflex ANA testing cost effective.

Anti-DNA Antibodies

Autoantibodies to DNA were first described in the 1950s. These are the best-recognized specific autoantibodies found in patients with SLE. Antibodies to DNA can be divided primarily into two groups: those reactive with denatured (single-stranded) DNA and those recognizing native (double-stranded) DNA.

Measurement of Anti-dsDNA Antibodies

There are currently three methods commonly used by most clinical laboratories to quantitate anti-dsDNA antibodies. Most of these tests measure both high- and low-avidity antibodies.

The Farr assay is based on the precipitation of radioactively labeled DNA–anti-DNA antibody complexes in 50% saturated ammonium sulfate. This assay primarily detects immune complexes consisting of histone and anti-DNA antibodies [17]. Approximately 50 to 78% of all patients with SLE have elevated titers of anti-DNA antibodies measured by this method; the titers appear to correlate closely with disease activity, especially with active proliferative nephritis [17, 18]. Because this method requires the use of a radioactive antigen, its routine use has been limited.

The *Crithidia luciliae* assay is an indirect immunofluorescent assay that makes use of the fact that the basal body of this unicellular flagellate is very rich in double-stranded DNA in the absence of other nuclear antigens [19]. This method, while of comparable sensitivity to the Farr assay, is more cumbersome to quantitate and the antibodies detected correlate less closely with active nephritis [20, 21].

A third method in routine use utilizes the ELISA technique [22, 23]. Double-stranded DNA adherent to polystyrene microwells, treated to increase their adhesiveness, serves as an antigen to capture antibodies. These antibodies are then quantitated using a second antiserum to human immunoglobulin conjugated to a detector enzyme. This method is positive in approximately 70% of patients with SLE. The IgG antibody titers correlate moderately well with active nephritis and, in the author's experience, there is a good correlation with disease activity in general.

Properties of Anti-dsDNA Antibodies

Anti-dsDNA antibodies can demonstrate different properties based on avidity that affects their usefulness as a diagnostic tool. As an example, high-affinity IgG anti-dsDNA antibodies can be demonstrated in 70 to 80% of patients with SLE when their disease is active [24]. In contrast, some patients with SLE have predominantly IgM or low-avidity IgG antibodies to dsDNA. These antibodies are less useful diagnostically, as they can be found in association with drug-induced lupus, rheumatoid arthritis, Sjogren's syndrome, other connective tissue diseases, chronic infection, chronic liver disease, and normal aging [18]; in these instances, the antibodies have no clinical significance. It is possible that lower avidity antibodies are actually reacting with ssDNA fragments in the DNA preparations used as antigenic substrates.

A number of properties of anti-dsDNA antibodies other than avidity also affect their pathogenicity, including the isoelectric point, isotype, and idiotype. Anti-DNA antibodies that are IgG, cationic, and bind with high affinity correlate best with renal activity.

These antibody properties may vary from individual to individual, over time in the same individual, or with disease activity, even in the face of a stable antibody titer. Thus, there are some patients with SLE who have persistent anti-dsDNA antibody activity despite an improvement in disease activity. Similarly, a small but significant minority of patients have active nephritis without elevations of anti-dsDNA antibody titer. These findings may be related in part to differences in the anti-DNA antibodies over time. Anti-DNA antibodies present in some patients during periods of active nephritis may differ idiotypically from the anti-DNA antibodies present in the same patient during periods of inactive disease [25].

TABLE 2 Anti ds DNA

	Sensitivity	Specificity	Positive LR	Negative LR
SLE vs normals & other diseases	0.573	0.974	16.4	0.49
SLE: active vs inactive	0.66	0.66	4.14	0.51
Lupus nephritis:				
present vs absent	0.65	0.41	1.7	0.76
active vs inactive	0.86	0.45	1.7	0.3

Clinical Relevance of Anti-dsDNA Antibodies

1. They are relatively specific (97%) for SLE, making them very useful for diagnosis (see Table 2) [18, 18a]. They have been found occasionally in autoimmune hepatitis and in a few patients receiving minocycline, etanercept, infliximab, and penicillamine.

2. There is a well-recognized association of high titers of IgG anti-dsDNA titers with active glomerulonephritis [24, 26, 27]; there also appear to be highly enriched amounts of anti-dsDNA antibodies in the glomerular deposits of immune complexes found in patients with lupus nephritis. These observations have led many investigators to believe that anti-dsDNA antibodies are of primary importance in the pathogenesis of lupus nephritis [26, 28].

3. Titers rise when disease is active and usually fall (generally into the normal range) when the flare subsides [26, 29–30]. Early studies reported a tight correlation between high-titer anti-dsDNA antibodies and nephritic activity [18, 26], particularly in the setting of hypocomplementemia [26, 29]. More recent studies, however, have reported exceptions to this correlation of titers and disease activity, e.g., some patients have elevated titers of anti-dsDNA antibodies in the setting of inactive or minimally active lupus [31]. A review of the literature is summarized in Table 2 [18a].

This phenomenon may be attributable to the current sensitive assays that are more likely to detect low-avidity antibodies that previously would have been missed. As mentioned earlier, clinical correlations that hold about high-avidity antibodies appear not to be applicable to those of lower avidity, and current assays are unable to distinguish between high- and low-avidity, i.e., pathogenic and nonpathogenic, antibodies. Thus, while the correlation between antibody titer and disease activity holds for the majority of patients with SLE, there may be some patients for whom this assay has limited clinical utility.

The association between anti-DNA antibodies and other disease manifestations of SLE is far less clear. As an example, there is no relationship between antibody titer and disease activity for lupus cerebritis [32].

Distinguishing active lupus from infectious complications (e.g., secondary to treatment with immunosuppressive agents), from toxic effects of drugs, and from unrelated disease is always a challenge. Anti-DNA antibodies may be helpful in some patients in making this distinction. Although, as noted earlier, there is clear variability among patients, the test becomes very useful once one has demonstrated that a given patient follows the characteristic pattern of rising DNA and falling complement in the setting of a flare. However, once a particular patient shows a disassociation between his or her anti-DNA antibody titer and clinical evidence of nephritis, future changes in anti-DNA antibody activity are unlikely to accurately reflect disease activity. In this setting, therapeutic decisions must be guided by the clinical picture and perhaps by other serological findings such as complement levels.

Anti-ssDNA Antibodies

Antibodies that identify denatured DNA are probably reacting with the purine and pyrimidine bases that are accessible on single-stranded DNA, but are buried within the β helix of double-stranded DNA. Thus, anti-denatured DNA antibodies do not cross-react with native DNA. Antibodies to ssDNA have the following general properties: (1) They have been eluted from the kidneys of patients with proliferative nephritis and may

therefore be of pathogenic significance [33]. (2) They are much less specific for SLE than antibodies to dsDNA. As an example, anti-ssDNA antibodies have been reported in rheumatoid arthritis, drug-related lupus, healthy relatives of patients with SLE [34], and, less commonly, in other rheumatic diseases. Thus, anti-ssDNA has limited usefulness for the diagnosis of SLE. (3) They do not correlate well with disease activity and are therefore not useful for disease management.

Anti-Smith Antibodies and Anti-RNP Antibodies

The anti-Sm and anti-RNP systems are considered together because they coexist in many patients with SLE and bind to related but distinct antigens.

Anti-Sm Antibodies

The Smith antigen is a nuclear nonhistone protein that was characterized in 1966 and was the first nuclear protein auto antigen to be described in SLE [35]. The antigen to which anti-Sm antibodies bind consists of a series of proteins: B; B'; D; E; F; and G, complexed with small nuclear RNAs: U1; U2; U4–6; and U5. These complexes of nuclear proteins and RNAs are called small nuclear ribonucleoprotein particles (snRNPs); they are important in the splicing of precursor messenger RNA [28], an integral step in the processing of RNA transcribed from DNA.

The anti-Sm immune reaction consists of multiple antibodies binding to multiple protein antigens. Thus, although we speak of the anti-Sm antibody, it is actually better described as an antibody system.

Methods of Measurement

While anti-Sm antibodies and anti-RNP antibodies can be detected by immunoprecipitation in agarose gels using radial immunodiffusion or counterimmunoelectrophoresis, these methods are relatively insensitive and difficult to quantitate [35, 36]. Most clinical laboratories now employ either ELISA or hemagglutination to detect these antibodies [37]. Both methods are sensitive, although the ELISA method is quantitated more easily. Because the titer of these antibodies has very limited clinical utility, this methodological difference has little practical significance. In a proficiency survey conducted by the College of American Pathologists, there was greater than 95% agreement in results between the two methods.

Anti-Sm antibodies are insensitive (18 to 30% depending on the assay used) [38, 39], but highly specific for SLE, and generally remain positive when titers of anti-DNA antibodies have fallen into the normal range and the clinical activity of SLE has waned. Thus, the measurement of anti-Sm titers may be useful diagnostically, particularly at a time when DNA antibodies are undetectable.

Anti-RNP Antibodies

The anti-RNP system binds to antigens that are different from but related to Sm antigens. These antibodies bind to proteins containing only U1-RNA. The U1-RNP particle is involved in splicing heterogeneous nuclear RNA into messenger RNA. Anti-RNP antibodies are not specific for SLE, but are a defining feature in the related syndrome, mixed connective tissue disease. The antibody is present in lower titers in several other rheumatic diseases, including scleroderma.

Usefulness

Anti-Sm antibodies occur more frequently in African-Americans and Asians than in Caucasians with SLE. As an example, one series reported a prevalence rate of 25% in African-Americans and 10% in Caucasians (via immunodiffusion) [40]. The prevalence of anti-Sm antibodies in SLE using the ELISA assay varies depending on whether Sm is measured alone or in combination with RNP. Generally accepted rates of anti-Sm antibody positivity in SLE are in the range of 10 to 30% in Caucasians and 30 to 40% in Asians and African-Americans [40].

Antibody titers may fluctuate somewhat over time, although this is a controversial issue. Levels occasionally drift into the negative range when the immunodiffusion technique is used, but this should not occur when more sensitive methods are employed.

Many studies have tried to correlate anti-Sm antibody with disease activity in general and with specific disease manifestations. These investigations have often yielded conflicting results.

- Several authors have reported an association between anti-Sm antibodies and a low frequency of progression to end-stage renal disease (i.e., milder renal disease) [41–43]. However, others have found that the severity of renal disease is similar in patients who do and do not make antibodies to Sm [44].
- One report concluded that the presence of anti-Sm antibodies in the absence of antibodies to DNA was associated with an increased incidence of serositis and a decrease in the incidence of hematologic abnormalities [45].

- Another study found an association between anti-Sm antibodies and the presence of central nervous system (CNS) disease occurring as an isolated clinical finding [31].

These often-contradictory results may be explained by the use of different methods of antibody assay by different investigators, as well as by the application of inappropriate statistical methods.

At this point, there is no evidence that anti-Sm antibodies will be useful for following or predicting disease activity in the way that anti-dsDNA antibodies are used. Antibodies to Sm do function as an important diagnostic marker for SLE. As an example, the presence of antibodies to the Sm antigen system in a patient in whom we suspect the diagnosis of SLE strongly supports the diagnosis. Similarly, in a patient with a less convincing clinical picture, we believe that the presence of these antibodies suggests that even a nonspecific symptom complex is likely to progress to SLE. Given their relatively low prevalence, however, a negative value in no way excludes a presumptive diagnosis of SLE.

Anti-Ro/Anti-SSA Antibodies

Anti-Ro/SSA and anti-La/SSB antibodies are ANAs that have been detected with high frequency in patients with SLE and in patients with Sjogren's syndrome [46].

Immunodiffusion, ELISA, Western blot, and RNA immunoprecipitation detect antibodies to Ro clinically. Most but not all sera react in all three assays. ELISA assays are 10 to 100 more sensitive than immunodiffusion assays; they are also quantitative [47]. The Ro antigen is extracted from human or bovine spleen, Wil-2 cell lines, or represents recombinant fusion proteins [47].

These antinuclear antibodies recognize cellular proteins with molecular masses of approximately 52 and 60 kDa. The 60-kDa protein is complexed with the hy1–5 species of small nuclear RNAs [48].

Relationship between Anti-Ro and Anti-SSA Antibodies

In 1969, Clark *et al.* [49] described the presence of antibodies in the sera of some patients with SLE that reacted with ribonucleoprotein antigens present in saline extracts of rabbit and human spleen. When purified preparations of this antibody were incubated with a human epithelial cell line (HEp2), both the nuclei and the cytoplasm were stained in a speckled pattern. These investigators named the antibody anti-Ro after the original patient in whom the antibodies were identified.

At about the same time, Alspaugh and Tan [50] noted antibody activity in sera from many patients with Sjogren's syndrome that gave the same immunofluorescent staining pattern as that reported for anti-Ro antibodies. These workers referred to this antibody as anti-SSA. It was soon shown that these two antibody systems (Ro and SSA) produced a line of identity on immunodiffusion, indicating that they were reacting with the same nuclear RNA protein [51]. Subsequent research found that anti-Ro antibodies in SLE and anti-SSA antibodies in primary Sjogren's syndrome actually react with different epitopes on the same 60-kDa particle [52].

Anti-Ro/SSA antibodies recognize at least two proteins: a 52 kDa (475 amino acids) and a 60 kDa (525 amino acids). Sera of all patients with anti-Ro/SSA activity detected by immunoprecipitation bind the 60-kD antigen a, whereas sera from only a subset bind the 52-kDa antigen. The additional antigenic reactivity appears to result from a molecular interaction between peptides of the 60- and 52-kDa proteins. No consistent disease association has been noted for any of the fine specificities of these antibodies.

Clinical Significance in SLE

Anti-Ro/SSA antibodies are found by immunodiffusion in approximately 38.6% of patients with SLE; the specificity is 99.986%; and the positive LR is 27571 and the negative LR is 0.4 (47). Anti-Ro/SSA antibodies are found by ELISA in 29.6% of patients with SLE; the specificity is 97.6%; and the positive LR is 12 and the negative LR is 0.7 [47]. Anti-Ro/SSA is found by immunoblotting in 40.6% of SLE patients; specificity 100%; and positive LR is 68 and the negative LR is 0.6 [47] (see Table 3). Anti-Ro/SSA has been associated with photosensitivity, a rash known as subacute cuta-

TABLE 3 Anti-Ro

	Sensitivity	Specificity	Positive LR	Negative LR
SLE vs normals by ID	38.6	99.986	27,571	0.4
SLE vs normals by ELISA	29.6	97.6	12	0.7
SLE vs normals by IB	40.6	100	>68	0.6
SS vs normals by ID	49	99.7	163	0.5
SS vs normals by ELISA	69	97.3	26	0.3
SS vs normals by IB	65	100	>108	0.4
PSS (vs normals)	7.9–21	98–100	4/6–11	0.8–0.9
DM/PM (vs normals)	23–24	98–100	12–14	0.8
RA (vs normals)	9.9–20	98–100	8.3–11	0.8–0.9

TABLE 4 Anti-Ro & Pregnancy

	Sensitivity	Specificity	Positive LR	Negative LR
Prevalence of anti-Ro in mothers of children with CCHB	85			
Predictive value at anti-Ro testing for NLB/CCHB in offspring of SLE patients	13	99.7	43	0.9
Predictive value of anti-Ro testing for fetal wastage/ prematurity in mothers with SLE	24	65	0.69	1.2

TABLE 5 Anti-Ro

	Sensitivity SLE	Sensitivity SS	Specificity (+)	Specificity (−)
Anti-52 kd alone	44	92	5.5	0.6
Anti-60 kd alone	3	88	0.3	1.1
Anti-52 + 60	28	75	1.1	1.0
All 52 kd	72	67	2.2	0.4
All 60 kd	31	63	0.8	1.1

neous lupus, cutaneous vasculitis (palpable purpura), interstitial lung disease, neonatal lupus, and congenital heart block (CCHB) [46, 53–56]. CCHB occurred in 35 of 278 anti-Ro/SSA-positive (immunodiffusion) SLE mothers and in 1 of 398 anti-Ro-negative mothers (sensitivity 13%; specificity 99.7%; positive LR 43) [47]. There appears to be no predisposition of mothers with anti-52-kDa vs 48-kDa Ro/SSA antibodies to develop CCHB [47] (Table 4). In addition, anti-Ro/SSA did not appear to predict either spontaneous abortions or stillbirths among SLE patients [47].

Association of Anti-Ro Antibodies with Other Disorders

Anti-Ro/SSA antibodies have been noted in 49–69% of patients with Sjogren's syndrome [47] and have occasionally also been noted in patients with rheumatoid arthritis (9.9–20%), progressive systemic sclerosis (7.9–21%), cutaneous vasculitis, dermatomyositis/ polymyositis (23–24%), undifferentiated connective tissue disease, mixed connective tissue disease, juvenile rheumatoid arthritis, chronic active hepatitis, primary biliary cirrhosis, and homozygous C2 or C4 deficiency [47, 48] (Table 3). They are also found in 0.1 to 0.5% of normal subjects; such individuals may have an enhanced sensitivity to ultraviolet light.

Relationship to "ANA-Negative SLE"

In the 1970s, there were several reports of patients who met the American College of Rheumatology criteria for SLE, but were persistently negative for ANA [57]. Although not recognized at the time, this consistently negative finding occurred because sera were tested using mouse and not human tissue as the substrate [48]. By comparison, anti-Ro/SSA antibodies were found in most of these patients when a human cell line extract was used as the substrate for the Ro antigens.

More recently, the substitution of HEp2 cells (a human cell line) for mouse tissue sections in the ANA test has resulted in only a small number of SLE patients who test persistently negative for ANA. Nevertheless, on rare occasions, the anti-Ro/SSA antibody test may be useful in suggesting a diagnosis of systemic autoimmune disease in the face of a negative ANA. In one study of 4025 sera sent for ANA testing, for example, 64 patients were ANA negative by immunofluorescence but anti-Ro positive by ELISA [58]. Of these, 12 and 5 patients were diagnosed with SLE and cutaneous LE, respectively.

Finding anti-60-kDa Ro is somewhat useful in distinguishing SLE from primary Sjogren's syndrome (sensitivity 12%; specificity 97%), whereas detecting anti-52-kDa Ro is more helpful in distinguishing primary Sjogren's syndrome from SLE (sensitivity 44%; specificity 92%) [47] (Table 5).

Anti-La/SSB Antibodies

Approximately 50% of sera from patients with SLE, which have anti-Ro antibody activity, also contain antibody to La, a closely related RNP antigen.

The antigen appears to be a nuclear phosphoprotein with a molecular mass of 48 kDa and is complexed with the Ro particle [59]. In addition to other functions, the La protein serves as a termination factor for RNA polymerase III.

Immunodiffusion, ELISA, Western blot, and RNA immunoprecipitation detect anti-La/SSB antibodies clinically. The La/SSB antigen is derived from rabbit thymus extracts or recombinant fusion proteins. ELISA assays are 10 to 100 more sensitive than immunodiffusion assays; they are also quantitative [47].

TABLE 6 Anti-La

	Sensitivity	Specificity	Positive LR	Negative LR
SLE vs normals ID	13	100	>33	0.9
SLE vs normals ELISA	24	99	20	0.8
SS vs normals ID	22	100	>17	0.8
SS vs normals ELISA	71	93	10	0.3
SS vs normals IB	50	100	28	0.5

Clinical Associations of Anti-La

Anti-La/SSB antibodies are found in the following circumstances.

- It is very unusual to encounter sera that contain anti-La/SSB activity without demonstrable antibodies to Ro/SSA in patients with SLE or Sjogren's syndrome.
- Antibodies to the La/SSB by immunodiffusion are present in 13% of patients with SLE (specificity 100% vs normals; positive LR >33; negative LR 0.9). Antibodies to La/SSB by ELISA are found in 24% of SLE patients (specificity 99% vs normals; positive LR 20; negative LR 0.8) [47] (Table 6).
- Anti-La/SSB antibody activity has also been detected in 22% (by immunodiffusion) to 71% (by ELISA) of patients with Sjogren's and has been seen in some patients with scleroderma (2–37%), dermato/polymyositis (3%), rheumatoid arthritis (1%), primary biliary cirrhosis, and autoimmune hepatitis [47] (Table 6).

Indications for Testing for Anti-RO/SSA and Anti-LA/SSB

In the author's opinion, indications for ordering an anti-Ro/SSA antibody test are as follows: women with SLE who have become pregnant; women who have a history of giving birth to a child with heart block or myocarditis; patients with a history of unexplained photosensitive skin eruptions; patients suspected of having a systemic connective tissue disease in whom the screening ANA test is negative; and patients with symptoms of xerostomia, keratoconjunctivitis sicca, and/or salivary and lacrimal gland enlargement.

Once a patient is either Ro/SSA and or La/SSB positive or negative this rarely changes. There is also little if any justification for performing serial determinations of anti-Ro/SSA and/or anti-La/SSB [47].

Nucleosome-Specific Autoantibodies

The first autoantibody described in systemic lupus erythematosus, the "LE factor," was a nucleosome-specific autoantibody. These antibodies are now termed nucleosome-restricted antibodies because they bind to the complex of DNA and histones, but not to the individual components.

Pathogenic Role

An original report suggested that nucleosomes might be critical antigens in the eventual generation of antinuclear antibodies in SLE [60]. Subsequent observations have provided some support for this hypothesis [61, 62]. The appearance of these antibodies may precede the emergence of antibodies to DNA or histones.

Methods of Detection

The detection of antinucleosome-specific antibodies remains problematic because the nucleosomal material used as a substrate also binds anti-dsDNA and antihistone antibodies. The traditional method of circumventing this problem, absorption techniques, is cumbersome and difficult to perform. An epitope recognized only by nucleosome-specific antibodies has not yet been identified.

Incidence and Accuracy

Because of the difficulties in detection, there is a paucity of data concerning the incidence and accuracy of antinucleosome-specific autoantibodies. A prevalence of 48 to over 80% has been noted in patients with SLE [63, 64]. They usually but not always are associated with anti-dsDNA and antihistone antibodies [63]. The specificity of these antibodies remains a source of controversy.

Associated Clinical Manifestations

Nucleosome-specific autoantibodies have been linked with lupus nephritis in murine models of SLE. In humans, however, the results are conflicting. Some series have found a weak but significant association [65, 66], whereas others have not [62, 64]. One study of a large cohort of patients with varied connective tissue disorders and control sera from those with hepatitis C virus infection found that IgG antinucleosomal antibodies were found exclusively in patients with SLE, systemic sclerosis, and MCTD, with prevalences of 72, 46, and 45%, respectively [66]. In addition, antinucleosomal

antibodies of the IgG3 subclass were observed only in patients with SLE and correlated more closely with active disease and particularly with nephritis than anti-DNA antibodies.

Summary

The clinical utility of antinucleosome-specific autoantibodies remains undefined, as their sensitivity, specificity, and association with particular manifestations of SLE remain undefined. Nevertheless, these antibodies are a source of interest because they may have a role in the pathogenesis of autoantibody production in SLE.

Anti-hnRP Antibodies

The spliceosome refers to a complex of nuclear RNA-binding proteins, which processes pre-mRNA into spliced mature mRNA. Among the subunits of the spliceosome are a group of 30 structurally related proteins known as heterogeneous nuclear ribonuclear proteins, which are called hnRNP [67].

Methods of Detection

Autoantibodies specific for the RNA-binding regions of two of the hnRNP proteins, A2 (also called RA33) and A1, produce a finely speckled pattern of staining in the nucleoplasm on indirect immunofluorescence microscopy. Their presence must be confirmed by either immunoblotting or dot blot assays [68].

Incidence and Accuracy

Anti-hnRNP antibodies against the A1 and A2 proteins have been reported with the following frequencies [69, 70]:

- Rheumatoid arthritis: 50% for the A1 protein and 35% for the A2 protein
- SLE: 22 to 38% for A1 and 23% for A2
- Mixed connective tissue disease: 40% for A1 and 38% for A2
- Scleroderma, myositis, Sjogren's syndrome: <5% for A2
- No connective tissue disease: <7% for A1 and A2

The clinical utility of anti-hnRNP antibodies is currently limited because they cross-react with multiple antigens, and antibodies directed against hnRNP antigens other than A1 and A2 may be found in different autoimmune disorders.

Summary

Anti-hnRNP antibodies have limited utility in the diagnosis of connective tissue diseases as they are found at significant levels in multiple disorders and their presence appears to correlate highly with other specific antibodies (such as anti-Sm antibodies).

Anti-PCNA Antibodies

A number of proteins accumulate in the nucleoplasm of cells during the G1/S phase of the cell cycle, which corresponds to the period of DNA synthesis. Approximately 2 to 10% of sera from patients with SLE sera contain antibodies, which react with one such protein: proliferating cell nuclear antigen (PCNA) [71].

Methods of Detection

Anti-PCNA antibodies can be detected by indirect immunofluorescence as a finely granular nuclear staining pattern on rapidly dividing cells. An enzyme-linked immunoassay for the detection of these antibodies has been reported. The dominant epitope of PCNA is an area of 14 contiguous amino acids [72].

Accuracy

Anti-PCNA antibodies appear to be specific (>95%) for SLE; they have not been found in sera from patients with RA or other connective tissue diseases or in normal sera [71]. The incidence of this antibody varies, as its production is rapidly inhibited by the administration of corticosteroids and immunosuppressive drugs.

Clinical Association

The presence of anti-PCNA antibodies in patients with SLE is not associated with any particular clinical manifestation other than arthritis.

Summary

Despite its high specificity, the anti-PCNA antibody test has limited clinical utility because of its very low sensitivity.

Antibodies to Ribosomal P Proteins

Antibodies to ribosomal P proteins are detected by either Western immunoblotting or an ELISA with the latter being the test of choice. The ELISA, which is more sensitive using affinity-purified human P protein rather than bovine P protein, is quantitative and more convenient [73].

The targets of these antibodies were three phosphoproteins (P proteins) located on the 60S subunit of ribosomes [74, 75]. The proteins were designated P0, P1, and P2, with molecular masses of 35, 19, and 17 kDa, respectively. They were thought to be involved in protein synthesis; specifically, in the interaction of EF-1 α and EF-2 with ribosomes.

Clinical Associations

All published studies agree on the specificity of antiribosomal P protein antibodies for SLE [76–78]. These antibodies have not been found using conventional assays in normal controls [77, 79] or in patients with other autoimmune diseases (e.g., rheumatoid arthritis, scleroderma, and myositis) [80].

The antibodies have been detected in 10 to 20% of patients with SLE [76, 77, 81, 82]; however, the incidence may approach 50% in Asian populations and children [80, 83, 84].

Ribosomal P Protein Antibodies and Lupus Cerebritis

The presence of ribosomal P protein antibodies has been strongly associated with lupus manifested by psychosis and/or depression by some [77, 79, 80] but not by others [15, 18]. Similarly, some [77, 79], but not others [82, 85, 86], have found associations between a rise and a fall of antibody titer in association with activity of psychosis.

The aforementioned observations were all based on studies of a relatively small number of patients. Therefore, only the results of a prospective study of large numbers of patients will properly determine whether there is a correlation between active SLE and antiribosomal P antibodies.

Antibodies to Ribosomal P Protein and Non-CNS Lupus

The presence of antiribosomal P protein antibodies has been linked with the involvement of other organ systems in SLE, specifically the liver and the kidney.

1. Liver disease: In a case-control study, liver disease was found in 7 of 20 patients with SLE who had antiribosomal P antibodies, but in only 1 of 20 patients without these antibodies [87]. Antibodies to ribosomal P proteins were not found in sera of patients with chronic active hepatitis, primary biliary cirrhosis, or sclerosing cholangitis; these findings suggest that these autoantibodies were not simply a by-product of immune-related hepatic injury. Others have made similar observations [88].

2. Renal disease: Antiribosomal P antibodies have also been associated with lupus nephritis in some studies [87], including fluctuations of antibodies levels with the activity of renal disease [89]; however, others have noted no such association [77, 82].

3. An association between antiribosomal P protein antibodies and antibodies to cardiolipin has been noted [90]. It is unknown whether the coexistence of such antibodies is associated with a higher risk of neuropsychiatric manifestations.

Variability of Antiribosomal P Protein Antibody Results

Any or all of the following possibilities may distort the results of studies evaluating the frequency of antibodies to ribosomal P proteins and their clinical associations: (1) Differences in methodology for detection of antibody, (2) variable ethnic composition of populations in different studies, (3) small sample sizes, and (4) problem of definition.

Perhaps the biggest confounding factor is the problem of definition. Lupus-related psychosis and depression remain exceedingly difficult to diagnose and still harder to quantify. In addition, authors use different criteria to diagnose these conditions. As examples, one study included in their group of lupus patients with depression only those requiring admission for their psychiatric manifestations [77], whereas another included patients with much less severe disease [85]. Results might therefore not be comparable.

Utility of Antiribosomal P Antibodies in SLE

It is believed that testing for antibodies to ribosomal P proteins may be useful in certain circumstances as they are quite specific, but not sensitive (12 to 16%), for SLE [76, 77, 91]. A positive antiribosomal P antibody might be used diagnostically in a fashion similar to that of antibodies to Sm. As an example, presence of an anti-Sm antibody suggests a diagnosis of SLE even in a patient with otherwise undifferentiated disease. Positive antibodies to ribosomal P proteins may also favor this diagnosis in this setting. In addition, the presence of these antibodies is independent of antibodies to dsDNA [79, 82]. They may therefore remain elevated when other marker antibodies have returned to normal and might again be helpful in diagnosing SLE even with quiescent disease. The utility of these antibodies in these scenarios, as well as their exact role in NPSLE, liver disease, and renal disease, requires further investigation.

Other Autoantibodies

Anti-C1q Antibodies (see Complement Section)

Coombs

Overt autoimmune hemolytic anemia (AIHA), characterized by an elevated reticulocyte count, low haptoglobin levels, an increased indirect bilirubin concentration, and a positive direct Coombs' test, has been noted in approximately 10% of patients with SLE. Even more patients have a positive Coombs' test without evidence of overt hemolysis. The presence of both immunoglobulin and complement on the red cell is usually associated with some degree of hemolysis, whereas the presence of complement alone (e.g., C3 and/or C4) is often not associated with hemolysis. The antibodies are "warm," IgG, and are directed against Rh determinants. IgM-mediated cold agglutinin hemolysis is uncommon. Anemia associated with a positive Coombs may result more from increased splenic sequestration than to direct hemolysis.

Rheumatoid Factors

Rheumatoid factors (RF) represent autoantibodies to the Fc portion of IgG. Most RF are IgM, although RF of all Ig classes have been described. RF, as detected by latex fixation, have been found in 20 to 60% (mean 33%) of patients with SLE [92]. While earlier observations suggested a negative correlation between a positive RF test and lupus nephritis, subsequent studies have failed to confirm this. While RF titers fluctuate little in patients with RA, they tend to both vary in titer and come and go in patients with SLE, to some extent in correlation with the activity of SLE. It is the author's opinion that there is no merit in testing for RF in patients with SLE.

Antiplatelet Antibodies

Antiplatelet antibodies demonstrable as platelet-bound IgG have been shown to be increased in virtually all SLE patients with thrombocytopenia [92]. While the serum antiplatelet antibodies in chronic ITP are IgG3, in SLE sera they are IgG1–4 [93, 94]. Serum antibody assays for antiplatelet antibodies generally, however, do not correlate well with the presence of thrombocytopenia. In contrast, all IgG subclasses are found bound to platelets (in patients with thrombocytopenia) in both SLE and ITP [95]. Testing for platelet-bound IgG may be helpful in evaluating SLE patients with thrombocytopenia. The presence of the antibody suggests an antibody-mediated thrombocytopenia, whereas an absence of the antibody suggests that thrombocytopenia is not due to an ITP antibody-mediated mechanism. The IgG bound to platelets mediates thrombobocytopenia by causing increased phagocytosis of opsonized platelets in the spleen.

Thrombocytopenia in a patient with SLE should also make one suspect the antiphospholipid antibody syndrome (APS) (see later).

Anti-Wbc Antibodies

Anti-white blood cell antibodies, especially antilymphocyte antibodies, have been noted in many SLE patients. This subject is reviewed in Chapter 36. Detection of these antibodies is performed primarily for research.

ANTIPHOSPHOLIPID ANTIBODIES

Antiphospholipid antibodies, such as lupus anticoagulants (LA) and anticardiolipin antibodies (aCL), are recognized causes of thromboembolic phenomenon, thrombocytopenia, and several adverse obstetrical outcomes. This disorder is referred to as the primary antiphospholipid antibody syndrome when these antibodies occur alone. It is called secondary antiphospholipid syndrome when they are found in association with systemic lupus erythematosus, other rheumatic diseases, and certain infections and drugs.

Four types of antiphospholipid antibodies (APA) have been described: (1) Antibodies that give a false-positive serologic test for syphilis (STS), (2) lupus anticoagulants, (3) anticardiolipin antibodies, and anti-β_2-glycoprotein 1 antibodies.

False-Positive Serologic Test for Syphilis

Some patients with SLE have a false-positive serologic test for syphilis. When patient sera contain anticardiolipin antibodies (see later), the false-positive STS occurs because the antigen used in the test is embedded in cardiolipin. As a result, a reaction against this molecule will be interpreted incorrectly as being directed against the treponemal antigen. STS should not be used to screen for APL because it has a low sensitivity and specificity [96].

Lupus Anticoagulants

Lupus anticoagulants are antibodies directed against plasma proteins (such as prothrombin or Beta-2-glycoprotein I) bound to anionic phospholipids [97–99]. The LA blocks *in vitro* assembly of the prothrombinase complex, resulting in a prolongation of *in vitro* clotting assays such as the activated partial thromboplastin time (aPTT), the dilute Russell viper venom time (dRVVT), the kaolin clotting time, and rarely the prothrombin

time. These abnormalities are not reversed when the patient's plasma is diluted 1:1 with normal platelet-free plasma, a procedure that will correct clotting disorders due to deficient clotting factors [97, 100]. Abnormal clotting test results can be largely reversed by incubation with a hexagonal phase phospholipid, which neutralizes the inhibitor [97, 100, 101]. Although these changes suggest impaired coagulation, patients with LA have a paradoxical increase in the frequency of arterial and venous thrombotic events [102, 103].

Anticardiolipin Antibodies

Anticardiolipin antibodies (aCL) react with proteins (e.g., β_2-glycoprotein 1 and annexin V) bound to anionic phospholipids, such as cardiolipin and phosphatidylserine. There is an approximate 85% concordance between the presence of a LA and aCL. In many cases, however, the LA is a separate population of antibodies from aCL [100, 101, 108]. Thus, testing should be performed for both LA and aCL if APS is clinically suspected. LA positivity incurs a somewhat greater risk for thrombosis than aCL.

Different immunoglobulin isotypes and subclasses are associated with aCL, including IgG, IgA, IgM, and IgG subclasses 1 to 4. Elevated levels of IgG aCL (particularly IgG2) incur a greater risk of thrombosis than other immunoglobulin isotypes [104].

IgA aCL is the most prevalent isotype in African-Americans [109]. The predominance of this isotype in combination with the low prevalence of significantly elevated levels of aCL may account for the low frequency of APS in these patients.

Anti-β_2 Glycoprotein I Antibodies

Anti-β2GP1 antibodies directly bind to β2GP1 as opposed to aCL, which frequently bind to a complex of anionic phospholipids and β2GPl. Antibodies to β_2-glycoprotein 1, a phospholipid-binding inhibitor of coagulation, are found in a large percentage of patients with primary or secondary APS [110]. Although antibodies to β_2-glycoprotein 1 are found commonly in those with other antiphospholipid antibodies, they are the sole antiphospholipid antibody found in approximately 11% of patients with APS [110].

Antiprothrombin Antibodies

Antiprothrombin antibodies have been described in association with both clotting and pulmonary hemorrhage [111].

Patients with antiphospholipid antibodies may also have antibodies directed against other proteins, including heparin/heparin sulfate, prothrombin, platelet-activating factor, tissue-type plasminogen activator, protein S, annexin IV and V, thromboplastin, oxidized low-density lipoproteins, thrombomodulin, and kininogen [112–122].

Prevalence of Antiphospholipid Antibodies

Although antiphospholipid antibodies are associated with a propensity for thrombosis and with various autoimmune disorders, they can sometimes be found in normal asymptomatic individuals as illustrated by the following observations.

- Normal individuals occasionally have elevated levels of either IgG or IgM aCL [123, 124]. In one study, for example, the prevalence was 5% on a first test but only 2% on retesting [124].
- Increased levels of IgG or IgM aCL have been observed in 12 to 52% of the elderly [123, 125].
- One study found that 40 of 499 normal blood donors had a positive LA test (frequently young women); however, only 3 had elevated levels of aCL [107].

Associated Disorders

Antiphospholipid antibodies have been noted in increased frequency in patients with SLE: approximately 31% of patients have a LA, 23 to 47% have an aCL, and 20% have antibodies to β_2-glycoprotein 1 [105, 126–128]. However, roughly 50% of patients with a LA have SLE [105, 108]. Antiphospholipid antibodies also occur with increased frequency (5–10%) in women with greater than three spontaneous recurrent abortions [106].

Both LA and aCL have also been found in patients with a variety of autoimmune and rheumatic diseases, including [105]:

- Hemolytic anemia
- Idiopathic thrombocytopenic purpura (up to 30%)
- Juvenile arthritis
- Rheumatoid arthritis (7 to 50%)
- Psoriatic arthritis (28%)
- Scleroderma (25%), especially with severe disease [129]
- Behcet's syndrome (20%)
- Sjogren's syndrome (25 to 42%)
- Mixed connective tissue disease (22%)
- Polymyositis and dermatomyositis
- Polymyalgia rheumatica (20%) [130]
- Osteoarthritis (less than 14%)
- Occasionally in gout and in multiple sclerosis
- Chronic discoid LE [131]

- Eosinophilia myalgia and toxic oil syndrome [132]
- Raynaud's phenomenon [133]

Antiphospholipid antibodies have also been noted in patients with infections and after the administration of certain drugs. These are usually IgM aCL antibodies, which may occasionally result in thrombotic events [105, 134]. Furthermore, the antibodies do not appear to have anti-β_2-glycoprotein 1 antibody activity [135]. The infections that have been associated with these antibodies include hepatitis A and C virus (see next section), mumps, bacterial septicemia, HIV infection, syphilis, HTLV-I, malaria, *Pneumocystis carinii*, infectious mononucleosis, and rubella. Among the drugs that have been implicated are phenothiazines (chlorpromazine), phenytoin, hydralazine, procainamide, quinidine, quinine, dilantin, interferon-α, amoxicillin, and propranolol [134, 136]. A discussion of the clinical manifestations of antiphospholipid antibodies is found in Chapters 39–41.

IMMUNOGLOBULINS

SLE is characterized by a polyclonal gammopathy. In fact, increased levels of Ig have been noted in up to 76% of patients [137]. Elevated levels tend to correlate with active disease. Elevations of IgG (including all four IgG subclasses), IgA, IgM, and IgE have been noted. Two series have suggested an increased frequency (3–4%) of IgA deficiency. Acquired hypogammaglobulinemia, especially of IgG subclasses, has rarely been noted, but should be suspected in patients with recurrent infections. The hypogamma IgG may relate to chronic immunosuppressive therapy.

IMMUNE COMPLEXES

SLE is considered to be the prototype for immune complex-mediated diseases. A number of methods have been developed for the detection of immune complexes in serum based on the (highly) variable biological and physical characteristics of these immune complexes. These methods include complement activation, C1q binding, rheumatoid factor binding, the Raji cell assay, conglutinin assay, assay with C3, and cryoprecipitation. This subject is discussed in depth in Chapter 14. While early results suggested good correlation with clinical activity in patients with SLE, especially lupus nephritis, subsequent studies showed highly variable results. These assays are therefore rarely employed nowadays.

COMPLEMENT

Considerable evidence shows that much of the pathology in patients with systemic lupus erythematosus can be attributed to immune complexes [138, 139] and, thereby, complement activation [138, 140]. These immune complexes may either form in the circulation and later deposit in tissues or form *in situ*. Immune complexes may cause tissue inflammation directly or through activation of the complement system. Complement activation causes the release of various mediators, promotes cell interaction, and ultimately results in inflammation. Evaluation of the complement system, which can serve as an indirect measure of the presence of immune complexes, often correlates with clinical aspects of SLE. Monitoring blood levels of complement may be useful in adjusting therapy for the patient.

Measurement of Complement

The complement system can be assessed by (1) the measurement of total hemolytic complement activity (CH50); (2) the immunochemical or hemolytic measurement of individual components; (3) measurements of complement fragments, activation-dependent neoepitopes, and complexes that arise from complement activation; and (4) the determination of complement metabolism. The total hemolytic complement level (CH50) represents the sum of all components of the system. By adding diluted serum to antibody-sensitized sheep erythrocytes and quantitating the amount of released hemoglobin, one can measure this level in units that represent the reciprocal of that dilution of serum that causes 50% of cells to lyse (i.e., CH50). A CH100 represents that dilution of serum that lyses 100% of sensitized cells; this measurement is not nearly as accurate as a CH50, which reflects actual hemolysis in a more linear fashion than a CH100 [141–145].

Separate complement components generally are measured by immunochemical means: radial immunodiffusion, electroimmunodiffusion, or nephelometry. However, such determinations yield no information about the functional biologic integrity or hemolytic potential of the components being measured. Such functional tests of complement components are rarely employed because of their difficulty in routine clinical assays.

When measuring CH50 or components, it is important to remember that some components are thermolabile. Serum stored at room temperature, even for a few days, is adequate for the immunochemical measurement of individual components as proteins (as, for instance, by immunodiffusion). However, it is essential to store samples (preferably EDTA plasma) as soon as possible

at –70°C when measurement of the hemolytic levels of individual components is desired. The CH50 will remain relatively stable for a few hours at room temperature or overnight at –20°C; however, samples are best stored at –70°C as soon as possible. Heating for 30 min at 56°C is also known to inactivate complement components and decrease/abolish CH50 activity.

These assays give but a glimpse of the dynamic state of the complement system. Serial values may be helpful in assessing this dynamic state: falling values suggest more catabolism (e.g., via immune complex fixation) than synthesis, whereas rising levels suggest more synthesis than catabolism is taking place.

Measurement of both the native components and the activation products of individual components is a way of assessing the catabolic state of the complement system without necessarily doing serial measurements. Activation products in EDTA plasma generally are measured by ELISA using monoclonal antibodies. However, the levels of complement activation factors can also be affected by binding to complement (fragment) receptors, degradation by serum proteases, and renal and hepatic clearance [142].

Complement metabolism is a better way of assessing the dynamic state (i.e., synthesis and catabolism) of individual complement components [146]. However, these studies are rarely performed because of the great difficulty both in isolating hemolytically and biologically active purified components and in maintaining their activity after radiolabeling. The liver is the primary source of synthesis of complement components, with the notable exception of C1q, which is probably produced primarily by macrophages [147]. A more complete discussion of complement abnormalities is found in Chapter 6.

ACUTE-PHASE REACTANTS

The acute-phase response is a major pathophysiologic phenomenon that accompanies inflammation [148, 149]. With this reaction, normal homeostatic mechanisms are replaced by new set points, which presumably contribute to defensive or adaptive capabilities. Focus on this phenomenon first occurred with the discovery of elevated serum concentrations of C-reactive protein (CRP) during the acute phase of pneumococcal pneumonia [150].

Despite its name, the acute phase response accompanies both acute and chronic inflammatory states. It can occur in association with a wide variety of disorders, including infection, trauma, infarction, inflammatory arthritides, and various neoplasms.

Acute-phase proteins are defined as those proteins whose plasma concentrations increase (positive acute-phase proteins) or decrease (negative acute-phase proteins) by at least 25% during inflammatory states [151]. These changes largely reflect their production by hepatocytes.

Increases in acute-phase proteins may vary from approximately 50% with ceruloplasmin and several complement components to 1000-fold for CRP and serum amyloid A. Other positive acute-phase proteins include fibrinogen, α_1-antitrypsin, haptoglobin, and ferritin, whereas negative reactants include albumin, transferrin, and transthyretin.

Clinical Relevance of Acute-Phase Reactants

Despite the lack of diagnostic specificity, the measurement of serum levels of acute-phase proteins is useful because it may reflect the presence and intensity of an inflammatory process. Currently, the most widely used indicators of the acute-phase protein response are the erythrocyte sedimentation rate (ESR) and CRP. The rate at which erythrocytes fall through plasma, the ESR, depends largely on the plasma concentration of fibrinogen [152].

These tests may be useful both diagnostically (1) in helping to differentiate inflammatory from noninflammatory conditions and (2) in patient management because they may generally reflect the response to and need for therapeutic intervention.

Comparison of ESR and CRP

Compared to the measurement of CRP, the ESR has the advantages of familiarity, simplicity, and an abundant literature compiled over the past seven decades. However, the ESR has a number of disadvantages compared to the CRP determination.

1. The ESR is only an indirect measurement of plasma acute-phase protein concentrations; it can be influenced greatly by the size, shape, and number of red cells, as well as by other plasma constituents, such as immunoglobulins. Thus, results may be imprecise and sometimes misleading.
2. As a patient's condition worsens or improves, the ESR changes relatively slowly; by comparison, CRP concentrations change rapidly.
3. The range of abnormal values for CRP is greater than for the ESR, with accompanying clinical implications: as an example, among patients with CRP concentrations greater than 10 mg/dl (100 mg/liter), 80 to 85% have bacterial infections [151].

4. Normal values for the ESR are slightly higher among women than men.

Population studies reveal a skewed, rather than normal, distribution of the plasma CRP concentration. Although most normal subjects have CRP concentrations of 0.2 mg/dl (2 mg/liter) or less, some individuals have concentrations as high as 1.0 mg/dl (10 mg/liter). These higher values have been attributed to a modest stimulation by minimally apparent low-grade processes such as gingivitis or trivial injury. This observation has led to the suggestion that values less than 1.0 mg/dl (10 mg/liter) should be regarded as clinically insignificant [151].

Information drawn from the third national health and nutrition evaluation survey indicates that the upper reference limits in a representative sample of the population vary with age, gender, and race [153]. The following formulas provide values for CRP levels that closely approximate the 95th percentile for subjects without identified inflammatory conditions:

- Females: For those 25 to 70 years of age, the upper limit of reference range = (age/65) + 0.7 mg/dl.
- Males: For those 25 to 70 years of age, the upper limit = (age/65) + 0.1 mg/dl.

For most clinical purposes, CRP values less than 0.1 or 0.2 mg/dl can be regarded as normal and values over 1.0 as indicating clinically significant inflammation. However, although CRP is a sensitive reflector of inflammation, it is not specific. Values between 0.2 and 1.0 mg/dl may reflect minor degrees of inflammation, but may also reflect obesity, cigarette smoking, diabetes mellitus, or other noninflammatory causes of modest elevations in the CRP.

Rationale for Employing Multiple Tests

Although elevations in multiple components of the acute-phase response commonly occur together, not all happen uniformly in all patients. Discordance between concentrations of different acute-phase proteins is common; some may be elevated whereas others are not. These variations may be explained by differences in the production of specific cytokines or their modulators in different diseases [150].

Discrepancies between ESR and CRP are found with some frequency. An elevated ESR observed together with a normal CRP is often a false-positive value for the ESR; this may reflect the effects of blood constituents that are not related to inflammation but that can influence the ESR. However, this conclusion is not always valid. As an example, the ESR may be markedly elevated in patients with active SLE, while the CRP is normal.

As there undoubtedly are a number of other clinical situations in which similar discrepancies occur, there probably is no single best laboratory test to reflect inflammation. Currently, the optimal use of acute-phase protein measurements may be to obtain several measurements rather than a single test, the results of which are interpreted in light of the clinical context and the considerations indicated previously.

ESR can be high with no obvious clinical activity and normal with active disease [154]. SLE represents an exception to the generalization that CRP concentrations correlate with the extent and severity of inflammation in patients with rheumatic disorders. Many patients with active SLE do not have elevated CRP concentrations, although they may have marked increases in CRP concentrations during bacterial infection [155]. This finding can be applied to the differential diagnosis of fever in patients with SLE, but one must remember that CRP concentrations are high in patients with active lupus serositis [156] or chronic synovitis [157].

Serum amyloid A levels also tend to be lower in SLE than in other chronic inflammatory disorders, which may explain why secondary amyloidosis is unusual in this disease [158].

HEMATOLOGICAL ABNORMALITIES

Abnormalities of the formed elements of the blood, and of the clotting, fibrinolytic, and related systems, are very common in SLE. The major clinical manifestations are anemia, leucopoenia, and thrombocytopenia. Thus whenever the author sees a patient with SLE he tends to order a CBC.

Anemia

Anemia is a frequent occurrence in SLE, affecting most patients at some time in the course of their disease. Multiple mechanisms contribute, including inflammation, renal insufficiency, blood loss, dietary insufficiency, medications, and immune hemolysis [159–165].

Chronic Inflammation

The most frequent cause of anemia in SLE is suppressed erythropoiesis from chronic inflammation (anemia of chronic disease) and/or renal insufficiency [162, 163]. The anemia is normocytic and normochromic with a relatively low reticulocyte count. Although serum iron levels may be reduced, bone marrow stores are adequate and the serum ferritin concentration is elevated.

As in other chronic illnesses, serum erythropoietin levels may be inappropriately low for the degree of

anemia. However, some of the apparent reduction in serum erythropoietin may be spurious [166].

Blood Loss

Anemia may reflect acute or chronic blood loss from the gastrointestinal tract, usually secondary to medications (nonsteroidal anti-inflammatory drugs or steroids), or may be due to excessive menstrual bleeding. Iron deficiency anemia is not uncommon, especially among teenagers or young women.

Red Cell Aplasia

Red cell aplasia, probably due to antibodies directed against bone marrow erythroblasts, has been rarely observed [163, 164]. This form of anemia may respond to steroids, although cyclophosphamide and cyclosporine have been employed successfully. Even rarer are isolated case reports of aplastic anemia that is presumably mediated by autoantibodies against bone marrow precursors; immunosuppressive therapy may also be effective in this setting [167–169].

In addition, bone marrow suppression can also be induced by medications, including antimalarials and immunosuppressive drugs.

Autoimmune Hemolytic Anemia

Overt autoimmune hemolytic anemia (AIHA), characterized by an elevated reticulocyte count, low haptoglobin levels, increased indirect bilirubin concentration, and a positive direct Coombs' test, has been noted in approximately 10% of patients with SLE [160–162, 170]. Other patients have a positive Coombs' test without evidence of overt hemolysis. The presence of both immunoglobulin and complement on the red cell is usually associated with some degree of hemolysis, whereas the presence of complement alone (e.g., C3 and/or C4) is often not associated with hemolysis [159–162]. The antibodies are "warm," IgG, and are directed against Rh determinants. IgM-mediated cold agglutinin hemolysis is uncommon.

Lupus has also been associated with a thrombotic microangiopathic hemolytic anemia [171]. Most affected patients also have thrombocytopenia, kidney involvement, fever, and neurologic symptoms, producing a picture of thrombotic thrombocytopenic purpura; some do not have fever or neurologic disease, producing a pattern of hemolytic–uremic syndrome. The pathogenesis of this syndrome is not completely understood. In one report of 4 patients plus 24 others identified from a literature review, antiphospholipid antibodies were searched for in 8 and found in 5 [171].

Leukopenia

Leucopoenia is common in SLE and may reflect disease activity. A white blood cell count of less than 4500/μl has been noted in approximately 50% of patients, especially those with active disease [161, 162], whereas lymphocytopenia occurs in approximately 20% [161]. In comparison, a white blood cell count below 4000/μl (the ARA criteria) occurs in only 15 to 20% of patients [161, 172].

Leucopoenia in patients with SLE can result from immune mechanisms, medications (e.g., cyclophosphamide or azathioprine), bone marrow dysfunction, or hypersplenism [161, 162, 172]. Functional defects of neutrophils have also been noted. They are thought to be induced by immune abnormalities (e.g., immune complexes, inhibition of complement-derived chemotactic factors) and/or medications (e.g., corticosteroids) [173, 174].

Although leucopoenia is more common, leukocytosis (mostly granulocytes) can occur in SLE. It is usually due to infection or to the use of corticosteroids (in high doses) [175], but may be seen during acute exacerbations of SLE. A shift of granulocytes to more immature forms (a "left" shift) suggests infection.

White cell constituents other than neutrophils can also be affected in SLE.

1. Lymphocytopenia (less than 1500 cells/μl), especially involving suppressor T cells, has been observed in 20 to 75% of patients, particularly during active disease [159–161, 176]. This finding is strongly associated with IgM, cold reactive, complement fixing, and presumably cytotoxic antilymphocyte antibodies. In one study, for example, these antibodies were noted in 26 of 29 patients with SLE; the antibody titer correlated directly with the degree of lymphopenia. Another potential mechanism of lymphopenia is increased apoptosis, as reflected by the increased expression of Fas antigen on T cells [177].
2. Steroid therapy may result in low absolute eosinophil and monocyte counts [178].
3. The number of basophils may also be decreased in SLE, particularly during active disease [179]. Basophil degranulation with release of platelet-activating factor and other mediators may play a role in immune complex deposition and vascular permeability.

Thrombocytopenia

Mild thrombocytopenia (platelet counts between 100,000 and 150,000/μl) have been noted in 25 to 50% of patients, whereas counts of less than 50,000/μl occur in only 10% [159, 161, 162, 172]. The major mechanism

is increased platelet destruction by antiplatelet antibodies, leading to phagocytosis via their Fc receptors in the spleen, as in idiopathic thrombocytopenic purpura (ITP) [180]. Other important mechanisms in selected patients include bone marrow suppression by immunosuppressive drugs (other than corticosteroids) and increased consumption due to a thrombotic microangiopathy [171] or the antiphospholipid antibody syndrome.

ITP may be the first sign of SLE, followed by other symptoms as long as many years later. It has been estimated that 3 to 15% of patients with apparently isolated ITP go on to develop SLE [181]. Evan's syndrome, i.e., both autoimmune thrombocytopenia and autoimmune hemolytic anemia, may also precede the onset of SLE.

Splenectomy in ITP was originally thought to predispose to the development of SLE [182]. However, this hypothesis was refuted in subsequent studies [183].

Antibodies to Clotting Factors

Antibodies to a number of clotting factors, including VIII, IX, XI, XII, and XIII, have been noted in patients with SLE [159, 160, 172]. These antibodies may not only cause abnormalities of *in vitro* coagulation tests, but may also cause bleeding. Much more common and more important are antiphospholipid antibodies, the presence of which has been associated with a prolongation of the partial thromboplastin time and an increased risk of arterial and venous thrombosis, thrombocytopenia, and fetal loss.

RENAL FUNCTION

This subject is covered in Chapter 30. The author requests a routine urinanlysis most times when lupus patients are seen. In a patient without a history of renal disease, it should be done at least annually. For those patients with renal disease, routine urinanlysis should be done on a more routine basis, the frequency depending on the severity of the renal disease.

Likewise, a serum creatinine should be done at least on an annual basis. In someone with renal disease, it should be measured more frequently depending on the severity of the disease.

The author tends to do periodic GFR (usually as creatinine clearances) in patients with renal disease to monitor their response to therapy as well as progression.

For patients with proteinuria, especially if they are clinically nephritic, the author monitors periodic 24-h urine for protein, although measuring and monitoring serum albumin is easier to do and is just as useful.

SYNOVIAL FLUID

Joint effusions are generally infrequent and small in volume. The synovial fluid viscosity is generally normal and mucin clots are normal [184]. The white blood cell count is usually under 2000 cells/mm^3 and is infrequently over 10,000. Most of the white blood cells tend to be mononuclear. Total protein tends to be elevated, but complement levels are usually depressed markedly, even more so than in blood, suggesting local activation of the complement system (the same is also found in rheumatoid arthritis). Sugar levels tend to be normal, in contrast to rheumatoid arthritis where they are often low. LE cells can be found, but are not diagnostic. ANAs reflect diffusion from plasma.

PLEURAL FLUID

Pleural effusion in SLE is a mild exudate characterized by an elevation in pleural fluid LDH but not signs of marked inflammation. The findings differ from those in other conditions, particularly rheumatoid arthritis.

- The total white cell count (with a predominance of either lymphocytes or polymorphonuclear cells) is lower in lupus-related effusions [185, 186]. However, because there is substantial overlap, the white count in an individual patient cannot be used to establish the diagnosis.
- Pleural glucose levels in lupus effusions are slightly lower than serum blood levels; by comparison, pleural glucose levels in rheumatoid effusions are reduced significantly [185].
- Low complement levels characterize lupus and rheumatoid arthritis effusions.
- The protein concentration tends to be lower in lupus effusions than in patients with RA, reflecting their transudative as well as slight inflammatory nature.

Although the presence of rheumatoid factor or ANA in pleural fluid suggests RA and SLE, respectively, these findings provide no additional diagnostic information beyond that obtained from the measurement of these autoantibodies in serum and should therefore not be performed. Additional causes of pleural effusions, such as infection, congestive heart failure, and uremia, must also be excluded.

PERICARDIAL FLUID

The pericardial fluid is a fibrinous exudate or transudate that may contain antinuclear antibodies, LE cells,

low complement levels, and immune complexes similar to those seen in lupus pleural effusions [187]. The glucose concentration is normal and the protein concentration is variable, being low with a transudate and elevated with an exudate. The pericardium may reveal foci of inflammatory lesions with immune complexes. There is usually a predominance of mononuclear cells, but scarring may be the primary finding in healed disease.

CEREBROSPINAL FLUID

Routine evaluation of the cerebrospinal fluid (CSF) is usually normal in patients with CNS lupus, except in cases of aseptic meningitis and transverse myelitis. Some reports, however, have noted some pleocytosis (usually mononuclear cells), as well as immunologic abnormalities, including elevated levels of total protein, anti-DNA antibodies, IgG, oligoclonal banding, immune complexes, and interleukin-6, which may roughly correlate with neuropsychiatric disease [188–190]. Other antibrain antibodies have been detected, but these assays should be considered strictly research. While considered useful in one paper, measuring complement levels has been shown not to be of any use clinically. CSF examination is performed primarily to exclude infection and/or bleeding.

Acknowledgment

Much of this chapter has been adapted from the relevant chapters in UPTODATE in Rheumatology.

References

1. Kavanaugh, A., Tomar, R., Reveille, J., *et al.* (2000). Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens: American College of Pathologists. *Arch. Pathol. Lab. Med.* **124**, 71.
2. Shen, G., Lal, N., Patnaik, M., and Dardashit, B. (2000). Comparison of EIA screen and IFA for antinuclear antibodies. *Arthritis Rheum.* **43**, S153.
3. Reiff, A., Haubruck, H., and Amos, M. (2002). Evaluation of a recombinant antigen enzyme linked immunosorbent assay (ELISA) in the diagnostics of antinuclear antibodies (ANA) in children with rheumatic disorders. *Clin. Rheum.* **21**, 103.
4. Reichlin, M., and Arnett, F. C., Jr. (1984). Multiplicity of antibodies in myositis sera. *Arthritis Rheum.* **27**, 1150.
5. Rosenberg, A. M. (1988). Clinical associations of antinuclear antibodies in juvenile rheumatoid arthritis. *Clin. Immunol. Immunopathol.* **49**, 19.
6. Petri, M., Karlson., E. W., Cooper, D. S., and Ladenson, P. W. (1991). Autoantibody tests in autoimmune thyroid disease: A case-control study. *J. Rheumatol.* **18**, 1529.
7. Czaja, A. J., Nishioka, M., Morshed, S. A., and Hachiya, T. (1994). Patterns of nuclear immunofluorescence and reactivities to recombinant nuclear antigens in autoimmune hepatitis. *Gastroenterology* **107**, 200.
8. Taylor, S. L., Dean, P. J., and Riely, C. A. (1994). Primary autoimmune cholangitis: An alternative to antimitochondrial antibody-negative primary biliary cirrhosis. *Am. J. Surg. Pathol.* **18**, 91.
9. Rich, S., Kieras, K., Hart, K., *et al.* (1986). Antinuclear antibodies in primary pulmonary hypertension. *J. Am. Coll. Cardiol.* **8**, 1307.
10. Kaplan, M., and Tan, E. (1968). Antinuclear antibodies in infectious mononucleosis. *Lancet* **1**, 561.
11. Burnham, T. (1972). Antinuclear antibodies in patients with malignancies. *Lancet* **2**, 436.
12. Seiner, M., Klein, E., and Klein, G. (1975). Antinuclear reactivity or sera in patients with leukemia and other neoplastic diseases. *Clin. Immunol. Immunopathol.* **4**, 374.
13. Tan, E. M., Feltkamp, T. E., Smolen, J. S., *et al.* (1997). Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum.* **40**, 1601.
14. Solomon, D. H., Kavanaugh, A., Schur, P., Lahita, R., Reveille, J. D., and Sherrer, Y. (2002). Evidence-based guidelines for the use of immunologic tests: Antinuclear antibody testing. *Arthr. Rheumat.* **97**, 434.
15. Buyon, J. P., Ben-Chetrit, E., Karp, S., *et al.* (1989). Acquired congenital heart block: Pattern of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La system in neonatal lupus. *J. Clin. Invest.* **84**, 627.
16. Cook, L. (1998). New methods for detection of antinuclear antibodies. *Clin. Immunol. Immunopathol.* **88**, 211.
17. Hylkema, M. N., van Bruggen, M. C., ten Hove, T., *et al.* (2000). Histone-containing immune complexes are to a large extent responsible for anti-dsDNA reactivity in the Farr assay of active SLE patients. *J. Autoimmun.* **14**, 159.
18. Hahn, B. H. (1998). Antibodies to DNA. *N. Engl. J. Med.* **338**, 1359.

18a. Kavanaugh, A., Solomon, D., *et al.* (2003). Guidelines for immunological laboratory testing in the Rheumatic Diseases: Anti-DNA antibody tests. Submitted for publication.

19. Aareen, L. A., deGroot, E. R., and Feltkamp, T. E. W. (1975). Immunology of DNA III *Crithidia luciliae*: A simple substrate for the determination of anti-dsDNA with the immunofluorescence technique. *Ann. N.Y. Acad. Sci.* **254**, 505.
20. Smeenk, R., Lily, G., and Aarden, L. (1982). Avidity of antibodies to dsDNA: Comparison of IFT on *Crithidia luciliae*, Farr assay and PEG assay. *J. Immunol.* **128**, 73.
21. Crowe, W., Kushner, I., and Clough, J. D. (1978). Comparison of the *Crithidia luciliae*, millipore filter, Farr and hemagglutination methods for detection of antibodies to DNA. *Arthritis Rheum.* **21**, 390.
22. Miller, T. E., Lahita, R. G., Zarro, V. J., *et al.* (1981). Clinical significance of anti-double stranded DNA

antibodies detected by a solid phase immunoassay. *Arthritis Rheum.* **24**, 602.
23. Avina-Zubieta, J. A., Galindo-Rodriguez, G., Kwar-Yeung, L., *et al.* (1995). Clinical evaluation of various selected ELISA kits for the detection of anti-DNA antibodies. *Lupus* **4**, 370.
24. Cervera, R., Kharmasta, M., Font, J., *et al.* (1993). Systemic lupus erythematosus: Clinical and immunological patterns of disease expression in a cohort of 1,000 patients. *Medicine (Baltimore)* **72**, 113.
25. Ronsuke, S., Evans, M., and Abou, N. (1991). Idiotypic and immunochemical differences of anti-DNA antibodies of lupus patients during active and inactive disease. *Clin. Immunol. Immunopathol.* **61**, 320.
26. Schur, P. H., and Sandson, J. (1968). Immunologic factors and clinical activity in systemic lupus erythematosus. *N. Engl. J. Med.* **278**, 553.
27. Rothfield, N. F., and Stollar, B. D. (1967). The relation of immunoglobulin class, pattern of antinuclear antibody, and complement-fixing antibodies to DNA in sera from patients with systemic lupus erythematosus. *J. Clin. Invest.* **46**, 1785.
28. Bush, H., Reddy, R., Ruthblum, L., and Choy, Y. C. (1982). SnRNAs, SnRNPs, and RNA processing. *Annu. Rev. Biochem.* **51**, 617.
29. Lloyd, W., and Schur, P. (1981). Immune complexes, complement and anti-DNA in exacerbations of systemic lupus erythematosus (SLE). *Medicine (Baltimore)* **60**, 208.

29a. Swaak, A. J., Groenwold, J., and Bronsveld, W. (1986). Predictive value of complement profiles and anti-dsDNA in systemic lupus erythematosus. *Ann. Rheum. Dis.* **45**, 359.

30. Ter Borg, E. J., Horst, G., Hummel, *et al.* (1990). Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. *Arthritis Rheum.* **33**, 634.
31. Gladman, D. D., Urowitz, M. B., and Keystone, E. C. (1979). Serologicially active clinically quiescent systemic lupus erythematosus. *Am. J. Med.* **66**, 210.
32. Winfield, J., Brunner, C., and Koffler, D. (1978). Serologic studies in patients with systemic lupus erythematosus and central nervous system dysfunction. *Arthritis Rheum.* **21**, 289.
33. Koffler, D., Agnello, V., Winschester, R., and Kunkel, H. G. (1973). The occurrence of single-stranded DNA in the serum of patients with systemic lupus erythematosus and other diseases. *J. Clin. Invest.* **52**, 198.
34. Miles, S., and Isenberg, D. A. (1993). A review of serological abnormalities in the relatives of patients with systemic lupus erythematosus. *Lupus* **2**, 145.
35. Tan, E. M., and Kunkel, H. G. (1966). Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. *J. Immunol.* **96**, 464.
36. Karata, N., and Tan, E. M. (1976). Identification of antibodies to nuclear acid antigens by counterimmunoelectrophoresis. *Arthritis Rheum.* **19**, 574.
37. Houtman, P. M., Kallenberg, C. G. M., Lumburg, P. C., *et al.* (1985). Quantitation of antibodies to nucleoribonucleoprotein by ELISA: Relations between antibody levels and disease activity in patients with connective tissue disease. *Clin. Exp. Immunol.* **62**, 696.
38. Barad, F. A., Jr., Andrews, B. S., Davis, J. S., and Taylor, R. P. (1981). Antibodies to Sm in patients with systemic lupus erythematosus: Correlation of Sm antibody titers with disease activity and other laboratory parameters. *Arthritis Rheum.* **24**, 1236.
39. Beaufils, M., Kouki, F., Mignon, F., *et al.* (1983). Clinical significance of anti-Sm antibodies in systemic lupus erythematosus. *Am. J. Med.* **74**, 201.
40. Arnett, F. C., Hamilton, R. G., Roebber, M. G., *et al.* (1988). Increased frequencies of Sm and nRNP autoantibodies in American blacks compared to whites with systemic lupus erythematosus. *J. Rheumatol.* **15**, 1773.
41. Homma, M., Mimori, T., Takeda, Y., *et al.* (1987). Autoantibodies to the Sm antigen: Immunological approach to clinical aspects of systemic lupus erythematosus. *J. Rheumatol.* **14**(Suppl. 13), 188.
42. Takeda, Y., Wang, G., Wang, R., *et al.* (1989). Enzyme-linked immunosorbent assay using isolated (U) small nuclear ribonucleoprotein polypeptides as antigens to investigate the clinical significance of autoantibodies to these polypeptides. *Clin. Immunol. Immunopathol.* **50**, 213.
43. Winn, D., Wolfe, J., Lindberg, D., *et al.* (1979). Identification of a clinical subset of systemic lupus erythematosus by antibodies to the Sm antigen. *Arthritis Rheum.* **22**, 1334.
44. Reinitz, E., Grayzel, A., and Barland, P. (1977). Specificity of Smith antibodies. *Arthritis Rheum.* **20**, 693.
45. Janwityanuchit, S., Verasertniyom, O., Vanichapuntu, M., and Vatanasuk, M. (1993). Anti-Sm: Its predictive value in systemic lupus erythematosus. *Clin. Rheumatol.* **12**, 350.
46. Sanchez-Guerrero, J., Lew, R. A., Fossel, A. H., and Schur, P. H. (1996). Utility of anti-Sm, anti-RNP, anti-Ro/SS-A, and anti-La/SS-B (extractable nuclear antigens) detected by enzyme-linked immunosorbent assay for the diagnosis of systemic lupus erythematosus. *Arthritis Rheum.* **39**, 1055.
47. Reveille, J. D., Solomon, D. H., Schur, P., and Kavanaugh, A. (2003). Evidence based guidelines for the use of immunological laboratory tests: Anti-Ro (SS-A) and La (SS-B). Submitted for publication.
48. Provost, T. T., Watson, R., and Simmons-O'Brien, E. (1996). Significance of the anti-Ro (SS-A) antibody in the evaluation of patients with cutaneous manifestations of a connective tissue disease. *J. Am. Acad. Dermatol.* **35**, 147.
49. Clark, G., Reichlin, M., and Tomeri, T. (1969). Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythematosus. *J. Immunol.* **102**, 117.
50. Alspaugh, M. A., and Tan, E. M. (1975). Antibodies to cellular antigens in Sjêgren's syndrome. *J. Clin. Invest.* **55**, 1067.

51. Alspaugh, M., and Maddison, P. (1979). Resolution of the identity of certain antigen-antibody systems in systemic lupus erythematosus and Sjêgren's syndrome: An interlaboratory collaboration. *Arthritis Rheum.* **22**, 796.
52. Barakat, S., Meyer, G., Torterotot, F., *et al.* (1992). IgG antibodies from patients with primary Sjêgren's syndrome and systemic lupus erythematosus recognize different epitopes in 60-Kd-A/Ro protein. *Clin. Exp. Immunol.* **89**, 38.
53. Maddeson, P., Mogavero, H., Provost, T. T., *et al.* (1979). The clinical significance of autoantibodies to a soluble cytoplasmic antigen in systemic lupus erythematosus and other connective tissue diseases. *J. Rheumatol.* **6**, 189.
54. Lockshin, M. D., Bonja, E., Elkon, K., and Druzen, M. L. (1988). Neonatal lupus risk to newborns of mothers with systemic lupus erythematosus. *Arthritis Rheum.* **31**, 697.
55. Buyon, J. P., Ben-Chetrit, E., Karp, S., *et al.* (1989). Acquired congenital heart block: Pattern of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La system in neonatal lupus. *J. Clin. Invest.* **84**, 627.
56. Garcia, S., Nascimento, J. H., Bonfa, E., *et al.* (1994). Cellular mechanism of the conduction abnormalities induced by serum from anti-Ro/SSA positive patients in rabbit heart. *J. Clin. Invest.* **93**, 718.
57. Maddison, P., Provost, T., and Reichlin, M. (1981). Serological findings in patients with "ANA-negative" systemic lupus erythematosus. *Medicine* **60**, 87.
58. Blomberg, S., Ronnblom, L., Wallgren, A. C., *et al.* (2000). Anti-SSA/Ro antibody determination by enzyme-linked immunosorbent assay as a supplement to standard immunofluorescence in antinuclear antibody screening. *Scand. J. Immunol.* **51**, 612.
59. Scofield, R. H., Farris, A. D., Horsfall, A. C., and Harley, J. B. (1999). Fine specificity of the autoimmune response to the Ro/SSA and La/SSB ribonucleoproteins. *Arthritis Rheum.* **42**, 199.
60. Hardin, J. (1986). The lupus autoantigens and the pathogenesis of systemic lupus erythematosus. *Arthritis Rheum.* **29**, 457.
61. Burlingame, R., Rubin, R., Balderas, R., and Theofilopoulos, A. (1993). Genesis and evolution of antichromatin autoantibodies in murine lupus implicates T-dependent immunization with self antigen. *J. Clin. Invest.* **91**, 1687.
62. Amoura, Z., Piette, J.-C., Bach, J.-F., and Koutouzov, S. (1999). The key role of nucleosomes in lupus. *Arthritis Rheum.* **42**, 833.
63. Chabre, H., Amoura, Z., Piette, J., *et al.* (1995). Presence of nucleosome-restricted antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **38**, 1485.
64. Massa, M., de Benedetti, F., and Pignatti, P. (1994). Anti-double-stranded DNA, antihistone and antinucleosome IgG reactivities in children with systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **12**, 219.
65. Tupchong, M., Wither, J., Hallett, D., and Gladman, D. (1997). Anti-nucleosome antibodies as markers for renal disease in lupus nephritis. *Arthritis Rheum.* **40**, 304.
66. Amoura, Z., Koutouzov, S., Chabre, H., *et al.* (2000). Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases: Antinucleosome antibodies of the IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 76.
67. Dreyfuss, G., Matunis, M. J., Pinol-Roma, S., and Burd, C. G. (1993). hnRNP proteins and the biogenesis of mRNA. *Annu. Rev. Biochem.* **62**, 289.
68. Montecucco, C., Caporali, R., Negri, C., *et al.* (1990). Antibodies from patients with rheumatoid arthritis and systemic lupus erythematosus recognize different epitopes of a single heterogeneous nuclear RNP core protein. *Arthritis Rheum.* **33**, 180.
69. Hassfeld, W., Steiner, G., Sludnicka-Benke, A., *et al.* (1995). Autoimmune response to the spliceosome: An immunologic link between rheumatoid arthritis, mixed connective tissue disease, and systemic lupus erythematosus. *Arthritis Rheum.* **38**, 777.
70. Biamonti, G., Ghigna, C., Caporali, R., and Montecucco, C. (1998). Heterogeneous nuclear ribonucleoproteins (hnRNPs): An emerging family of autoantigens in rheumatic diseases. *Clin. Exp. Rheum.* **16**, 317.
71. Fritzler, M. J., McCarty, G. A., Ryan, J. P., and Kinsella, T. D. (1983). Clinical features of patients with antibodies directed against proliferating cell nuclear antigen. *Arthritis Rheum.* **26**, 140.
72. Roos, G., Landberg, G., Huff, J. P., *et al.* (1993). Analysis of the epitopes of proliferating cell nuclear antigen recognized by monoclonal antibodies. *Lab. Invest.* **68**, 204.
73. Rayno, K., and Reichlin, M. (2000). Evaluation of assays for the detection of autoantibodies to the ribosomal P proteins. *Clin. Immunol.* **95**, 99.
74. Elkon, K. B., Parnassa, A. P., and Foster, C. L. (1985). Lupus autoantibodies target ribosomal P proteins. *J. Exp. Med.* **162**, 459.
75. Francour, A. M., Peebles, C. I., Heckman, K. J., *et al.* (1985). Identification of ribosomal protein autoantigens. *J. Immunol.* **135**, 2878.
76. Bonfa, E., and Elkon, K. B. (1986). Clinical and serologic associations of the antiribosomal P protein antibody. *Arthritis Rheum.* **29**, 981.
77. Schneebaum, A. B., Singleton, J. D., West, S. G., and Blodgett, J. K. (1991). Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am. J. Med.* **90**, 54.
78. Koffler, D., Miller, T. E., and Lahita, R. G. (1979). Studies on the specificity and clinical correlation of antiribosomal antibodies in systemic lupus erythematosus sera. *Arthritis Rheum.* **22**, 463.
79. Bonfa, E., Golombek, S., Kaufman, L., *et al.* (1987). Association between lupus psychosis and antiribosomal P protein antibodies. *N. Engl. J. Med.* **317**, 265.
80. Sato, T., Uchiumi, T., Ozawa, T., *et al.* (1991). Autoantibodies against ribosomal proteins found with high frequency in patients with systemic lupus erythematosus with active disease. *J. Rheumatol.* **18**, 1681.
81. Koren, E., Reichlin, M. W., Koscec, M., *et al.* (1992). Autoantibodies to the ribosomal P proteins react with a

plasma membrane-related target on human cells. *J. Clin. Invest.* **89**, 1236.
82. Van Dam, A., Nossent, H., de Jong, J., *et al.* (1991). Diagnostic value of antibodies against ribosomal phosphoproteins: A cross sectional and longitudinal study. *J. Rheumatol.* **18**, 1026.
83. Nojima, Y., Minota, S., Yamada, A., *et al.* (1992). Correlation of antibodies to ribosomal P protein with psychosis in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 1053.
84. Reichlin, M., Broyles, T. F., Hubscher, O., *et al.* (1999). Prevalence of autoantibodies to ribosomal P proteins in juvenile-onset systemic lupus erythematosus compared with the adult disease. *Arthritis Rheum.* **42**, 69.
85. Teh, L., Bedwell, A., Isenberg, D., *et al.* (1992). Antibodies to protein P in systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 489.
86. Derksen, R. H., van Dam, A. P., Gmelig Meyling, F. H., *et al.* (1990). A prospective study on antiribosomal P proteins in two cases of familial lupus and recurrent psychosis. *Ann. Rheum. Dis.* **49**, 779.
87. Hulsey, M., Goldstein, R., Scully, L., *et al.* (1995). Antiribosomal P antibodies in systemic lupus erythematosus: A case-control study correlating hepatic and renal disease. *Clin. Immunol. Immunopathol.* **74**, 252.
88. Arnett, F. C., and Reichlin, M. (1995). Lupus hepatitis: An under-recognized disease feature associated with autoantibodies to ribosomal P. *Am. J. Med.* **99**, 465.
89. Chindalore, V., Neas, B., and Reichlin, M. (1998). The association between anti-ribosomal P antibodies and active nephritis in systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **87**, 292.
90. Ghirardello, A., Doria, A., Zampieri, S., *et al.* (2000). Anti-ribosomal P protein antibodies detected by immunoblotting in patients with connective tissue diseases: Their specificity for SLE and association with IgG anticardiolipin antibodies. *Ann. Rheum. Dis.* **59**, 975.
91. Miyachi, K., and Tan, E. (1979). Antibodies reacting with ribosomal ribonucleoprotein in connective tissue diseases. *Arthritis Rheum.* **22**, 981.
92. Quismorio, F. P. (1993). Other serologic abnormalities in SLE. *In* "Dubois' Lupus Erythematosus" (D. J. Wallace and B. H. Hahn, eds.), 4th Ed., Lea & Febiger, Philadelphia. pp. 264–276.
93. Karpatkin S., Schur, P. H., Strick, N., and Siskind, G. W. (1973). Heavy chain subclass of human antiplatelet antibodies. *Clin. Immunol. Immunopathol.* **2**, 1.
94. Dixon, R. H., and Rosse, W. F. (1975). Platelet antibody in auto-immune thrombocytopenia. *Br. J. Hematol.* **31**, 129.
95. Hymes, K., Schur, P. H., and Karpatkin, S. (1980). Heavy-chain subclass of bound antiplatelet IgG autoimmune hemolytic anemia. *Blood* **56**, 84.
96. Sammaritano, L. R., Gharavi, A. E., and Lockshin, M. D. (1990). Antiphospholipid antibody syndrome: Immunologic and clinical aspects. *Semin. Arthritis Rheum.* **20**, 81.
97. Santoro, S. A. (1994). Antiphospholipid antibodies and thrombotic predisposition: Underlying pathogenetic mechanisms. *Blood* **83**, 2389.
98. Roubey, R. A. (1994). Autoantibodies to phospholipid-binding plasma proteins: A new view of lupus anticoagulants and other "antiphospholipid" autoantibodies. *Blood* **84**, 2854.
99. Rauch, J. (1998). Lupus anticoagulant antibodies: Recognition of phospholipid-binding protein complexes. *Lupus* **7**(Suppl. 2), S29.
100. Roubey, R. A. (1996). Immunology of the antiphospholipid antibody syndrome. *Arthritis Rheum.* **39**, 1444.
101. Ginsberg, J. S., Wells, P. S., Brill-Edwards, P., *et al.* (1995). Antiphospholipid antibodies and venous thromboembolism. *Blood* **86**, 3685.
102. Asherson, R. A., Khamashta, M. A., Ordi-Ros, J., *et al.* (1989). The "primary" antiphospholipid syndrome: Major clinical and serological features. *Medicine* **68**, 366.
103. Mackworth-Young, C. G., Loizov, S., and Walport, M. J. (1989). Antiphospholipid antibodies and disease. *Q. J. Med.* **72**, 767.
104. Sammaritano, L. R., Ng, S., Sobel, R., *et al.* (1997). Anticardiolipin IgG subclasses: Association of IgG2 with arterial and/or venous thrombosis. *Arthritis Rheum.* **40**, 1998.
105. McNeil, H. P., Chesterman, C. N., and Krilis, S. A. (1991). Immunology and clinical importance of antiphospholipid antibodies. *Adv. Immunol.* **49**, 193.
106. Melk, A., Mueller Eckhardt, G., Polten, B., *et al.* (1995). Diagnostic and prognostic significance of anticardiolipin antibodies in patients with recurrent spontaneous abortions. *Am. J. Reprod. Immunol.* **33**, 228.
107. Shi, W., Krilis, S. A., Chong, B. H., *et al.* (1990). Prevalence of lupus anticoagulant in a healthy population: Lack of correlation with anticardiolipin antibodies. *Aust. N. Z. J. Med.* **20**, 231.
108. Triplett, D. A., Brandt, J. T., and Musgrave, K. A. (1988). Relationship between lupus anticoagulants and antibodies to phospholipids. *JAMA* **259**, 550.
109. Cucurull, E., Gharavi, A. E., Diri, E., *et al.* (1999). IgA anticardiolipin and anti-beta(2)-glycoprotein I are the most prevalent isotypes in African American patients with systemic lupus erythematosus. *Am. J. Med. Sci.* **318**, 55.
110. Day, H. M., Thiagarajan, P., Ahn, C., *et al.* (1998). Autoantibodies to beta2-glycoprotein I in systemic lupus erythematosus and primary antiphospholipid antibody syndrome: Clinical correlations in comparison with other antiphospholipid antibody tests. *J. Rheumatol.* **25**, 667.
111. Galli, M. (2000). Should we include anti-prothrombin antibodies in the screening for the antiphospholipid syndrome? *J. Autoimmun.* **15**, 101.
112. Puurunen, M., Vaarala, O., Julkunen, H., Aho, K., *et al.* (1996). Antibodies to phospholipid-binding plasma proteins and occurrence of thrombosis in patients with SLE. *Clin. Immunol. Immunopathol.* **80**, 16.
113. Shibata, S., Harpel, P. C., Gharavi, A., *et al.* (1994). Autoantibodies to heparin from patients with antiphospholipid syndrome inhibit formation of antithrombin III-thrombin complexes. *Blood* **83**, 2532.

114. Vaarala, O. (1996). Antiphospholipid antibodies and atherosclerosis. *Lupus* **5**, 442.
115. Galli, M. (1996). Non beta 2-glycoprotein cofactors for antiphospholipid antibodies. *Lupus* **5**, 388.
116. Wu, R. H., Nityanand, S., Berglund, L., *et al.* (1997). Antibodies against cardiolipin and oxidatively modified LDL in 50-year-old men predict myocardial infarction. *Arterioscler. Thromb. Vasc. Biol.* **17**, 3159.
117. Satoh, A., Suzuki, K., Takayama, E., *et al.* (1999). Detection of anti-annexin IV and V antibodies in patients with antiphospholipid syndrome and systemic lupus erythematosus. *J. Rheumatol.* **26**, 1715.
118. Song, K. S., Park, Y. S., and Kim, H. K. (2000). Prevalence of anti-protein S antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **43**, 557.
119. Munoz-Rodriguez, F. J., Reverter, J, C., Font, J., *et al.* (2000). Prevalence and clinical significance of antiprothrombin antibodies in patients with systemic lupus erythematosus or with primary antiphospholipid syndrome. *Haematologica* **85**, 632.
120. Tektonidou, M. G., Petrovas, C. A., Ioannidis, J. P. A., *et al.* (2000). Clinical importance of antibodies against platelet activation factor in antiphospholipid syndrome manifestations. *Eur. J. Clin. Invest.* **30**, 646.
121. Cugno, M., Dominguez, M., Cabibbe, M., *et al.* (2000). Antibodies to tissue-type plasminogen activator in plasma from patients with primary antiphospholipid syndrome. *Br. J. Haematol.* **108**, 871.
122. Gromnica-Ihle, E., and Schlosser, W. (2000). Antiphospholipid syndrome. *Int. Arch. Allergy. Immunol.* **123**, 67.
123. Fields, R. A., Toubbeh, H., Searles, R. P., and Bankhurst, A. D. (1989). The prevalence of anticardiolipin antibodies in a healthy elderly population and its association with antinuclear antibodies. *J. Rheumatol.* **16**, 623.
124. Vila, P., Hernandez, M. C., Lopez-Fernandez, M. F., and Battle, J. (1994). Prevalence, follow-up and clinical significance of the anticardiolipin antibodies in normal subjects. *Thromb. Haemost.* **72**, 20.
125. Manoussakis, M. N., Tzioufas., A. G., Silis, M. P., *et al.* (1987). High prevalence of anticardiolipin and other autoantibodies in a healthy elderly population. *Clin. Exp. Immunol.* **68**, 557.
126. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and non-SLE disorders. *Ann. Intern. Med.* **112**, 682.
127. Abu-Shakra, M., Gladman, D., Urowitz, M. B., and Farewell, V. (1995). Anticardiolipin antibodies in systemic lupus erythematosus: Clinical and laboratory correlations. *Am. J. Med.* **99**, 624.
128. Sebastiani, G. D., Galeazzi, M., Tincani, A., *et al.* (1999). Anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus: Prevalence and clinical associations. *Scand. J. Rheumatol.* **28**, 344.
129. Picillo, U., Migliaresi, S., Marcialis, M. R., *et al.* (1995). Clinical significance of anticardiolipin antibodies in patients with systemic sclerosis. *Autoimmunity* **20**, 1.
130. Chakravarty, K., Pountain, G., Merry, P., *et al.* (1995). A longitudinal study of anticardiolipin antibody in polymyalgia rheumatica and giant cell arteritis. *J. Rheumatol.* **22**, 1694.
131. Ruffatti, A., Veller-Fornasa, C., Patrassi, G. M., *et al.* (1995). Anticardiolipin antibodies and antiphospholipid syndrome in chronic discoid lupus erythematosus. *Clin. Rheumatol.* **14**, 402.
132. Carreira, P. E., Montalvo, M. G., Kaufman, L. D., *et al.* (1997). Antiphospholipid antibodies in patients with eosinophilia myalgia and toxic oil syndrome. *J. Rheumatol.* **24**, 69.
133. Vayssairat, M., Abuaf, N., Baudot, N., *et al.* (1998). Abnormal IgG cardiolipin antibody titers in patients with Raynaud's phenomenon and/or related disorders: Prevalence and clinical significance. *J. Am. Acad. Dermatol.* **38**, 555.
134. Triplett, D. A. (1998). Many faces of lupus anticoagulants. *Lupus* **7**(Suppl. 2), S18.
135. McNally, T., Purdy, G., Mackie, I. J., *et al.* (1995). The use of anti-beta 2-glycoprotein-I assay for discrimination between anticardiolipin antibodies associated with infection and increased risk of thrombosis. *Br. J. Haematol.* **91**, 471.
136. Merrill, J. T., Shen, C., Gugnani, M., *et al.* (1997). High prevalence of antiphospholipid antibodies in patients taking procainamide. *J. Rheumatol.* **24**, 1083.
137. Wallace, D. J. (1993). Serum and plasma protein abnormalities and other clinical laboratory determinations in SLE. *In* "Dubois' Lupus Erythematosus" (D. J. Wallace and B. H. Hahn, eds.), 4th Ed., pp. 457–460. Lea & Febiger. Philadelphia.
138. Gatenby, P. A. (1991). The role of complement in the aetiopathogenesis of systemic lupus erythematosus. *Autoimmunity* **11**, 6166.
139. Porcel, J. M., and Vergani, D. (1992). Complement and lupus: Old concepts and new directions. *Lupus* **1**, 343.
140. Pickering, M. C., and Walport, M. J. (2000). Links between complement abnormalities and systemic lupus erythematosus. *Rheumatology* **39**, 133.
141. Schur, P. H. (1977). Complement testing in the diagnosis of immune and autoimmune disease. *Am. J. Clin. Pathol.* **68**, 647.
142. Oppermann, M., Hopken, U., and Gotze, O. (1992). Assessment of complement activation in vivo. *Immunopharmacology* **24**, 119.
143. Porcel, J. M., Peakman, M., Senaldi, G., and Vergani, D. (1993). Methods for assessing complement activation in the clinical immunology laboratory. *J. Immunol. Methods* **157**, 19.
144. Walport, M. J. (2001). Advances in Immunology: Complement. *N. Engl. J. Med.* **344**, 1140.
145. Walport, M. J. (2001). Advances in Immunology: Complement. *N. Engl. J. Med*. **344**, 1140.
146. Ruddy, S., Carpenter, C. B., Chin, K. W., Knostman, J. N., Soter, N. A., Gotze, O., Muller-Eberhard, H., and Austen, K. F. (1975). Human complement metabolism: An analysis of 144 studies. *Medicine* **54**, 165.

147. Colten, H. R. (1976). Biosynthesis of complement. *Adv. Immunol.* **22**, 67.
148. Kushner, I. (1982). The phenomenon of the acute phase response. *Ann. N. Y. Acad. Sci.* **389**, 39.
149. Gabay, C., and Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340**, 448.
150. Tillet, W. S., and Francis, T. J. (1930). Serological reactions in pneumonia with a non-protein somatic fraction of pneumococcus. *J. Exp. Med.* **52**, 561.
151. Morley, J. J., and Kushner, I. (1982). Serum C-reactive protein levels in disease. *Ann. N. Y. Acad. Sci.* **389**, 406.
152. Bedell, S. E., and Bush, B. T. (1985). Erythrocyte sedimentation rate: From folklore to facts. *Am. J. Med.* **78**, 1001.
153. Wener, M. H., Daum, P. R., and McQuillan, G. M. (2000). The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. *J. Rheumatol.* **27**, 2351.
154. Wallace, D. J. (1993). Serum and plasma protein abnormalities and other clinical laboratory determinations in SLE. *In* "Dubois' Lupus Erythematosus" (D. J. Wallace and B. H. Hahn eds.), 4th Ed., pp. 457–460. Lea and Febiger, Philadelphia.
155. Pepys, M. B., Lanham, J. G., and De Beer, F. C. (1982). C-reactive protein in SLE. *Clin. Rheum. Dis.* **8**, 91.
156. ter Borg, E. J., Horst, G., Limburg, P. C., *et al.* (1990). C-reactive protein levels during disease exacerbations and infections in systemic lupus erythematosus: A prospective longitudinal study. *J. Rheumatol.* **17**, 1642.
157. Moutsopoulos, H. M., Mavridis, A. K., Acritidis, N. C, and Avgerinos, P. C. (1983). High C-reactive protein response in lupus polyarthritis. *Clin. Exp. Rheumatol.* **1**, 53.
158. De Beer, F. C., Mallya, R. K., Fagan, E. A., *et al.* (1982). Serum amyloid-A protein concentration in inflammatory diseases and its relationship to the incidence of reactive systemic amyloidosis. *Lancet.* **2**, 231.
159. Laurence, J., Wong, J. E., and Nachman, R. (1992). The Cellular hematology of systemic lupus erythematosus. *In* "Systemic Lupus Erythematosus" (R. G. Lahita, ed.), 2nd Ed. Churchill Livingstone, New York.
160. Shoenfeld, Y., and Ehrenfeld, M. (1996). Hematologic manifestations. *In* "The Clinical Management of Systemic Lupus Erythematosus" (P. H. Schur, ed.), 2nd Ed. Lippincott, Philadelphia.
161. Nossent, J. C., and Swaak, A. J. (1991). Prevalence and significance of haematological abnormalities in patients with SLE. *Q. J. Med.* **80**, 605.
162. Keeling, D. M., and Isenberg, D. A. (1993). Haematological manifestations of SLE. *Blood Rev.* **7**, 199.
163. Liu, H., Ozaki, K., Matsuzaki, Y., *et al.* (1995). Suppression of haematopoiesis by IgG autoantibodies from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **100**, 480.
164. Cavalcant, J., Shadduck, R. K., Winkelstein, A., *et al.* (1978). Red-cell hypoplasia and increased bone marrow reticulin in systemic lupus erythematosus: Reversal with corticosteroid therapy. *Am . J. Hematol.* **5**, 253.
165. Voulgarelis, M., Kokori, S. I., Ioannidis, J. P., *et al.* (2000). Anaemia in systemic lupus erythematosus: Aetiological profile and the role of erythropoietin. *Ann. Rheum. Dis.* **59**, 217.
166. Schett, G., Firbas, U., Fureder, W., *et al.* (2001). Decreased serum erythropoietin and its relation to anti-erythropoietin antibodies in anaemia of systemic lupus erythematosus. *Rheumatology (Oxford)* **40**, 424.
167. Winkler, A., Jackson, R. W., Kay, D. S., *et al.* (1988). High dose intravenous cyclophosphamide treatment of systemic lupus erythematosus associated aplastic anemia. *Arthritis Rheum.* **31**, 693.
168. Brooks, B. J., Jr., Broxmeyer, H. E., Bryan, C. F., and Leech, S. H. (1984). Serum inhibitor in systemic lupus erythematosus associated with aplastic anemia. *Arch. Intern. Med.* **144**, 1474.
169. Roffe, C., Cahill, M. R., Samanta, A., *et al.* (1991). Aplastic anemia in systemic lupus erythematosus: A cellular immune mechanism? *Br. J. Rheumatol.* **30**, 301.
170. Kokori, S. I., Ioannidis, J. P., Voulgarelis, M., *et al.* (2000). Autoimmune hemolytic anemia in patients with systemic lupus erythematosus. *Am. J. Med.* **108**, 198.
171. Nesher, G., Hanna, V. E., Moore, T. L., *et al.* (1994). Thrombotic microangiopathic hemolytic anemia in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **24**, 165.
172. Budman, D. R., and Steinberg, A. D. (1977). Hematologic aspects of systemic lupus erythematosus: Current concepts. *Ann. Intern. Med.* **86**, 220.
173. Perez, H. D., Lipton, M., and Goldstein, I. M. (1978). A specific inhibitor of complement (C5)-derived chemotactic activity in serum from patients with systemic lupus erythematosus. *J. Clin. Invest.* **62**, 29.
174. Abramson, S. B., Gren, W. P., Eddson, H. S., and Weissman, G. (1983). Neutrophil aggregation induced by sera from patients with active systemic lupus erythematosus. *Arthritis Rheum.* **26**, 630.
175. Boumpas, D. T., Chrousos, G. P., Wilder, R. L., *et al.* (1993). Glucocorticoid therapy for immune-mediated diseases: Basic and clinical correlates. *Ann. Intern. Med.* **119**, 1198.
176. Rivero, S. J., Diaz-Jouanen, E., and Alarcon-Segovia, D. (1978). Lymphopenia in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 295.
177. Amasaki, Y., Kobayashi, S., Takeda, T., *et al.* (1995). Up-regulated expression of Fas antigen (CD 95) by peripheral naive and memory T cell subsets in patients with SLE: A possible mechanism for lymphopenia. *Clin. Exp. Immunol.* **99**, 245.
178. Isenberg, D. A., Patterson, K. G., Todd-Pokropek, A., *et al.* (1982). Haematological aspects of SLE: A reappraisal using automated methods. *Acta Haematol.* **67**, 242.
179. Camussi, G., Tetta, C., Coda, R., and Benveniste, J. (1981). Release of platelet-activating factor in human pathology. I. Evidence for the occurrence of basophil degranulation

and release of platelet-activating factor in systemic lupus erythematosus. *Lab. Invest.* **44**, 241.
180. Pujol, M., Ribera, A., Vilardell, M., *et al.* (1995). High prevalence of platelet autoantibodies in patients with SLE. *Br. J. Haematol.* **89**, 137.
181. Karpatkin, S. (1980). Autoimmune thrombocytopenic purpura. *Blood* **56**, 329.
182. Dameshek, W., and Reeves, W. H. (1956). Exacerbation of lupus erythematosus following splenectomy in "idiopathic" thrombocytopenic purpura and autoimmune hemolytic anemia. *Am. J. Med.* **21**, 560.
183. Best, W. R., and Darling, D. R. (1962). A critical look at the splenectomy SLE controversy. *Med. Clin. North Am.* **46**, 19.
184. Ziminski, C. M. (1996). Musculoskeletal manifestations. *In* "The Clinical Management of Systemic Lupus" (P. H. Schur, ed.), 2nd Ed., pp. 47–65. Lippincott, Philadelphia.
185. Quismorio, F. P., Jr. (1988). Clinical and pathologic features of lung involvement in systemic lupus erythematosus. *Semin. Respir. Dis.* **9**, 297.
186. Turner-Stokes, L., and Turner-Warwick, M. (1982). Intrathoracic manifestations of SLE. *Clin. Rheum. Dis.* **8**, 229.
187. Hunder, G. G., Mullen, B. J., and McDuffie, F. C. (1974). Complement in pericardial fluid of lupus erythematosus. *Ann. Intern. Med.* **80**, 453.
188. West, S. G., Emlen, W., Wener, M. H., and Kotzin, B. L. (1995). Neuropsychiatric lupus erythematosus: A 10-year prospective study on the value of diagnostic tests. *Am. J. Med.* **99**, 153.
189. Hirohata, S., and Miyamoto, T. (1990). Elevated levels of interleukin-6 in cerebrospinal fluid from patients with systemic lupus erythematosus. *Arthritis Rheum.* **33**, 644.
190. McLean, B. N., Miller, D., and Thompson, E. J. (1995). Oligoclonal banding of IgG in CSF, blood-brain barrier function, and MRI findings in patients with sarcoidosis, SLE, and Behcet's disease involving the nervous system. *J. Neurol. Neurosurg. Psych.* **58**, 548.

22

LUPUS PREGNANCY

Michael D. Lockshin
Lisa R. Sammaritano
Sergio Schwartzman

NORMAL AND ABNORMAL PREGNANCY

Definition of Pregnancy

Normal pregnancy differs from nonpregnant normality in signs, symptoms, and hormonal and imaging tests. A woman has *presumptive* pregnancy when she has nausea, disturbances in urination, fatigue, and cessation of menses. Later she will have enlargement of the abdomen, changes in the cervix and uterus, and positive tests for human chorionic gonadotropin (hCG). *Conclusive* signs of pregnancy are the identification of fetal heart action on ultrasound, recognition of the embryo and fetus at any time in pregnancy by ultrasound (vaginal ultrasound in early pregnancy), and perception of fetal movements.

False-positive diagnosis of pregnancy occurs with tumors of the pelvis or uterus (myomas, adenomyosis). A false-positive elevated level of hCG occurs with an hCG-secreting tumor, self-injection of hCG, and systemic lupus erythematosus (SLE) itself [1]. Hyperprolactinemia due to drug induction (e.g., phenothiazines), pituitary tumor, or lymphocytic hypophysitis may simulate pregnancy by causing amenorrhea; hyperprolactinemia also occurs in SLE patients. Amenorrhea with normal hCG and normal prolactin may result from anovulation, emotional disorders, or menopause. Imaginary pregnancy, or pseudocyesis, is usually seen in women nearing menopause or in women with an intense desire to become pregnant. Patients develop typical subjective symptoms, increased abdominal size (due to fat deposition or increased bowel gas), breast changes, and morning sickness. The delusion of pregnancy may persist for years in emotionally distressed women [2].

Special Issues

Ectopic Pregnancy

Ectopic (extrauterine) pregnancy occurs in 1% of all pregnancies; of these, more than 95% are in the oviduct. Mechanical causes of ectopic pregnancy include salpingitis, adhesions, and other anatomic abnormalities. Functional causes include external migration of the ovum, uterine reflux, and altered tube mobility, especially in the setting of progestin-only contraceptives [3] and possibly nonsteroidal anti-inflammatory drugs (NSAID) [4]. Ectopic pregnancy is the second leading cause of maternal morbidity in the United States and the leading cause of maternal morbidity in the first trimester [5]. Signs and symptoms include pelvic pain, amenorrhea with spotting, a hCG value that is lower than expected, and confirmation by abdominal and/or vaginal sonography. Treatment is surgical, often requiring removal of the oviduct [3].

Twin Pregnancy

Dizygotic twin pregnancy results from fertilization of two separate ova; monozygotic twin pregnancy, which occurs one-third as often, is the result of a division of a single fertilized ovum. Diagnosis is almost always based

on ultrasound. With the use of assisted reproductive techniques, rates of twinning have increased. Twin pregnancies have greater than normal rates of fetal malformation, restricted fetal growth, preterm delivery, and spontaneous abortion. Preterm delivery is the major reason for the increased risk of neonatal death and morbidity in twins.

On occasion, one twin succumbs early in pregnancy, but pregnancy continues with one living fetus. At delivery the dead fetus and placenta may be compressed (*fetus compressus*) or flattened (*fetus papyraceous*). Disseminated intravascular coagulation and hypofibrinogenemia may be complications. Incidence of twin resorption after 20 weeks gestation is about 5%, with high likelihood for continuation of the pregnancy and viability of the remaining twin [6, 7].

Pregnancy at the Extremes of Reproductive Life

The mean age of menarche is now 12.8 years [8]. In the older teenage population, obstetrical risks are related to poverty, inadequate nutrition, and poor health before pregnancy. For teenagers 15 years old and less, complications are also related to age. The major risks are preeclampsia, low birth weight infants, and poor neonatal outcome [9].

Maternal complications of women over 35 include chronic and pregnancy-induced hypertension, diabetes, thrombosis, and postpartum pulmonary edema. Late pregnancy bleeding is increased. The risk of Cesarean section doubles after age 35. The maternal mortality rate for this group is increased fourfold because of preexisting medical conditions. The risk of ectopic pregnancy increases with age as well.

Fetal complications of women over 35 include macrosomia, congenital malformations and chromosomal abnormalities, spontaneous abortion, stillbirths, preterm delivery, and restricted fetal growth. Perinatal and infant mortality is also somewhat higher [10].

Other Special Problems

While the dates of conception (the start of pregnancy) may be easily ascertained, the precise point at which a woman is no longer *postpartum* is not at all well defined. The *puerperium* is the period of confinement during and just after birth. By popular use, this term describes the 6 weeks following delivery during which uterine involution occurs and the female reproductive tract returns anatomically to a nonpregnant state. All body systems do not return to the prepregnancy status during this period, however. For example, most women return to their prepregnancy weight 6 months after delivery. Menses may return in 6 to 8 weeks in the nonlactating woman, but ovulation is less frequent in women who breast feed. *Postpartum* depression, felt to be intrinsically related to hormonal shifts of pregnancy, is defined as such if it begins up to 6 months after childbirth [11]. Bone recovery may take up to 2 years. Return of the immune system to its prepregnancy state is not defined.

Physiologic Adaptations in Pregnancy Influencing the Course of Lupus

Gestational changes relevant to lupus fall into four major groups: (1) volume and blood pressure (leading to cardiac, renal, or hypertensive complications), (2) coagulation control mechanisms (thrombocytopenias and circulating anticoagulants), (3) immunologic (complement kinetics, immune complexes, humoral and cellular immunity), and (4) hormonal [12]. These changes may alter the interpretation of phenomena otherwise attributed to SLE or may make an SLE patient unusually susceptible to complications of pregnancy (Table 1).

Volume and Blood Pressure Changes

Lupus patients with renal, vascular, or cardiac compromise may not be able to tolerate the increase in intravascular volume (about 30%) that occurs during normal pregnancy.

TABLE 1 Phenomena of Pregnancy Influencing the Interpretation or Management of Lupus

Normal pregnancy change	Effect on management of lupus
Hematologic	
Hemodilutional anemia	Suggests disease exacerbation
Decreased platelet survival	Worsens disease-induced thrombocytopenia
Increased fibrinogen	Suggests inflammation
Increased factor VIII	Suggests vasculitis
Immunologic	
Immune complexes present	Suggests disease exacerbation
Increased complement	Modifies interpretation of disease-induced hypocomplementemia
Endocrine	
Increased cortisol	?Induces remission, decreased cortisol *postpartum* results in flare
High estrogen	?Worsens SLE
Renal	
Increased clearance	If clearance does not increase, fluid overload occurs; preeclampsia *versus* disease exacerbation may be hard to distinguish
Joints	
Ligamentous loosening	Effusion mimics active arthritis

Thrombocytopenia and Coagulation Factors

In normal pregnancy, platelet survival decreases and plasma levels of fibrinogen and factors V, VIII, and X increase, as does von Willebrand factor, platelet activation inhibitor-1, thrombin–antithrombin complex, prothrombin fragment 1 and 2, d-dimer, and tissue plasminogen activator. Free and total protein S and possibly antithrombin III decrease. Normally, platelet production increases, usually resulting in no net change in the peripheral platelet count, but thrombocytopenia may occur even in uncomplicated pregnancy [13]. In pathologic pregnancy, intravascular coagulation systems may be activated [14]. Thus thrombocytopenia accompanying lupus may be difficult to interpret during pregnancy.

Pregnancy-Induced Immunologic Changes

In pregnancy, cell-mediated immunity is generally depressed, as reflected by abnormal lymphocyte stimulation and other functional tests, decreased T-cell–B-cell ratios, increased suppressor–helper T-cell ratios, and decreased lymphocyte–monocyte ratios [15]. Cutaneous and humoral immune responses, including responses to specific microbial antigens, are selectively depressed, as are cellular measures of inflammation, such as leukocyte chemotaxis and adhesion. Autoantibodies occur in apparently normal young women, particularly in those with complicated gestations, but they do not appear to be specifically induced by pregnancy [16–18]. The number of immunoglobulin-secreting cells increases, inflammatory responses decrease, some plasma proteins increase and others decrease, and circulating immune complexes are often present.

Pregnancy-specific proteins such as α-fetoprotein, β_1-glycoprotein, α_2-macroglobulin, and others suppress lymphocyte function, as do increases in endogenous corticosteroids. Although their roles are not completely understood, interleukins-1 and -3 (IL-1, IL-3), tumor necrosis factor α (TNF-α), interferon-γ (IFN-γ), granulocyte–macrophage colony-stimulating factor (GM-CSF), and other cytokines may be critical in sustaining pregnancy [19]. IL-3, for instance, is diminished in women with repeated pregnancy loss [20]. This is relevant in that the production or metabolism of some cytokines is thought to be abnormal in rheumatic illness.

In SLE, circulating and local levels of cytokines, including IL-1, IL-2, and IFN-γ, may be constitutively or secondarily abnormal [21]. In SLE pregnancy, T-cell function may paradoxically normalize.

In normal pregnancy, total C3, C4, and CH_{50} are usually similar or raised relative to nonpregnant levels. These increments likely reflect an increase in complement synthesis rather than a decrease in activation because the measurement of some classic pathway activation products (C3a, C4a, C5a, but not of the C1s–C1 inhibitor complex) suggests that low-grade classic pathway activation, i.e., increased degradation, occurs in normal pregnancy. Disease-associated decreased complement synthesis may not return to normal during pregnancy [22, 23]. Changes induced by gestation can either falsely normalize (if synthesis does increase) or exaggerate (if synthesis does not increase) disease-related hypocomplementemia. In a mouse model, complement activation *in situ* in the placenta must be inhibited for pregnancy to progress normally [24].

Pregnancy-Induced Hormonal Changes

Estrogen, prolactin, and other hormone levels rise in pregnancy (Fig. 1) [25]. Estrogens upregulate and androgens downregulate T-cell responses and immunoglobulin synthesis [26]. IL-1, IL-2, IL-6, and TNF-α can be regulated by sex hormones. How or if these changes affect lupus in a pregnant woman is unknown.

Non-SLE Pregnancy Complications: Maternal

Hypertensive Disorders: Preeclampsia, Eclampsia, Pregnancy-Induced Hypertension, HELLP Syndrome

Hypertension in pregnancy may have several presentations. The impact of hypertension may be negligible or severe.

Pregnancy-induced hypertension is defined by a blood pressure of 140/90 that develops as a consequence of pregnancy and that regresses *postpartum*. It may include proteinuria, edema, proteinuria, and convulsions.

Coincidental hypertension refers to chronic hypertension that antedates pregnancy or persists *postpartum*. Chronic hypertension is one of the most common medical complications of pregnancy. Risks of coincidental hypertension include preeclampsia, eclampsia, and *abruptio placentae*. The fetus is at risk of growth restriction and death. Underlying hypertension that is worsened by pregnancy, including preeclampsia and/or eclampsia, is termed *pregnancy-aggravated hypertension*. It usually occurs after 24 weeks gestation. Preeclampsia in a chronic hypertensive patient is termed superimposed preeclampsia and is usually severe, developing earlier and often accompanied by fetal growth restriction.

Transient hypertension develops after the middle trimester of pregnancy. It is characterized by mild

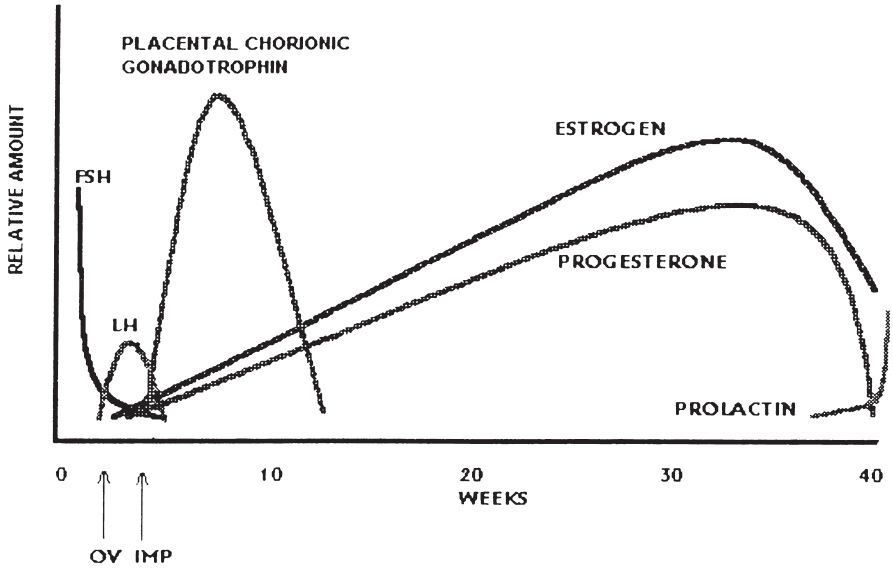


FIGURE 1 Hormonal changes of pregnancy. The pituitary (FSH, LH) controls early pregnancy and lactation (prolactin). Placental hormones (chorionic gonadotropin) are dominant early after implantation, and ovarian hormones (estrogen, progesterone) are dominant in late pregnancy. Ovulation (OV) occurs 2 weeks after the last menstrual period, implantation (IMP) at 3 weeks.

TABLE 2 Common Pregnancy Complications Unrelated to SLE

Complication	Clinical manifestations
Preeclampsia, eclampsia	Proteinuria, hypertension, edema, hyperuricemia, thrombocytopenia, renal insufficiency, abdominal pain, encephalopathy, convulsion
Pregnancy-induced hypertension	Hypertension, proteinuria, edema, renal insufficiency
HELLP syndrome	Hemolysis, elevated liver enzymes, thrombocytopenia (low platelets), abdominal pain, hepatic failure
Thrombocytopenia	Thrombocytopenia
Erythema nodosum gravidarum	Erythema nodosum, leukocytoclastic vasculitis
Chorea gravidarum	Chorea
Herpes gestationis	Pruritic skin rash superficially similar to discoid lupus, IgG and complement at dermal–epidermal junction

elevations of blood pressure that do not compromise the pregnancy. It usually regresses *postpartum* but may return in subsequent gestations. By definition, transient hypertension is diagnosed retrospectively [27, 28].

Preeclampsia, also known as toxemia, is the condition in which an apparently normal woman develops, usually late in pregnancy, hypertension, proteinuria, edema, and hyperuricemia. Untreated, preeclampsia can progress to seizures, in which case the syndrome is named *eclampsia*. Patients with advanced preeclampsia appear systemically ill. They may be vasoconstricted, diaphoretic, and encephalopathic. They may develop other visceral disease, e.g., hepatic infarction or congestive heart failure. Thombocytopenia, often severe, is common. Complement levels may be low. In its eclamptic phase, preeclampsia may be lethal. Antihypertensives, sedatives, and delivery are the cornerstones of treatment.

Preeclampsia can be confused with active SLE. In both preeclampsia and SLE, the patient feels generally ill. Urinary protein rises rapidly a 24-h excretion of 2 to 25 g is common. A patient may go from normal to nephrotic range proteinuria within days. In a preeclamptic lupus patient, proteinuria, abdominal pain, neurologic symptoms, and thrombocytopenia may be interpreted correctly or incorrectly as active lupus.

The acronym of the *HELLP syndrome* stands for *h*emolysis, *e*levated *l*iver enzymes, *l*ow *p*latelets. Experts in this field disagree about the relationship between the HELLP syndrome and preeclampsia. Both occur in late pregnancy and both are associated with hypertension, thrombocytopenia, and liver abnormalities. Patients with this syndrome suffer prominent hepatic enzyme abnormalities (AST and ALT often in the thousands), fever, hepatic pain, and encephalopathy. Often they are very ill. The thrombocytopenia can be life-threatening. As in preeclampsia, fetal and, in severe cases, maternal death may occur. The HELLP syndrome may worsen *postpartum* for several weeks. Some have suggested that

severe preeclampsia and/or the HELLP syndrome is especially frequent in patients with lupus and antiphospholipid syndrome, but detailed epidemiologic studies have not yet been done [29]. (An association of antiphospholipid antibody with thrombotic microangiopathic hemolytic anemia is generally accepted.) Short of ending the pregnancy and providing general supportive care, there is no effective treatment. Cases of HELLP syndrome that continued after delivery have resolved with plasmapheresis [30].

Thrombocytopenia (Anemia, Leukocytosis)

Normal pregnant women become modestly anemic (hematocrit approximately 33) in the third trimester. The apparent lowered blood count is due to hemodilution as the plasma volume increases. Modest leukocytosis may occur, particularly near the normal end of pregnancy and not be meaningful. More problematic is the regular occurrence of thrombocytopenia in late normal pregnancy. This happens in approximately 8% of normal women. Usually of trivial importance, it may reach levels in which hemorrhage is threatened. It self-corrects upon delivery and usually needs no specific treatment. Evaluation for other causes of thrombocytopenia may be necessary.

Hypercoagulability

Pregnancy is associated with increased intravascular coagulation [31]. Pregnancy induces increases in concentrations of the coagulation factors I (fibrinogen), II (prothrombin), VII, VIII, IX, and X. Protein S levels decrease, and the fibrinolytic system is inhibited in the third trimester [32]. As a result, and coupled with increased venous stasis, venous compression by the gravid uterus, and bed rest, the risk of venous thromboembolism is increased five times higher in pregnancy, especially in the third trimester and immediately postpartum. Particularly after a difficult labor, pelvic thrombophlebitis and pulmonary embolization are feared complications. In pelvic thrombophlebitis, clinical indicators in the legs do not occur. Pelvic discomfort and persistent fever within the first few days following delivery are the most reliable symptoms. In contrast, the risks of both cerebral infarction and intracerebral hemorrhage are reported to be increased in the 6 weeks *after* delivery but not during pregnancy itself [33]. "Silent" deficiencies in antithrombin III, protein C, protein S, and the presence of the factor V_{Leiden} mutation all add to the risk of thrombosis in pregnancy [34, 35]. Procoagulant states other than APS also predispose women to recurrent fetal loss, including factor V Leiden, prothrombin 20210 mutation, antithrombin III, and heritable deficiencies of protein C and protein S [36]. Hyperhomocysteinemia due to the C677T MTHFR (homozygous) genotype has resulted in placental thrombosis with stillbirth [37]. Any of these in combination with aPL may increase risk in an additive manner. A recent case report, for example, describes a patient with antiphospholipid and anti-protein S antibodies, factor V Leiden, and recurrent thromboses and abortion [38]. The presence of antiphospholipid antibody may impose an additional risk on pregnant patients.

Increased indicators of platelet activation, prothrombin fragments 1 and 2, and d-dimer all suggest low-grade activation of the coagulation system in normal pregnancy. This seldom is clinically evident, but in cases of amniotic fluid embolization, dead fetus, or retained placenta or fetus, lethal disseminated intravascular coagulation may occur.

Cardiac Disease

Heart disease complicates up to 1% of pregnancies. Previously, rheumatic heart disease was most common but now *congenital heart lesions* constitute at least half of all heart disease encountered in pregnancy [39]. The spectrum of heart disease in lupus pregnancy includes surgically corrected heart disease, valve disease, septal defects, cyanotic heart disease, and pulmonary hypertension. In SLE patients, Libman–Sacks endocarditis is a risk. *Peripartum cardiomyopathy* may occur (during or shortly after labor), often with no apparent etiology. Prognosis is generally poor [40].

Ischemic heart disease is a relatively rare complication of pregnancy, usually associated with classic risk factors but occasionally associated with the antiphospholipid syndrome [41]. Prognosis for a myocardial infarction during the course of pregnancy itself is serious, with 40% mortality in the third trimester. The advisability of undertaking pregnancy after a myocardial infarction is unclear [42].

Diabetes Mellitus

Diabetes during pregnancy may be preexisting or may develop during the course of gestation. A standard classification scheme aids in diagnosis and treatment. *Classes A_1 and A_2* are gestational in onset and are treated with diet alone or diet with insulin, depending on the fasting and 2-h postprandial glucose. *Classes B, C, D, F, R, and H* represent diabetes that predates pregnancy. Classification depends on age of onset, duration of disease, and presence of associated complications. All are treated with insulin and diet [43].

Gestational diabetes is usually a disorder of late pregnancy. Hyperglycemia during the first trimester

reflects overt or preexisting diabetes. Risk factors for a mother to develop gestational diabetes include age over 30 years, history of a prior macrosomic infant, obesity, hypertension, and glucosuria. Screening is with a 1-h (50 g) glucose tolerance test done between 24 and 28 weeks. A plasma value of greater than 140 mg/dl at 1 h indicates the need for a 3-h, 100-g glucose tolerance test [44].

Preexisting, overt diabetes has a more significant impact on pregnancy outcome. The likelihood of successful outcome is related to the degree of underlying maternal cardiovascular or renal disease. Effects on the fetus include spontaneous abortion, malformation, unexplained fetal demise, oligohydramnios, neonatal death and morbidity, respiratory distress, hypoglycemia, hypocalcemia, hyperbilirubinemia, cardiac hypertrophy, long-term cognitive developmental abnormalities, and altered fetal growth. Maternal effects include diabetic nephropathy, retinopathy and neuropathy, preeclampsia, ketoacidosis, and predisposition to infection [45].

Erythema Nodosum Gravidarum, Herpes Gestationis, and Other Rare Complications

Rashes of pregnancy mimic rashes of rheumatic disease and may suggest lupus activation. *Erythema nodosum gravidarum* in clinical appearance, evolution, and histology is identical to erythema nodosum occurring in women who are not pregnant. It is a legitimate question whether this entity is a coincidental appearance of an otherwise common disease. Evidence supporting pregnancy as a specific trigger of erythema nodosum is recurrence in sequential pregnancies. Usually mild, it requires little or no treatment. If treatment is necessary, aspirin or a low-dose steroid for a brief period is sufficient. *Leukocytoclastic vasculitis* in a pregnant woman differs in no important way from leukocytoclastic vasculitis in a woman who is not pregnant; treatment in most cases is optional.

Herpes gestationis is an immunological illness of uncertain cause. It causes a pruritic rash, usually on the proximal extremities. The rash may be extensive. Clinically recognizable by an experienced physician, it resembles discoid lupus erythematosus and may confuse persons who have not seen it before. On biopsy, herpes gestationis demonstrates immunoglobulin deposits at the dermal–epidermal junction, a further point of confusion. These deposits result from antibody directed at the 180-kDa extracellular domain of the bullous pemphigoid antigen of the basement membrane zone. The antibody is strikingly IgG4, which can be identified by a blood or immunofluorescence test. Pathologists unaware of this rare illness may wrongly interpret dermal–epidermal deposits as being indicative of lupus. There is no satisfactory treatment; the condition disappears when pregnancy ends. It recurs with subsequent pregnancies. In one report, a patient had *chronic* herpes gestationis in association with antiphospholipid antibody syndrome. The patient did not respond to corticosteroid, but both conditions responded well to cyclophosphamide administered *postpartum* [46].

Liver Disease in Pregnancy

Many liver conditions induced by pregnancy disappear following delivery. *Intrahepatic cholestasis* of pregnancy, characterized by cholestasis and/or icterus, usually occurs late in pregnancy and is likely stimulated by the high estrogen concentrations in susceptible individuals [47]. *Acute fatty liver of pregnancy* is a rare (1 in 10,000 pregnancies) complication of pregnancy that may be fatal for both mother and fetus [48]. Gross examination of the liver in fatal cases reveals a small soft yellow greasy organ, with swollen hepatocytes filled with microvesicular fat and minimal hepatocellular necrosis. The pathogenesis is unknown. Mean gestational age at onset is 27.5 weeks. It is more common in nulliparous women, women with a male fetus, and women with multifetal gestations. Clinical symptoms include an onset of anorexia, nausea and vomiting, epigastric pain and jaundice, accompanied by hypofibrinogenemia, prolonged clotting, hyperbilirubinemia, and elevation of serum transaminases [49]. Hepatic coma occurs in up to 60% of cases, severe coagulopathy in 55%, and renal failure in 55%. Fetal death is common at this severe stage. Recovery, when it occurs, is almost complete. Management assumes that delivery is essential for cure, in addition to intensive medical support. If hepatic failure does not resolve, liver transplantation is an alternative. The condition does not usually recur in subsequent pregnancies.

Maternal Bone Health; Bone Physiology in Pregnancy and Lactation

Both pregnancy and lactation have a significant impact on bone metabolism. Calcium metabolism is altered as a consequence of a change in the maternal hormonal milieu, the needs of the developing fetus and neonate, weight gain and exercise, and compensatory mechanisms known to affect bone turnover.

The parathyroid hormone remains normal or decreases during pregnancy [50]. Osteocalcin remains in the normal range during the first trimester, declines during the second trimester, and returns to normal during the third trimester [51]. Serum alkaline phosphatase increases gradually in the first and second trimesters and rises rapidly in the third trimester, in part because of the addition of placental alkaline phos-

phatase [52]. Parathyroid hormone-related protein (PTHrP) levels during pregnancy are approximately double those observed during the nonpregnant state [53]. Free 1,25-dihydroxyvitamin D, vitamin D-binding proteins, and bound dihydroxyvitamin D levels all increase during pregnancy [54].

The clinical implications of these changes are controversial. Studies examining bone density during pregnancy have been inconsistent and have demonstrated either a decrease in bone density or no change. Osteoporosis of pregnancy may exist as an uncommon idiopathic entity with resultant fractures [55]. The characteristic risk factors for this group of women, however, are unknown.

Lactation places a significant calcium demand on maternal bone. Approximately 168 to 280 mg/day of calcium are expended during breast feeding. Studies on bone density and fracture risk during lactation, however, have been inconsistent with some studies demonstrating no change [56], others demonstrating a protective effect [57], and yet others demonstrating a significant decrease in bone mass [58].

Placenta and Abruptio Placentae

An understanding of *normal placental physiology and morphology* is critical to understanding the pathological changes that occur in the placenta during pregnancy.

The placenta brings maternal and fetal blood in close relationship. The placenta is partly of fetal origin (the *trophoblast*) and partly of maternal origin (transformation of the uterine mucosa). The fetal trophoblast ensures implantation; it is composed of two layers; the inner cellular layer (*cytotrophoblast*) and the exterior syncytial layer (*syncytiotrophoblast*). The placenta is well defined by the third month. At term it weighs 500 g and is 20 cm in diameter. The human placenta is *villous, hemochorial*, and *chorioallantoic*, meaning that the placental villi are bathed directly in maternal blood and are traversed by vessels coming from the allantoic circulation of the fetus. While maintenance of pregnancy prior to implantation is assured by both ovarian and pituitary hormones, after implantation the placenta assumes an important role in hormone production: chorionic gonadotrophins are discernible several days after nidation, peak at the 60th day, and then fall to low levels until completion of pregnancy [59].

Antepartum bleeding occurs in many otherwise normal pregnancies but puts the patient at increased risk for poor outcome, even though the bleeding may be self-limited [60]. *Abruptio placentae* is premature separation of the normally implanted placenta, usually presenting as a vaginal hemorrhage. However, bleeding may be retained between the placenta and the uterus as a concealed hemorrhage. The frequency of placental abruption is estimated at 1 in 150 deliveries. The perinatal fetal mortality rate is 20%. Etiology is unknown, but incidence increases with maternal age and is higher in women of greater parity. Placental abruption is associated most commonly with pregnancy-induced or chronic hypertension. The risk of recurrent abruption in a subsequent pregnancy is high. Severe abruption may be accompanied by maternal shock, consumption coagulopathy, and renal failure. Management varies with gestational age and status of the mother and the fetus [61].

Placenta previa is a separation of the placenta implanted in the immediate vicinity of the cervical canal. The degree of previa depends largely on cervical dilatation at the time of examination. Digital palpation of the cervix with progressive dilatation may provoke severe hemorrhage. Although serious, the condition is uncommon (0.3%) [62]. As with placental abruption, multiparity and advancing maternal age increase the likelihood of this condition. Clinical symptoms include painless hemorrhage, often at the end of the second trimester and without warning. Associated coagulopathy is rare. Diagnosis is best made with a sonogram. Management depends on gestational age, onset of labor, and severity of hemorrhage. Delivery is always by Cesarean section. The prognosis has improved dramatically in recent years [63].

THE PREGNANT LUPUS PATIENT

Planning for Pregnancy

Communicating with the Obstetrical and Perinatal Team

Except in large centers with special interests, obstetricians, perinatalogists, and rheumatologists generally do not have great experience with lupus pregnancies. It is therefore wise for all interested parties, including the parents, to discuss together predictable complications and outcomes early during the pregnancy, before conception if possible, and to maintain close contact during the pregnancy. In particular, points of conflicting treatment philosophies should be explored and resolved. Common differences between obstetrical and rheumatological opinions include the use of corticosteroids; use of aspirin and heparin; treatment thresholds for proteinuria, hypertension, and thrombocytopenia; frequency and quality of fetal monitoring; and prophylaxis for antiphospholipid antibody. There should be clear prospective agreement about physician aggressiveness in the event of maternal flare near the point of fetal viability. The parents, rheumatologist, obstetrician, and

perinatalogist should also discuss in advance what will be done if there is a choice to deliver a 25-week fetus, with complete maternal safety, or to try to hold to 28 weeks at high risk to the mother. Trying to discuss these points, particularly if there are important differences of opinion, is extremely difficult at the point of crisis.

When Is Pregnancy Best Undertaken?

Advising couples about pregnancy is always difficult and very individualized. Considerations include the mother's current health, the mother's anticipated future health, her physical and emotional ability to care for a child, her desires, her partner's desires, her parents' desires, and many other factors. As a general rule, the longer the patient is in remission, the better her chances for completing a pregnancy without exacerbation. Whether flare rates in pregnant patients are higher, the same as, or lower than flare rates in patients who are not pregnant is still debated (see later).

Patients currently in flare become pregnant. While flare does considerably complicate pregnancy for both mother and child, it is possible for a woman to complete pregnancy even when she is in extreme flare or otherwise seriously ill. The issues to discuss when a woman in flare contemplates or becomes pregnant are her survival, the infant's survival, the doctor's ability to use a full complement of available drugs, fetal damage that may occur with extreme prematurity, and ability of the mother subsequently to care for her (well or ill) child. Included in the latter consideration is the mother's mental and physical state on high-dose prednisone, e.g., the likelihood of her having labile mood or psychosis.

Other than to say that well patients do better than do ill patients, there is no best answer for timing of pregnancy in a lupus patient. Full and very individualized discussion and consensus among all the treating physicians (including obstetricians, nephrologists, hematologists, rheumatologists, perinatalogists, orthopedists, and primary care physicians), both parents, and other concerned relatives is the best way to plan a pregnancy. The discussion should include open statements of known and perceived risks, advantages of proceeding at this or a subsequent point, and the values and priorities of each participant. For instance, if the mother anticipates needing cyclophosphamide within 2 years, is it an advantage to attempt pregnancy now? Do both parents, the obstetrician, and the treating physician agree about the value of attempting to save a 500-g, 24-week infant if the mother is rapidly worsening?

When Is Pregnancy Inadvisable?

Even in full remission, pregnancy "rocks the boat," i.e., introduces a new variable into an already complex situation. Thus pregnancy should always be considered to be adding risk, albeit in many cases the risk is small. Inadvisability is a relative issue. A physician should take a neutral stance, explaining the risks, but not judging a patient's motives, when faced with a patient with bad prognostic indicators who has inflexible convictions regarding her proposed or current pregnancy.

Pregnancy is truly inadvisable only when there is high probability of maternal or fetal death or disability and very little likelihood of a healthy child. Circumstances of this type include severe hypertension, progressive renal failure, patient's current use of teratogenic or abortifacient drugs such as cyclophosphamide or methotrexate, severe central nervous system disease, severe thrombocytopenia, and moderate-to-severe cardiopulmonary disease. Another consideration might be severe thrombosis, usually in women with antiphospholipid antibody, during a previous pregnancy or during use of contraceptive pills.

Initial Assessment

When a patient first announces her wish to consider pregnancy, it is incumbent on her physician to survey expeditiously for pertinent autoantibodies and for dysfunction of relevant organ systems. Table 3 indicates recommended initial and subsequent laboratory investigations.

Clinical evaluation should assure competency or normality of blood pressure, kidneys (by creatinine clearance and 24-h urine protein), cardiac and pulmonary function (by clinical examination), platelet count, and musculoskeletal system, particularly the pelvis and hips. To anticipate and prevent complications, and to advise the patient, the physician should consider the effects on the patient of pregnancy's normal increase in blood volume and cardiac output, diminution of lung volume, and increase in platelet utilization. The clinician should also consider the effect of ligamentous loosening on unstable joints. Testing for anti-Ro/SSA and anti-La/SSB antibodies (predictors of neonatal lupus syndrome) and antiphospholipid antibody and lupus anticoagulant (predictors of pregnancy loss) is also appropriate at this time.

Discontinuing Drugs

There are no absolute rules regarding discontinuation of drugs, even those known to cause fetal harm, prior to pregnancy. Intense bargaining with the patient, weighing the potential danger of recurrent disease in the mother against potential fetal toxicity, is mandatory. Controlled trial data regarding the recurrence rate of symptoms following elective drug withdrawal in

TABLE 3 Monitoring of the Pregnant SLE Patient[a]

Recommended frequency	Monitoring test
First visit	Complete blood count, including platelets Urinalysis Creatinine clearance 24-h urine protein Anticardiolipin antibody Lupus anticoagulant Anti-SSA/Ro and anti-SSB/La antibodies Anti-dsDNA antibody Complement (C3 and C4 or CH_{50})
Monthly	Platelet count[b]
Each trimester	Creatinine clearance[b] 24-h urine protein if screening urinalysis abnormal[b] Anticardiolipin antibody Complement[b] Anti-dsDNA antibody[b]
Weekly (last trimester, mothers with antiphospholipid antibody)	Antenatal fetal heart rate testing ("nonstress test"), periodic biophysical profile
At 18 and 25 weeks (mothers with anti-SSA/Ro and anti-SSB/La antibodies)	Fetal echocardiogram, ?fetal electrocardiogram

[a] The erythrocyte sedimentation rate is often abnormal in uncomplicated pregnancy and is not useful to follow.
[b] More frequently if abnormal.

asymptomatic patients exist only for hydroxychloroquine [64] and for warfarin [65] (Table 4).

In deciding to withdraw a medication, the physician must have knowledge of its persistence in the body. Antimalarial drugs last for months. If the physician *and the patient* agree that a medication is no longer necessary, and that withdrawal is appropriate prior to attempting pregnancy, then an interval between complete withdrawal and pregnancy should be long enough to accomplish the withdrawal and to watch for a possible flare. Thus, ideally, hydroxychloroquine should be discontinued 6 months in advance; 3 months to allow the drug to be fully excreted and 3 months to observe for disease exacerbation. Special care must be taken with drugs such as cyclophosphamide, methotrexate, and warfarin that are teratogenic, as discontinuation after confirmation of pregnancy may be too late. A patient taking warfarin might consider switching to heparin while she is attempting to conceive. As with other aspects of patient care, careful review of all options and individualization of advice is the best plan. General advice regarding common drugs is displayed in Table 5.

Prophylactic Treatment

There is no documented evidence that increasing corticosteroid therapy solely because of pregnancy improves maternal or fetal prognosis. The authors do not employ such prophylactic treatment.

TABLE 4 Risk of Discontinuing Therapy, Two Studied Drugs

Drug	Study interval	Symptom	Recurrence rate after drug withdrawn	Recurrence rate, drug continued
Hydroxychloroquine [64]	6 months	Flare	73%	36%
Warfarin [65]	12 months	Thrombosis	130%	10%

TABLE 5 Advice Regarding Discontinuation of Commonly Used Drugs before and during Pregnancy

Drug	Prior to pregnancy	During pregnancy
Prednisone	Continue at lowest effective dose	Continue at lowest effective dose
Aspirin (anti-inflammatory dose)	Continue at lowest effective dose	Discontinue or use low-dose prednisone
NSAIDs	Continue at lowest effective dose	Discontinue or use low-dose prednisone
Azathioprine	Consider withdrawing	Consider withdrawing
Cyclosporine	Consider withdrawing	Consider withdrawing
Mycophenolate mofetil	Consider withdrawing	Consider withdrawing
Cyclophosphamide	Discontinue	Discontinue
Methotrexate	Discontinue	Discontinue
Hydroxychloroquine	Consider discontinuing 6 months before	Consider discontinuing
Warfarin	Switch to heparin when pregnancy attempted	Switch to heparin

Measurement of Flare in Pregnancy

Global Flare

Identifying the flare of SLE [66] during pregnancy is particularly difficult because pregnancy-induced thrombocytopenia and proteinuria resemble SLE disease activity. A new or increasing rash, lymphadenopathy, arthritis, fever, or anti-dsDNA antibody are the strongest reasons to attribute urinary or hematologic abnormalities to SLE rather than to preeclampsia.

There have been several recent attempts to answer the specific question of whether pregnancy causes SLE to flare, but there is no agreement as yet [67–70]. The most recent study found higher flare rates in pregnant lupus patients compared to nonpregnant controls and to the patients themselves after pregnancy. Flares were most frequent in the second and third trimesters and in the puerperium.

When pregnant patients were prospectively matched with demographically and clinically similar controls, there was no difference of flare frequency between pregnant and nonpregnant SLE patients, either during pregnancy or in the first *postpartum* year; exacerbation occurred in less than 15% of all pregnancies. In another clinic, which prospectively quantifies flare in all patients four times per year, flare was twice as common in pregnant patients as in nonpregnant ones. In a third clinic, in which pregnant patients are stratified according to prepregnant disease status, pregnancy protects against flare. Different populations and entry criteria among these clinics are likely responsible for the different conclusions [71]. Despite the lack of consensus regarding the relationship between SLE flare and pregnancy, it is clear both that many patients do well during pregnancy and that vigilance for flare on the part of the treating physician is always necessary. Modifications have been suggested [72] for measuring SLE flare during pregnancy using the SELENA SLEDAI (SLEPDAI, systemic lupus erythematosus pregnancy disease activity index), the LAI (LAI-P, lupus activity index pregnancy), or the SLAM-R (m-SLAM, modified systemic lupus activity measure) and have been tested [73].

Single Organ Flare

Hematologic

Thrombocytopenia Thrombocytopenia is common in SLE pregnancy, especially in those patients with antiphospholipid antibody. Thrombocytopenia during gestation in patients with SLE occurs in five patterns.

1. Thrombocytopenia occurring early, usually before 15 weeks, usually reaching a nadir of greater than 50×10^9/liter, and remaining constant, occurs in association with antiphospholipid antibody. This type of thrombocytopenia remits after delivery, whether or not treatment is administered, and tends to recur with subsequent pregnancies. It is unaccompanied by signs of SLE activity in other organ systems; fragmented erythrocytes and increased lactic dehydrogenase (LDH) do not occur. Aspirin may reverse the thrombocytopenia [74].
2. A second type of thrombocytopenia, usually occurring after 25 weeks, is most often associated with impending or present preeclampsia. It tends to worsen as pregnancy progresses, is associated with worsening fetal health, and remits after delivery. It is not associated with other signs or symptoms of active SLE. Biochemical and clinical manifestations of preeclampsia or the HELLP syndrome may be present. Treatment directed at early delivery is usually indicated.
3. Severe (often $<10 \times 10^9$/liter) thrombocytopenia of the idiopathic thrombocytopenic purpura type occurs most often with no other signs of active SLE and is not thought to be related to pregnancy. Drug-induced marrow toxicity and hematologic malignancy must usually be excluded by bone marrow examination. It is our experience that this type of thrombocytopenia generally responds to high-dose corticosteroid within 2 to 3 weeks. Intravenous γ globulin has been used successfully as a therapeutic (temporizing) measure. In a minority of patients splenectomy is necessary. Aspirin should not be prescribed to these patients because the risk of hemorrhage, if platelet aggregation is hindered in the face of severe thrombocytopenia, is severe.
4. A moderate ($50–120 \times 10^9$/liter) thrombocytopenia, persistent over long periods of time and responding to prednisone, is accompanied by other manifestations of active SLE: evidence of vascular disease, renal or neurologic worsening, vasculitic skin rashes may be present, erythrocyte fragmentation is common, and serum LDH is often elevated. This thrombocytopenia reflects clinically active SLE.
5. The final type is the common benign thrombocytopenia of late pregnancy, unrelated to SLE.

No specific test clearly differentiates one type of thrombocytopenia from another in pregnant patients with SLE. Tests for platelet-associated immunoglobulin or for antiplatelet antibodies seldom help differentiate among the types of thrombocytopenia. In our experience, approximately one-fourth of all SLE patients experience thrombocytopenia during pregnancy. Antiphospholipid antibody syndrome, active SLE, and preeclampsia are equally distributed causes.

Anemia Anemia is a common complication of SLE. The anemia "of chronic disease" is the most

frequent attributed cause. A true autoimmune hemolytic anemia is uncommon, but does occur, sometimes in association with antiphospholipid antibody [75]. SLE-induced anemia amplifies the normal dilutional anemia of pregnancy and usually indicates a need to use (higher doses of) corticosteroid for protection of the fetus even if the mother tolerates the anemia well. The alternative treatment, maternal transfusion, is only temporarily effective and may carry more risk. Erythropoeitin may be used (see later).

Renal Disease and Preeclampsia

Proteinuria may increase during pregnancy in women with underlying renal disease, the increments being "physiologic" and unrelated to disease activity. It is always prudent to assume that increased proteinuria indicates a problem that requires careful evaluation and perhaps treatment. The differential diagnosis is between renewed or worsened activity of lupus nephritis and superimposed preeclampsia. Increasing urinary protein, clinical signs of active SLE, rising anti-dsDNA antibody, and the appearance of erythrocyte casts favor the diagnosis of lupus glomerulonephritis. Normal serum complement favors preeclampsia (Table 6) [76, 77, 78].

Rapid worsening over days is more common in preeclampsia than in active SLE. Hypertension, thrombocytopenia, hyperuricemia, and, in some cases, hypocomplementemia occur in both lupus nephritis and preeclampsia. Renal biopsy is helpful in distinguishing between lupus nephritis and preeclampsia, but is often not feasible. In women with preexisting renal disease who develop preeclampsia, renal function may not return to its prepregnancy baseline [79].

The interpretation of hypocomplementemic proteinuria in lupus pregnancy is difficult. Low-grade activation of the classic pathway may be attributable to pregnancy alone [80]. Measures of alternative pathway complement activation (Ba and Bb) are abnormal in active lupus nephritis and normal in lupus pregnancies with proteinuria not due to active SLE. Thus evaluation of the alternative complement pathway may be the clearest way to determine whether new proteinuria in the pregnant patient is due to active SLE or to preeclampsia. In the absence of a single definitive test, distinctions between lupus nephritis and superimposed preeclampsia must be made on clinical criteria.

Rash

Normal pregnancy erythemas, particularly of the face and hands, resemble active SLE rashes. Skin areas where there has been prior lupus rash frequently become more erythematous as cutaneous blood flow increases in pregnancy. With experience, distinguishing between active SLE rash and pregnancy-induced change is not difficult. Patients who suddenly discontinue hydroxychloroquine often have an exacerbation of rash and may have exacerbation of other disease manifestations [64, 81, 82].

Arthritis

Joints damaged previously by lupus arthritis often ache and develop noninflammatory effusions during pregnancy, particularly late in gestation as ligamentous loosening occurs. The decision that a given complaint is due to active SLE rather than to physiologic changes of pregnancy depends on the demonstration that the arthritis is inflammatory. Rarely, joint fluid examination is required. If extraarticular manifestations of active SLE are present, it is likely that the effusion represents active SLE.

Neurologic SLE

Neurologic events during the course of pregnancy are rare. There are isolated reports but no cogent evidence that SLE neurologic events, including chorea and transverse myelitis, are induced or exacerbated by pregnancy [83–85]. There are also occasional reports

TABLE 6 Comparison between Preeclampsia and Lupus Nephritis

Clinical measure	Preeclampsia		Lupus nephritis
C3, C4, CH_{50}	May be low		Almost always low
Urinalysis	Red blood cell (RBC) casts rare		RBC casts frequent
Onset of proteinuria	Commonly abrupt		Gradual or abrupt
Hepatic aminotransferases	May be increased		Rarely abnormal
Quantity of proteinuria		Will not differentiate	
Thrombocytopenia		Will not differentiate	
Hyperuricemia		Will not differentiate	
Hypertension		Will not differentiate	

of SLE patients suffering stroke *postpartum*, usually in the setting of coexisting antiphospholipid antibody.

One of the most difficult diagnostic decisions concerns the occurrence of seizures late in pregnancy, when hypertension and renal failure are also present. The circumstances in which the events occur, the presence of other clinical and serologic (rising anti-dsDNA antibody) evidence of active SLE, and response to therapy may all be required to make the distinction between active neurologic SLE and eclampsia. Often, treatment for both is indicated.

Summary of Data: Does Pregnancy Influence the Course of Disease?

Because pregnancy-induced abnormalities resembling lupus flare may occur, the patient's health may worsen, although, in a technical sense, her disease does not. The distinction between disease-induced and pregnancy-induced complications is critical because treatment directed against the primary illness (on the assumption that it has become "active") rather than toward the pregnancy event may be misguided and potentially harmful. Whether pregnancy induces flare remains controversial. Relevant controlled studies are summarized in Table 6. It is likely that referral selection bias, differences in the ways in which different clinics diagnose flare, demographic differences of studied patients, inclusion of patients first diagnosed during pregnancy, and disagreements about the distinctions between preeclamptic and lupus renal and hematologic abnormalities account for the different conclusions reached in different clinics. Regardless of population statistics, every patient should be followed during pregnancy under the assumption that there is a risk of flare.

MANAGEMENT OF PREGNANCY: MOTHER

Monitoring

Women entering pregnancy in full remission should be fully evaluated by their treating medical physician at intervals no less than each trimester. Detailed history and physical examination, looking for signs of lupus activation, are indicated. Laboratory analysis for anti-DNA antibody, complement, 24-h urinary protein excretion, creatinine clearance, and complete blood count including platelets is also indicated, as is repeat tests for antiphospholipid antibody and lupus anticoagulant (if previously negative). Table 3 indicates suggested frequency of monitoring for a patient with an uncomplicated pregnancy. The clinical activity of disease and any pregnancy abnormality mandate more frequent observations.

Treatment of Lupus during Pregnancy

With qualifications, criteria for treatment of the pregnant patient do not differ from criteria for the nonpregnant patient. Clinical flares of skin, joints, blood, kidneys, mucosae, serosae, and nervous systems, *if attributable to SLE activity rather than to pregnancy*, always merit the same increase in corticosteroid therapy that these symptoms would merit if the patient were not pregnant. The choice to use antimalarial, nonsteroidal, and immunosuppressive drugs, however, is restricted by weighing potential fetal injury against control of maternal disease.

Anemia (hemoglobin less than 8.0 g/dl), fever (sustained fever greater than 38.5°C), and hypoalbuminemia (albumin less than 3.0 g/dl) merit more aggressive treatment in the pregnant patient than they might in the nonpregnant patient because these abnormalities threaten the growing fetus. Worsening fetal health, in the absence of clinical exacerbation of SLE, does not justify treatment for SLE.

Pregnancy Complications in Patients Entering Pregnancy with Preexisting Abnormalities

Severe *multisystemic* SLE will seriously complicate pregnancy, primarily through its manifestations of high fever, hypertension, renal failure, and, on occasion, cardiac and respiratory failure, as well as through the treatments administered.

Any *renal* disease, including lupus glomerulonephritis, predisposes patients to preeclampsia. There is some disagreement whether patients with renal SLE do less well than those with other forms of glomerulonephritis. In general, those with preserved renal function and minimal hypertension do well. Even those with severe renal disease often have surprisingly good pregnancy outcomes. Normal urinary protein in pregnancy may be as high as 500 mg/day. Modest increases in urinary protein (particularly in patients with fixed proteinuria), accompanied by proportional increases in glomerular filtration in mid- to late pregnancy and not accompanied by other signs of active lupus, likely do not indicate lupus nephritis and need not be treated. In patients with marked increases in urinary protein, with or without hypertension, it may be impossible to distinguish between preeclampsia and active lupus nephritis, and

may therefore be necessary to treat if delivery is not likely in the immediate future. Hypocomplementemia alone, without renal worsening, is not an indication to treat.

In our experience with 125 completed SLE pregnancies, only 15% of SLE patients entered pregnancy with preexisting renal disease. Of this 15%, two-thirds (10% of the total) developed preeclampsia, a complication that also developed in 14% of the 107 women without preexisting kidney disease. Our experience is similar to that of Nossent and Swaak [86] but differs from the prognoses reported by Petri *et al*. [67]. The latter reported that more than half of women entered pregnancy with preexisting lupus nephritis, and while the authors did not comment on the frequency of preeclampsia, they did report a high frequency of renal flare.

High-dose (60 mg/day) oral corticosteroid therapy is the initial treatment of SLE glomerulonephritis. Corticosteroid therapy may cause hypertension or elevated blood glucose; in the pregnant patient, the risk of hypertension may be greater than in the nonpregnant patient. Thus proactive means to prevent hypertension, and its attendant preeclampsia, are indicated.

Obstetricians frequently use low-dose aspirin (60–100 mg/day) to prevent preeclampsia in women identified as being at high risk [87–90]. Most frequently, preexisting hypertension is the characteristic for which high risk is assigned. Aspirin does not reduce the incidence of preeclampsia in women at low risk for this complication [91]. All forms of renal disease predispose to preeclampsia. Lupus glomerulonephritis is no exception. There is no systematic study of the use of aspirin to prevent preeclampsia in SLE patients. Nonetheless, some physicians, including us, do prescribe this drug.

Both prolonged labor and increased peripartum bleeding may occur when aspirin is used close to term [92, 93]. Premature closure of the *ductus arteriosus* is a risk of use of indomethacin [94]. The aspirin effects are quantitatively minor. Aspirin and other nonsteroidal anti-inflammatory agents may decrease glomerular filtration rates in patients with renal disease.

Patients with severe hypertension, on hemodialysis, or with renal transplants may have successful pregnancies. Management in these situations is very individualized and requires expert multidisciplinary care.

Thrombocytopenia, independent of antiphospholipid antibodies or preeclampsia, is not an independent predictor of fetal death. Women who enter pregnancy with preexisting modest thrombocytopenia may experience a further fall as the pregnancy progresses. While platelet counts must be monitored carefully, a decrease in platelet count from 120 to 80×10^9/ml need not *necessarily* require additional therapy. Treatment of thrombocytopenia in a pregnant lupus patient depends on a clear definition of the cause of the thrombocytopenia. Thrombocytopenia associated with antiphospholipid antibody may respond within several days to 81 mg/day ("baby") aspirin. However, aspirin should not be used in ITP or antiplatelet antibody thrombocytopenia. Prednisone, 30 to 60 mg/day, should be prescribed if the cause of the thrombocytopenia is uncertain. The response to prednisone takes several days to 3 weeks to occur. There is no documented evidence that the treatment of modest thrombocytopenia ($>70 \times 10^9$/liter) improves either maternal or fetal prognosis, but many physicians interpret modest thrombocytopenia as evidence of disease activity and do treat it. When thrombocytopenia is life-threatening ($<30 \times 10^9$/liter) or if delivery is imminent, treatment is indicated.

A temporary remission of thrombocytopenia may be obtained by infusing platelets (response within minutes; duration, hours to 2 days) or intravenous aggregate-free immunoglobulin G (response within days, duration, 2–3 weeks) [95–97]. Neither of these treatments can be used on a long-term basis. Intravenous immunoglobulin adds to the volume load of patients already at risk for hypertension or congestive cardiomyopathy and late in pregnancy could theoretically add a volume load to the fetus, increase fetal blood viscosity, and inhibit fetal immunoglobulin synthesis. However, normal children have been delivered of mothers receiving immunoglobulin therapy. Splenectomy and cyclophosphamide are last resorts in the treatment of thrombocytopenia; both have been used successfully in pregnancy [98]. The potential roles of argatroban or lepirudin in the pregnant lupus patient are unknown; both have FDA pregnancy category B ratings.

Neuropsychiatric disease, so long as it and its treatment do not affect the mother's general health, has little direct influence on pregnancy outcome. Fetal injury from hypoxia during seizures, self-abuse, or neglect during psychosis is possible. Sedation or addiction of the fetus from maternal phenobarbital or other anticonvulsant drugs is also possible, as is drug-related teratology (see later). Corticosteroids, anticonvulsants, and, for stroke, anticoagulation are the primary forms of treatment for neurologic events. These events are rare in pregnancy; they are treated similarly in pregnant and nonpregnant patients, except that warfarin is contraindicated in pregnancy.

Osteonecrosis, with or without total hip replacement, is a common event in SLE. Pregnancy occasionally occurs in a woman suffering this complication. With a few exceptions, neither osteonecrosis nor hip

replacement alters the course of pregnancy or its management. The exceptions are as follows: *protrusio acetabulae* narrows the pelvic inlet, possibly obstructing the passage of a child and thus mandating operative delivery. A markedly restricted range of motion of the hip, especially abduction and abduction in flexion, will interfere with vaginal delivery from the lithotomy position. If the delivery room staff is unaware of the limited motion, they may try to force abduction and risk hip fracture or dislocation. It is wise for the rheumatologist or orthopedist to advise the obstetrical staff well in advance of anticipated delivery of all musculoskeletal disabilities a patient has. Antibiotic coverage is advisable during labor and delivery for women with prosthetic joints. A suitable regimen is cephalosporin or vancomycin plus gentamicin.

Case reports of fatalities due to *pulmonary hypertension* developing or worsening during pregnancy suggest that patients with important lung involvement should avoid conceiving [99, 100]. Because of the growing fetus, diaphragms are high in late pregnancy. Patients with "shrinking lung," or with recurrent pleurisy, may find breathing further compromised. *Arthritis* does not as a rule influence the course of the pregnancy. In nonpregnant patients, full doses of NSAIDs are the first treatment option for arthritis. Although they appear to be generally safe, antiinflammatory doses of aspirin or nonsteroidal antiinflammatory drugs are not recommended in pregnancy because there is little published information concerning safety to the fetus. For treatment of arthralgia or arthritis, low-dose prednisone is preferred [101]. Corticosteroid therapy is the primary treatment for SLE-caused *anemia* (either hemolytic or due to chronic disease), if treatment is necessary, and if anemia is not due to vitamin or iron deficiency, sickle cell disease, glucose-6-phosphatase deficiency, or other Coombs-negative hemolysis. As most patients are minimally symptomatic, the indication for treatment is usually fetal rather than maternal; a hemoglobin concentration of less than 8 g/dl is often used for the decision to treat. Transfusions are occasionally also necessary. Safety and efficacy of human recombinant erythropoietin in pregnancy have been shown, including at least one case of SLE pregnancy with nephritis [102, 103]. There is no transfer of erythropoietin across the placenta [104]. *Rashes* may be treated with topical or systemic corticosteroid or may be left untreated. Some authors comment on the benignity of antimalarial drugs during pregnancy (see later) [105, 106]. Discontinuation of antimalarials in pregnant women or in women contemplating pregnancy is common practice. The only available controlled trial in pregnancy suggests that patients do better taking rather than discontinuing hydroxychloroquine [107].

DRUG TREATMENT IN LUPUS PREGNANCY

Physiology of Drug Metabolism during Pregnancy

Physiologic changes during pregnancy alter maternal pharmacokinetics, which in turn may affect the amount of drug reaching the mother, the placenta, and the fetus [108, 109]. For example, gastrointestinal reflux, with a decrease in gastric emptying, reduces both drug absorption and peak concentration.

Placental drug transfer by diffusion resembles transfer across a lipid bilayer. Transfer is favored by lipid solubility, a large concentration gradient of unbound drug, and maintenance of the drug in a nonionized state in the maternal circulation. Differences in maternal and fetal pH influence the maternal/fetal concentration ratio.

In SLE, hypertension, diabetes mellitus and other conditions, disordered maternal and fetal physiology may produce changes in the placenta that impact drug transfer from mother to fetus. Drug distribution varies significantly during the course of pregnancy through the continuing expansion of maternal blood volume by 30%. In most cases there are no guidelines for adjusting dose. Measurement of blood levels when appropriate and observation for clinical effects and toxicity are necessary.

Common Drugs Used during Pregnancy (Non-SLE)

Non-SLE patients take an average of 3.1 drugs other than vitamins during pregnancy [110]. Commonly used drugs include antiemetics, antacids, antihistamines, antimicrobials, analgesics, and antihypertensives. Issues of teratogenicity are common. Teratogens (agents or factors to which embryo–fetal exposure produces a permanent alteration in the form or function of the child) [111] have different effects at different time periods. Teratogens administered after the vulnerable period usually will not cause structural malformations. Gestational periods are fertilization to implantation (0–2 weeks), embryonic (2–8 weeks, *the most critical period*), and fetal, from 8 weeks to term.

In 1979, the Food and Drug Administration established five categories of possible adverse fetal effects. Category A includes drugs for which controlled studies in human subjects show no fetal risk. Categories B, C, and D have progressively fewer data or greater risk, and category X describes proven fetal risks that clearly outweigh benefits [112].

Penicillins, cephalosporins, and macrolide *antibiotics* (erythromycin and others) are considered fairly safe

(category B). Drugs to avoid include tetracyclines, which cause yellow–brown discoloration of deciduous teeth and of the long bones; aminoglycosides, for which early studies showed associations with fetal eighth nerve damage; and ciprofloxacin, which causes fetal bone malformations. Griseofulvin, an oral antifungal, should be avoided as well.

Antihypertensives may be used in patients with chronic or pregnancy-induced hypertension and are often required in the SLE patient. Methyldopa, hydralazine, and clonidine have a long history of safe use. β-Adrenergic blocking agents do not have the same long record of use but are often recommended in preeclampsia. Calcium channel antagonists are also used, although verapamil (but not nifedipine or diltiazem) may decrease uterine blood flow. Angiotensin-converting enzyme inhibitors (ACE inhibitors) are contraindicated in pregnancy because they are associated with renal anomalies, nephrotoxicity, neonatal anuria, oligohydramnios, and death [113]. Diuretics are commonly used and little studied, with no major problems reported.

Anticonvulsant medications must continue during pregnancy in women with epilepsy or SLE-related seizures, even though the children of epileptic women taking these drugs during the first trimester have an increased frequency of congenital malformations. Hydantoins (including phenytoin) cause fetal hydantoin syndrome (craniofacial and limb malformations accompanied by mental deficiency). Carbamazepine, trimethadione, and valproic acid produce similar syndromes. No reports of malformations exist for phenobarbital or ethosuximide.

Antidepressant drugs used during the first trimester may be associated with some malformations. The newer selective serotonin reuptake inhibitors, however, are category B drugs. Antipsychotic medications, used in pregnancy most commonly for the treatment of hyperemesis, do not show an increased risk of malformation.

Drugs Used for Treating Lupus

Increased drug metabolism in pregnancy may mandate larger than normal doses of all drugs.

Corticosteroids

Prednisone and methylprednisolone have minimal placental transfer and are the drugs of choice for patients requiring corticosteroid therapy. In conventional doses, they are safe for the fetus. Fluorinated corticosteroids (dexamethasone and betamethasone) should not be used unless there is intent to treat the fetus, as these drugs easily cross the placenta [114]. There is no accumulated analyzed experience with "pulse" bolus corticosteroid in pregnancy. High-dose intravenous methylprednisolone does reach the fetus [115–117].

Immunosuppressives

Azathioprine is widely used in renal transplant patients and is thought to be generally safe in pregnancy [118, 119], but fetal cytopenias and malformations have occurred [120, 121]. Cyclophosphamide is contraindicated in early pregnancy because of its teratogenicity and abortifacient properties. There are reports of women given cyclophosphamide before and during pregnancy whose infants appear normal, but experience is small [122]. It may be possible to administer this drug late in pregnancy with less risk. Methotrexate is similarly contraindicated in the first trimester of pregnancy. It is abortifacient; it causes a characteristic fetal syndrome of craniosynostosis and other malformations. Cyclosporine may cause significant maternal toxicity, especially nephrotoxicity, but appears to be safe for the fetus. Mycophenolate mofetil is FDA category C—insufficient information is available.

Nonsteroidal Anti-inflammatory Drugs

Salicylates have no proven association with fetal anomaly [123]. NSAIDs are generally considered nonteratogenic, yet experience with these agents in pregnancy is limited. Aspirin in a therapeutic dose, but not at 80–150 mg/day, has been associated with oligohydramnios, premature closure of the ductus arteriosus, and pulmonary hypertension. Indomethacin, used to treat preterm labor, is also associated with premature ductus closure and fetal pulmonary hypertension. For this reason, anti-inflammatory doses of aspirin or NSAID use should be terminated after 34 weeks.

In general, aspirin is preferred to NSAIDs because of its longer history of use and greater accumulated literature. In many cases of minor arthritis or serositis during SLE pregnancy, low-dose prednisone is preferable to either aspirin or NSAIDs because of its known safety and minimal placental transfer.

Antimalarial Medications

Evidence regarding the teratogenicity of both chloroquine and hydroxychloquine is sparse [124]; the safety of antimalarials is not established. In one publication, 36 infants of 33 mothers had no apparent abnormalities attributable to hydroxychloroquine [125]. No harm to the fetus in treated pregnancies, but maternal flares in untreated pregnancies were shown in a double-

blind, placebo-controlled trial [107]. Concerns with these drugs, based on laboratory animal data, include ototoxicity and ophthalmotoxicity, which have not been reported in humans to date. It seems prudent to avoid use of these agents during pregnancy if possible, although scattered small series report successful pregnancies. Flare after the discontinuation of hydroxychloroquine is a real concern. Flare should be anticipated; the physician and the patient together should weigh the relative risks of continuing the antimalarial against the possibility of having to control an exacerbation with higher dose prednisone.

Intravenous Immunoglobulin (IVIG)

Intravenous immunoglobulin is used for several specific problems during pregnancy. Thrombocytopenia, whether from idiopathic thrombocytopenic purpura, lupus, or antiphospholipid antibody syndrome, generally responds well, although transiently, to this therapy. Several studies have reported successful pregnancy outcomes in patients with aPL treated with IVIG, usually in combination with other therapies. Anticardiolipin antibody titers may decrease with IVIG therapy, possibly related to anti-idiotype antibodies to antiphospholipid antibody present in commercial immunoglobulin preparations and demonstrable in patient plasma after treatment [126, 127].

Apheresis

While plasmapheresis has been used during pregnancy, its efficacy for any single indication is yet to be determined. Reports include case reports of patients carrying a fetus with neonatal lupus and congenital heart block, antiphospholipid antibody-related pregnancy losses, and antiphospholipid antibody-related refractory HELLP syndrome with postpartum renal insufficiency [128].

Drugs Used for Pregnancy

The following drugs are often used by obstetricians for pregnancy or menometrorrhagia management. Their effects on SLE, if any, are unknown.

Hormones

Both androgens (including danazol) and the nonsteroidal synthetic estrogen diethylstilbestrol (DES) may cause congenital anomalies. Oral contraceptives, if taken during pregnancy, do not cause fetal genital malformations [129]. Progesterone suppositories may be prescribed for patients with a presumed luteal phase defect (insufficient progesterone secretion by the corpus luteum or placenta associated with an increased incidence of spontaneous abortion). Progesterone intramuscular injections may also be used in patients undertaking assisted reproduction (see later). This drug is not associated with an increased risk of lupus flare.

Tocolytic Medications

Tocolytic medications are those used to prevent or control preterm labor. These include β-*adrenergic agonists*, *magnesium sulfate*, *prostaglandin inhibitors*, *calcium channel-blocking* drugs, and *nitric oxide donor* drugs.

Before medication, most patients with preterm labor are given a trial of bed rest and hydration. β-agonists decrease contractions but may be accompanied by troublesome cardiovascular effects, including pulmonary edema, palpitations, and glucose intolerance. Traditionally, the β-mimetics most used are *ritodrine* and *terbutaline*. Because of questions of efficacy of oral preparations, only parenteral forms of ritodrine are now available. Terbutaline is used with a subcutaneous pump, but its efficacy is unproven; oral terbutaline therapy is not efficacious [130].

Magnesium sulfate, in high concentration, can alter myometrial contractility, possibly functioning as a calcium antagonist *in vivo*. Its efficacy is controversial. Signs of hypermagnesemia of both mother and fetus must be sought. Prostaglandin inhibitors reduce the frequency of preterm labor, but these agents cause premature closure of the *ductus arteriosus*, necrotizing enterocolitis, and intracranial hemorrhage in the fetus [131].

Calcium channel blockers inhibit contractility of the myometrium but also uterine and systemic blood vessels. Thus there is a risk of decreased uteroplacental perfusion. Other agents include oxytocin inhibitors (atosiban) and nitric oxide donor drugs, as nitric oxide is a potent smooth muscle relaxant. Efficacy and risks of these are not established.

MANAGEMENT: TREATMENT OF FETUS DURING PREGNANCY

Fetal Monitoring

Fetal growth and development in SLE patients may be threatened by disease activity, by abnormality of maternal renal function, by antiphospholipid antibody, and by anti-SSA/Ro and anti-SSB/La antibodies.

Triple Screen False-Positive Tests

Unexplained elevation of maternal α-fetoprotein occurs in patients with systemic lupus erythematosus. The elevation correlates with preterm delivery, high prednisone dosage, and presence of antiphospholipid antibody [132]. Unexplained elevation of α-fetoprotein in patients with antiphospholipid antibody identifies an increased risk of fetal and perinatal death. Presumably, abnormalities in the fetoplacental barrier lead to an increased transport of α-fetoprotein from fetus to mother [133, 134]. It usually does not imply fetal malformation. False-positive elevations in human chorionic gonadotrophin, another component of the "triple screen" for Down's syndrome (α-fetoprotein, hCG, and unconjugated estriol), may also be seen [135].

Antibodies Transmitted

Maternal IgG-mediated thrombocytopenia may be transmitted to the fetus, but most infants born of thrombocytopenic mothers with SLE have normal platelet counts. Occasionally the IgG Coombs' hemolytic antibody may be transmitted to and cause hemolysis in the fetus and newborn. The anti-dsDNA antibody, also transmitted to the infant, has no apparent pathologic effect. The antiphospholipid antibody is associated with placental insufficiency, intrauterine growth restriction and fetal death; it does not usually cause abnormalities in the infant, but fetal thrombosis has occurred (see later). In women with anti-SSA/Ro or anti-SSB/La antibodies, neonatal lupus is a risk. Fetal echocardiography, seeking evidence of heart block or other cardiac dysfunction, is recommended between 15 and 25 weeks [136, 137].

Monitoring

Fetal growth rate and placental volume and appearance may also be monitored by sonography. Weekly antepartum fetal heart rate testing may begin as early as 25 weeks [138, 139]. In the 25- to 30-week period, nonreactive tests are relatively commonly seen. Occurrence of sinus bradycardia is predictive of potential fetal compromise. Later in gestation any nonreactive test is abnormal; sinus bradycardia may indicate a need to deliver, even at early gestational ages. The potential role of umbilical artery waveform determinations is not yet known, but it assists in timing induced delivery [140, 141].

Deciding to Intervene

The decision to end a viable pregnancy requires a yes answer to either of two questions: Is the mother's health likely to deteriorate *irreversibly* if the pregnancy continues? Is the baby likely to grow better delivered than *in utero*? The decision to use a fetotoxic drug requires a yes answer to the first question and an agreement that the risk to the fetus justifies the intervention.

Active lupus, of itself, does not mandate ending a pregnancy, although the decision that cyclophosphamide must be used before the fetus reaches 36 weeks may be reason to terminate or deliver early. Maternal complications that call for urgent termination of a pregnancy, regardless of fetal maturity, are more often pregnancy than lupus related: progressive preeclampsia, in any of its manifestations, progressive hepatic failure from fatty liver, or HELLP syndrome, uncontrollable heart failure, and, sometimes, severe thrombocytopenia. Pregnancies may continue in the face of severe renal failure (including dialysis and transplant), advanced nephrosis, thrombocytopenia, and maternal brain disease. The effects of these specific conditions on an individual child are unknown, but the authors have cared for pregnant patients with each of these conditions who have delivered apparently normal children at or near term. The parents' choice, the length of the pregnancy at the time the complication arises, medications taken by the mother, and individualized predictors of maternal and fetal health all weigh in the final decision.

Obstetrical rules regarding intervention to preserve fetal well-being are reasonably standard. Complete cessation of fetal growth, resorption of amniotic fluid, and poor performance on tests of fetal well-being [antepartum fetal heart rate testing (nonstress test), biophysical profiles, measurements of umbilical artery wave form] all are warning signs that the pregnancy has ceased nurturing the child. Spontaneous fetal sinus bradycardia is a forerunner of fetal death. If there is intent to salvage the child, i.e., if the child is potentially viable (greater than 24 weeks' gestational age), delivery in the face of these findings must be accomplished immediately. Another indication for immediate delivery is the occurrence of cardiac failure or pericarditis, especially in an infant with congenital heart block. Heart block alone, if cardiac function is good, is not an absolute reason to deliver.

Unrelated to lupus, *hydrops fetalis*, incompetent cervix, amnionitis, and other common obstetrical illnesses are reasons to deliver before term.

As a general rule, delivery of a child at 30 weeks or more, or who weighs more than 1500 g, followed by care in an experienced neonatal intensive care unit, gives acceptable levels of risk for complications of prematurity. The frequency of damaged children rises markedly the smaller the child (Table 7) [142, 143].

TABLE 7 Frequency of Major Impairment as a Function of Birth Weight

Birth weight (g)	% with major impairment (range)	% of survivors with major impairment at 75 months
1501–2500	8 (5–20)	—
1001–1500	15 (5–30)	—
≤1000	25 (8–40)	21–29

[a] Data from non-SLE populations [142, 143].

Prematurity, Intrauterine Growth Restriction, and Fetal Death

Fetal death, intrauterine growth restriction, and prematurity (birth before 38 weeks) are common in SLE pregnancies. They result from maternal disease severity, antiphospholipid antibody, or both. SLE activity by itself does not alter the outcome of pregnancy.

Prematurity most commonly results from a decision to deliver because of maternal or fetal distress. Depending on the mix of patients seen and upon variables of diagnostic and obstetrical thresholds, centers differ in the frequency of premature delivery. Rates of 50% are usual. Obstetrical reasons such as placenta previa are rare, but premature rupture of membranes, often associated with corticosteroid therapy, is common.

Congenital Abnormalities

Other than neonatal lupus (see Chapter 17), there are no congenital, genetic, or developmental abnormalities associated with SLE. Brain and other damage resulting from prematurity, placental insufficiency, toxemia, or neonatal lupus are not considered to be congenital abnormalities.

Miscellaneous

Men with SLE may become fathers. Other than the slight increased risk of eventual SLE in the child common to this familial illness, there is no known effect of paternal SLE on the infant, regardless of the clinical condition or serology of the father. Medications may alter male fertility: cyclophosphamide markedly diminishes sperm count and high-dose corticosteroid therapy alters both libido and potency.

Long-Term Outcomes of Lupus Children

There are no large-scale, long-term published follow-ups of offspring of lupus mothers, but data comparing such infants with sex- and gestational age-matched controls suggest that, although intelligence is normal, there may be a high frequency of learning disabilities, particularly in males. It is not clear whether autoantibodies are a cause [144, 145].

TABLE 8 Lupus-Specific Issues That May Force Operative Delivery

- Severe hypertension
- Severe thrombocytopenia
- Severe renal disease
- Immediate need to institute life-saving treatment to the mother that will be harmful to the fetus
- Severe maternal cardiac disease
- Severe pulmonary disease
- Deteriorating heart function in a fetus with congenital heart block
- Orthopedic indication (e.g., protrusio acetabuli blocking the pelvic outlet)

THE PUERPERIUM

Route of Delivery

The decision to deliver vaginally or operatively is an obstetrical one. Criteria that influence this decision are immediacy of the need to deliver (i.e., is there sufficient time for normal labor to proceed?), hemorrhage risk (fetal or maternal thrombocytopenia), placental position, fetal health, position, and size, and maternal pelvic size. Specific lupus issues rarely determine delivery route, although they may upgrade urgency and thus force operative delivery (Table 8). These issues can be generally categorized: severe organ failure, immediate need to institute treatment which may harm the infant, and fetal cardiomyopathy. There is never a need to insist upon operative delivery for the single reason of diagnosis of (uncomplicated) lupus. Other than blood pressure rises during uterine contraction, venous distention during repeated Valsalva maneuvers, and duration of physical activity, there are no characteristics of labor that are necessarily more stressful to the mother than anesthesia and major surgery. The internist may monitor and advise the obstetrician about the patient's organ competencies and stamina but otherwise should not attempt to direct the choice of delivery route.

Treatment in the Peripartum Period

The patient's usual medications continue during the peripartum period as they would during any surgical procedure. In particular, it is advisable to give "stress"

steroid doses beginning with the onset of labor and continuing 24 h after delivery concludes. Anticoagulation, including aspirin, must cease with the onset of labor to resume once the risk of hemorrhage is over *postpartum*. Observation of lochia (bloody uterine discharge after delivery) is a good guide to hemorrhage risk. Reinstituting anticoagulation 1 to 2 days after delivery is usual. Antihypertensive and anticonvulsant medications continue in parenteral forms during the peripartum period. Medications that have been discontinued for pregnancy, such as cyclophosphamide or hydroxychloroquine, may resume as soon as the patient has begun eating again, *unless the mother intends to breast feed and the medication is one that passes to the newborn*.

The authors recommend against, and themselves do not use, routine increases in corticosteroid dose to prevent postpartum flare.

The treating physician should alert the delivering physician and staff to any physical and orthopedic limitations a patient might have. Previously thrombosed femoral or iliac veins in antiphospholipid antibody syndrome patients may develop or release new clots as the pelvic veins become congested and occluded during fetal descent.

It is also useful to alert the neonatalogy staff about the mother's anti-Ro/SSA and anti-La/SSB status. Neonatalogists may be unfamiliar with the rash, thrombocytopenia, and, sometimes, heart block of neonatal lupus. Susceptible infants may first develop the rash when treated with ultraviolet light for hyperbilirubinemia. Alerting the pediatric staff may prevent much unnecessary testing and undue alarming of the parents. (Grave systemic infections, such as cytomegalovirus infection, which threatens mental retardation and blindness, are commonly suggested alternative diagnoses of the rash.)

The treating physician should note that the pregnancy complications of preeclampsia, HELLP syndrome, and fatty liver of pregnancy may continue to progress for several days to weeks *postpartum* before they eventually regress. Treatment decisions should therefore consider the possibility of insufficient hepatic or renal clearance of drugs and the presence of serious confounding illness in immediate *postpartum* patients.

Placentae

Lupus placentas are often small in size. They demonstrate ischemic–hypoxic change, decidual vasculopathy, decidual thrombi, fetal thrombi, chronic villitis, and perivillous fibrin. Antiphospholipid antibody placentae demonstrate decreased weight, ischemic–hypoxic change, decidual vasculopathy, and extensive infarction [146]. It is often difficult to distinguish these changes from those of preeclampsia. A preliminary study suggests that SLE placentas exhibit intense deposits of type IV collagen and laminin, whereas preeclampsia and normal placentas do not [147].

Several processes may determine the extent of placental damage or dysfunction [148]. Occlusive vasculopathy, due to placental intraluminal thrombosis, to endothelial cell proliferation, or to other placental vasculopathy, has been suggested [149–156]. The antiphospholipid antibody competes with the natural placental anticoagulant proteins for phospholipid substrate, possibly interdicting their anticoagulant function and permitting pathologic coagulation to occur in the placenta [157–159]. Antiphospholipid antibody, β_2-glycoprotein-1, and placental anticoagulant protein I are identifiable in the same histologic location in placentas of mothers with fetal death; neither antiphospholipid antibody nor β_2-glycoprotein-1 are present in placentas of women with normal births [160]. Other, putatively immunologic, placental lesions have been described in patients with SLE [161, 162].

Breast Feeding

Definitions

Colostrum is the deep lemon-colored liquid secreted by the breasts for the first several *postpartum* days. Compared with mature milk, colostrum contains more minerals and protein, but less sugar and fat. Antibodies are demonstrable in colostrum; immunoglobulin A may protect the newborn against enteric pathogens. Other host resistance factors, including complement, macrophages, lymphocytes, lactoferrin, lactoperoxidase, and lysozymes, are in human colostrum and milk.

Human *milk* contains α-lactalbumin, β-lactoglobulin, and casein. Most milk proteins are unique [163]. Whey contains large amounts of interleukin-6, with peak levels in colostrum. Prolactin is actively secreted into breast milk [164]. Milk also contains all vitamins, except vitamin K, and little iron. The breast concentrates iodine, gallium, technetium, indium, and sodium. Radioactive scanning materials appear rapidly in breast milk.

Hormonal Status during Lactation

Progesterone, estrogen, and placental lactogen, as well as prolactin, cortisol, and insulin, appear to act in concert to stimulate the milk-secreting apparatus. At delivery an abrupt decrease in the levels of estrogen and progesterone results in unopposed prolactin stimulation of α-lactalbumin production. The intensity and duration of lactation are controlled by the repetitive stimulus of

nursing. Oxytocin, released in pulsatile fashion from the neurohypophysis, stimulates the contraction of myoepithelial cells in the alveoli and ducts, allowing reflex milk ejection.

Drugs in Breast Milk

Drugs in maternal blood cross the capillary endothelium, extracellular space, and hydrophobic barrier created by the alveolar cell membrane cell membrane before entering the milk. Most drug transfer is by passive diffusion.

Compounds with high molecular weights, such as heparin, are unable to pass through the cell membranes into the milk. Because breast milk is mildly acidic, weak bases pass most readily into breast milk, creating a higher concentration in milk than in plasma. Drugs that are weak acids may have lower concentrations in breast milk, and highly lipophilic drugs may partition into milk fat and achieve a high concentration in milk than in plasma [165].

The amount of drug in the milk and *consumed by the infant* depends on the dose, frequency, and duration of exposure, route of administration, and time of administration relative to infant feedings. It is critical to counsel patients not to feed at peak milk levels of the drug but to administer medications after infant feedings or just before the infant's longest sleep period.

The nursing infant is constantly maturing, a fact that also affects its exposure to drugs. The manner in which an infant absorbs, tolerates, and metabolizes drugs can vary within a short period of time due functional readiness of the gastrointestinal tract and development of renal function.

Aspirin and *NSAIDs* are highly protein-bound weak acids that do not pass easily into breast milk. Because infant elimination of such drugs is slow, accumulation can occur if the mother takes high chronic doses. The most reasonable NSAID to use is ibuprofen, 400 mg every 6 h, as this has not been detected in breast milk [166]. Most *antibiotics* appear in breast milk, although concentrations vary. Sulfonamides rarely cause hyperbilirubinemia in the infant. Penicillins and cephalosporins are excreted in low concentrations. Tetracyclines appear in significant concentration, but are usually undetected in the infant's plasma. Nonetheless, because tetracyclines stain teeth in growing children, it is best to avoid their use. Aminoglycosides are surprisingly safe in lactation due to poor gastrointestinal absorption in the infant. Some antibiotics concentrate in breast milk and are contraindicated: these include metronidazole, chloramphenicol, and isoniazid. The major *anticoagulants* are safe during breast feeding. Heparin is too large to pass into breast milk, and warfarin (highly bound to plasma proteins and weakly acidic) is not detectable. Controversy exists regarding the use of *oral contraceptives* during lactation, both with regard to amount of hormone absorbed into the infant's circulation and with effect of the contraceptive steroids on maternal milk composition and yield. Estrogen directly inhibits lactation.

For patients with SLE, the major concern is the safety of *corticosteroid* therapy during lactation. Overall, especially with low doses and appropriate corticosteroid choice (prednisone or prednisolone), very little steroid is transmitted to breast milk. The neonatal effect (plasma level) in a woman taking a maternal dose of prednisolone of 20 mg/day or less is insignificant. Infant corticosteroid exposure is further minimized by feeding just prior to ingestion of the medication.

Complications of Breast Feeding

Breast problems during pregnancy and lactation include infection (mastitis, abscess formation) and galactoceles (noninfected milk-filled cysts that resolve with aspiration). Benign fibroadenomas may enlarge significantly during pregnancy. SLE rash may occur on the breast and around the nipple area and may be initially confused with simple mastitis; it may make breast feeding painful [167].

Suppression of Lactation

The simplest method of lactation suppression is support of the engorged breasts, application of cold packs, and appropriate analgesia if necessary. Symptoms usually disappear within 2 to 3 days.

Medical suppression of lactation is possible with estrogens or bromocriptine. Estrogen therapy, however, may increase thromboembolic risk. Bromocriptine, a dopamine agonist, stimulates the production of prolactin inhibitory factor, leading to a fall in prolactin and cessation of lactation. Adverse effects have been reported with this therapy, including hypertension, seizures, stroke, and myocardial infarction. Although no causative relationship has been established, use of this medication is now uncommon [168]. Oral contraceptives, due to their estrogen content, may inhibit lactation. For women desiring to continue nursing, a progestin-only contraceptive is preferable. There are minimal effects on milk production, neonatal growth, and early development. Smoking reduces breast milk volume and decreases infant growth rate [169]. Corticosteroids have a minimal effect on lactation.

ANTIPHOSPHOLIPID ANTIBODY SYNDROME

Criteria have been established [170] and validated [171] for the antiphospholipid antibody syndrome. Criteria require a clinical event (thrombosis or pregnancy loss) and a laboratory confirmation (moderate or high titer IgG or IgM anticardiolipin antibody or lupus anticoagulant, repeated on two occasions 6 weeks apart). Pregnancy-related criteria include three or more otherwise unexplained embryonic losses before 10 weeks or one or more otherwise unexplained fetal losses after 10 weeks, with or without severe early onset preeclampsia [172], placental infarction, or fetal growth restriction.

Identification of the Patient at Risk

The risk for current pregnancy loss due to antiphospholipid antibody is a function both of prior maternal pregnancy history and of titer of anticardiolipin antibody and/or lupus anticoagulant. Whether a mother is diagnosed as having the antiphospholipid antibody because she has lupus anticoagulant, ELISA-defined antiphospholipid antibody, or both does not alter outcome. A false-positive test for syphilis, in an unselected population of otherwise healthy pregnant women and in the absence of the other antibodies, does not predict outcome [173], but it does have predictive value in SLE patients. Most studies find that a high-titer antiphospholipid antibody is more predictive than a low titer and that the IgG isotype is more predictive of fetal loss than IgM [174–185]. There are few data on IgA antibody in pregnancy. There is some disagreement about absolute risk, probably because patients referred for an evaluation of recurrent fetal loss differ from normal pregnant women and from other women with SLE. The importance of the anti-β2GPI antibody in predicting the risk of APS obstetric complications is controversial. Most but not all studies suggest that while anti-β2GPI antibodies are present in many patients with complications, they do not predict pregnancy outcome with any greater accuracy than do aCL or lupus anticoagulant [186] and do not define a separate set of recurrent aborters [187]. Antiphospholipid antibodies other than anticardiolipin or lupus anticoagulant may occasionally be found [188].

The mechanism by which the antiphospholipid antibody induces fetal loss is not completely clear. Histology of placentas and success of anticoagulant therapy suggest that placental thrombosis is the most likely pathogenesis, possibly induced by neutralization of a protective placental anticoagulant [157, 158]. However, the antiphospholipid antibody may also interrupt trophoblast growth and differentiation or prostacyclin metabolism [189, 190].

Current risk estimates offer no justification for screening apparently normal pregnant women for antiphospholipid antibody [191]. Because low-titer IgM and IgG anticardiolipin antibody are as a rule not associated with poor fetal outcome, there is a legitimate argument for closely monitoring, but not arbitrarily treating, women with low-titer antibodies. Because primiparas and women with prior live-born children and no history of fetal loss, even with a high-titer antiphospholipid antibody, have at least a 50% probability of having a live-born child, an argument can be made for close monitoring, without treatment, of these women, although treatment with low-dose (81 mg/day) aspirin is common. First-trimester miscarriages have many causes, and second-trimester fetal deaths have been considered to be more characteristic of antiphospholipid antibody syndrome [192–196]. Data, however, suggest that a significant number of antiphospholipid antibody-related deaths do occur in the first trimester. There is general agreement that women *both* positive for antiphospholipid antibody *and* having prior fetal losses should be treated.

The antiphospholipid antibody is present in approximately 10% of apparently normal women with recurrent spontaneous abortion. The presence or absence of clinical SLE or of prior thromboses does not influence antiphospholipid antibody-related pregnancy outcome but does, of course, alter the risk for maternal complications and fetal complications related to treatment of the mother. Livedo reticularis, duration or persistence of antibody, hemolysis, and cardiac valve disease have not been examined as predictors of fetal outcome.

Effect of Pregnancy on the Course of Antiphospholipid Antibody Syndrome

There is no evidence that the frequency of thrombosis in antiphospholipid antibody syndrome patients changes in either direction during pregnancy, but anticoagulation of most patients for either fetal or maternal indications may hide a change in rate. Only a few patients not treated with heparin during pregnancy suffer thromboses; comparison rates with normal pregnancies are unavailable [197]. Stroke occurred in two *postpartum* antiphospholipid antibody syndrome patients 8 days after the discontinuation of aspirin therapy, emphasizing the need for *postpartum* as well as antepartum treatment [198]. Ischemic cardiomyopathy and myocardial infarction have also been thought to be *postpartum* complications of the antiphospholipid antibody syndrome [199, 200].

Thrombocytopenia regularly occurs in antiphospholipid antibody syndrome pregnancies and usually remits at the conclusion of a pregnancy. Livedo reticularis does not change during pregnancy. Libman–Sacks endocarditis during pregnancy has resulted in fatal thromboembolism with cerebral infarction [201, 202]. Severe thrombocytopenia may occur requiring IVIG and/or corticosteroid therapy. In one refractory case, laparoscopic splenectomy at 18 weeks gestation was successful [98]. The catastrophic antiphospholipid syndrome (CAPS), with multiorgan involvement, may occur during or immediately following pregnancy [203].

Mechanism of Antiphospholipid Antibody Pregnancy Loss

In pregnancies confirmed by the presence of a fetal heartbeat, the course of events is stylized: mild thrombocytopenia occurs within the first several weeks, but otherwise early pregnancy is uneventful. After 15 weeks fetal growth rate slows. Monitoring studies show, in succession, a nonreactive fetal heart rate pattern, spontaneous bradycardia, diminished fetal motion, decreased amniotic fluid, reduced placental size, and, if delivery is not accomplished, fetal death. During the course of these events, the mother, whether or not she has SLE, generally manifests no evidence of illness other than thrombocytopenia; some authors believe that the likelihood and severity of preeclampsia are greater in women who have antiphospholipid antibody than in those who do not [204]. An association between refractory HELLP syndrome and antiphospholipid antibodies has been proposed. Plasmapheresis was beneficial [205]. Hepatic infarction and renal thrombotic microangiopathy in association with early preeclampsia have occurred in several antiphospholipid antibody patients [206–210]. The probability that fetal death will occur is loosely related to the titer of IgG anticardiolipin antibody and to maternal history of prior fetal death. In carefully selected patients, fetal survival (complicated by prematurity and intrauterine growth restriction) rates of up to 80% are possible.

Lupus anticoagulant is a more specific, but less sensitive, predictor of fetal death than the anticardiolipin antibody. Pregnancy prognosis does not improve if the anticoagulant activity is corrected or if the anticardiolipin antibody titer falls.

Fetal death, prematurity, or intrauterine growth restriction is the effect of antiphospholipid antibody-associated placental insufficiency. Infants usually do not themselves have antiphospholipid antibody syndrome, but transplacental passage, leading to thrombosis, may occur [211]. Eight cases of thrombosis in a fetus carrying antiphospholipid antibody have been described, with varied location of thrombosis, many associated with neonatal death. Following delivery, growth-restricted infants gain weight rapidly. No long-term follow-up studies of infant survivors of antiphospholipid antibody syndrome have been published. Short-term surveys suggest that these children develop normally compared to children of similar prematurity [212].

While widely recognized as a significant factor in fetal distress and fetal loss, the antiphospholipid antibody may have a role in some cases of infertility. Increased levels of autoantibodies in general, and antiphospholipid antibody in particular, occur in patients with failed *in vitro* fertilization or embryo transfer cycles [213, 214]. Impairment of embryonic implantation and abnormal blastocyst development occur in a murine antiphospholipid antibody syndrome model [215].

Monitoring the Mother during Pregnancy

Maternal monitoring consists of measuring platelet count, lupus anticoagulant, and anticardiolipin antibody in early pregnancy. In women with strongly positive antiphospholipid antibody tests, serial testing is unnecessary, as a decrease in titer does not permit relaxation of vigilance. In women with low titer or negative tests, repetition at least once each trimester is useful, as an occasional patient demonstrates an increasingly abnormal test as pregnancy progresses, and the overall prognosis is that of the highest titer seen during the pregnancy. Even when initially normal, the platelet count should be repeated monthly, as thrombocytopenia may occur at any time. The risk of clotting may be increased in the immediate *postpartum* period.

Monitoring the Fetus during Pregnancy and the Puerperium

Monitoring of fetal growth and development, as outlined for patients with SLE, is essential for women with antiphospholipid antibody syndrome. Slowed fetal growth or other monitoring abnormalities indicate a need to intervene and, for viable infants, to deliver if fetal distress is clearly identified. Routine serological monitoring may also be of prognostic importance: several studies have demonstrated unexplained elevations of α-fetoprotein in patients with antiphospholipid antibody and, in these patients, have identified a higher risk of fetal and perinatal death. Presumably, abnormalities in the fetoplacental barrier associated with antiphospholipid antibody lead to an increased transport of α-fetoprotein. False-positive elevation in maternal hCG also occurs. There are no other special fetal risks than those of prematurity and growth restriction.

Premature closure of the *ductus arteriosus* is a theoretical concern in infants of aspirin-treated women but it has not been reported in this group of patients and is thought to be infrequent if it occurs at all. The choice of route of delivery is determined by standard obstetric criteria, including maternal and fetal platelet count.

Treatment

Our treatment recommendations are presented in Table 9. *In women with the secondary antiphospholipid syndrome, in whom SLE mandates the use of prednisone, prednisone should be part of the treatment of pregnancy*. The information provided in Table 9 applies to women with primary antiphospholipid syndrome or in those with the secondary syndrome in whom SLE is in remission.

In the earliest papers, low therapeutic levels of *heparin* (10,000 units BID and higher) were chosen as the optimum regimen. More recent studies suggest that even lower doses (5000 to 7000 units BID) are equally effective [216–218]. There are as yet no controlled clinical trials using low molecular weight heparin. Uncontrolled trials suggest no loss of efficacy but no substantial advantage in side effects or success rates. Most treating physicians also use one "baby" *aspirin* per day in conjunction with heparin.

Physicians offering heparin treatment should be aware of, and review with their patients, the substantial risk of bone loss [219]. No study published to date is definitive, but a loss of bone mineral density of up to 10% has been reported in women receiving heparin for an entire pregnancy [220]. In follow-ups up to 6 months, recovery did not occur. Nonsystematic and conflicting data suggest that recovery may occur by 2 years; breast feeding delays recovery. In one study, 2.2% of women receiving heparin for cardiac reasons suffered symptomatic vertebral fractures [221]. It is unclear whether low molecular weight heparin reduces this risk compared to unfractionated heparin [222, 223]. The risk may be lower for low-dose regimens compared to high dose. Animal data suggest that efficacy may be greater than with unfractionated heparin.

To minimize maternal risk, the safest treatment program for women not taking warfarin is to begin aspirin when the patient plans to conceive and to begin heparin only after pregnancy is confirmed. Heparin then should continue until immediately before delivery. It may be useful to obtain a pretreatment bone mineral density study and to reevaluate after delivery. Low

TABLE 9 Treatment Recommendations for Pregnant Women with Antiphospholipid Antibody

Patient characteristic	Recommendation
Medium or high titer IgG or IgM aPL antibody	
Primipara	Consider aspirin, 81 mg/day, or no therapy initially; if modest (>50 × 10^9/liter) thrombocytopenia occurs, aspirin[a]
Multipara, most recent pregnancy live born without complication	Consider aspirin, 81 mg/day, or no therapy initially; if modest (>50 × 10^9/liter) thrombocytopenia occurs, aspirin[a]
Multipara, most recent pregnancy failure <10 weeks (1 loss)	Aspirin
Multipara, most recent pregnancy failure ≥10 weeks without other explanation, or early or severe preeclampsia or fetal growth restriction	Aspirin while trying to conceive; add heparin, 5000 units BID at confirmation of fetal heartbeat, continue for duration of pregnancy
Low-titer IgG or IgM aPL antibody	
Primipara	No therapy
Multipara, no prior fetal loss	No therapy
Multipara, most recent pregnancy failure <15 weeks (1 loss)	Aspirin
Multipara, most recent pregnancy failure ≥15 weeks without other explanation, or >1 loss	Aspirin while trying to conceive; add heparin, 5000 units BID at confirmation of fetal heartbeat, continue for duration of pregnancy
Multipara, prior preeclampsia, IUGR, hypertension, or renal disease	Aspirin, beginning after first trimester
Normal aPL antibody	
Primipara	No therapy
Multipara, prior preeclampsia, IUGR, hypertension, or renal disease	Aspirin, beginning after first trimester
Multipara, all others	No therapy indicated by antiphospholipid antibody

[a] For thrombocytopenia <50 × 10^9/liter, consider intravenous immunoglobulin and/or prednisone.

molecular weight heparin has been used safely and successfully [224], but anecdotes of potential teratogenicity suggest caution. While evidence-based treatment of patients who fail aspirin and heparin therapy is not clear, almost all practitioners empirically add monthly intravenous immunoglobulin. A large number of small studies and case reports suggest success with the addition of IVIG [225]; however, no large-scale prospective randomized study has yet shown benefit, and a small study failed to show benefit [226]. The usual monthly dosage is a total of 1 to 2 g/kg infused over 1–5 days each month. The mechanism is not understood; it may act *via* an anti-idiotype directed against aCL; IVIG reduces aCL titers *in vivo* [227].

In some published treatment trials, *prednisone*, 40 to 60 mg/day (with low-dose aspirin), has been credited with saving pregnancies of women with lupus anticoagulant and prior fetal deaths [228, 229]. High-dose prednisone in the absence of aspirin is unhelpful. These experiences were small and uncontrolled, they included growth-restricted and premature infants as successes and allowed concomitant aspirin therapy. A controlled trial of pregnant women with at least two prior losses and any titer and isotype of antiphospholipid antibody demonstrated that a regimen consisting of low-dose aspirin (81 mg/day) plus subcutaneous heparin, 10,000 to 12,000 units twice daily, begun after ultrasonographic confirmation of a viable pregnancy (6.5 to 8 weeks) was less toxic and equally effective as prednisone, 40 mg/day, plus aspirin [230]. Prednisone-treated mothers suffered more hypertension, weight gain, diabetes, and premature rupture of membranes. Babies suffered amnionitis and prematurity.

In a group of women more rigorously selected but not randomized to different treatment regimens, prednisone plus aspirin, heparin, and aspirin alone were all effective when current, treated pregnancies were compared with prior pregnancies, but success in one treated pregnancy did not guarantee success with the same treatment in a subsequent pregnancy [231].

Several studies have reported a successful pregnancy outcome in patients treated with *intravenous immunoglobulin*. These studies have been limited to patients who have failed more traditional therapies; immunoglobulin is usually used in conjunction with other therapies rather than alone [232–234].

Warfarin is teratogenic and so is not used in pregnancy; subcutaneous heparin or low molecular weight heparin in full therapeutic doses is the anticoagulant of choice in women with prior thrombotic events. Warfarin may be used *postpartum*, even in women who breast feed. Anecdotes concerning the treatment of antiphospholipid antibody syndrome patients with *immunosuppressive* agents are not definitive and do not justify nonexperimental use of these agents [235].

Apheresis, to remove a pathogenic antibody, has been used in the catastrophic vascular occlusion syndrome and has been considered for *experimental* use in recurrent pregnancy loss, but no published experience on this method has yet appeared. In mouse models of pregnancy loss, interleukin 3, ciprofloxacin (which is thought to increase levels of interleukin 3), and a variety of experimental anticoagulants have all had success [236, 237]. There are no human trials with these agents. Ciprofloxacin damages fetal bone and cartilage and should not be used in pregnant women.

In the absence of clinical SLE, anticoagulation is the primary treatment for recurrent fetal loss due to antiphospholipid antibody. Early studies advocating high-dose prednisone, low-dose prednisone, aspirin, and aspirin plus prednisone have now been superseded by controlled clinical trials from several countries that uniformly support the use of unfractionated heparin (with or without aspirin). Fetal survival rates of more than 80% can be achieved even in women with a high-titer antibody and repeated fetal loss (Table 10), and low-dose regimens are effective.

TABLE 10 Randomized Controlled Trials of Treatment for Recurrent Fetal Loss in Women with Antiphospholipid Antibody

Author	No. A/B	Regimen A	Regimen B	Fetal survival A	Fetal survival B
Cowchock *et al*. [230]	12/8	Heparin 12,000 U BID + ASA 81 mg	Prednisone 40 mg + ASA 81 mg	80	75[a]
Kutteh [216]	25/25	Heparin 13300 U BID + ASA 81 mg	ASA 81 mg	80	44
Rai *et al*. [218]	45/45	Heparin 5000 U BID + ASA 81 mg	ASA 81 mg	70	40
Kutteh and Ermel [217]	25/25	Heparin 8100 U + ASA 81 mg	Heparin 13300 U BID + ASA 81 mg	76	80

[a] Marked increase in fetal and maternal morbidity compared to regimen A.

The rate of later thrombosis in the nonanticoagulated patient with a history only of pregnancy morbidity is high. In one study of patients referred for obstetrical management, 48% developed a new, vaso-occlusive symptom in an average follow-up of 3.2 years [238]. In one retrospective study, more than 59% of women with no prior thrombosis who had fetal loss due to aPL and did not subsequently receive low-dose aspirin suffered a thrombotic event within the next decade, compared to only 10% of similar women who did receive aspirin prophylaxis [239]. It is likely that low-dose aspirin will become standard long-term *postpartum* therapy.

Patients with Prior Thrombotic events; Thrombocytopenic Patients

Patients suffering thrombotic events during pregnancy receive full anticoagulant doses of heparin. Similarly, conventional hematology rules apply for thrombocytopenic patients. In general, it is acceptable to continue anticoagulation if the platelet count is above 100×10^9/ml, and possibly if the platelet count is above 50×10^9/ml.

Treatment for Infertility in Antiphospholipid Antibody Syndrome Patients

There are few studies addressing the issue of fertility in patients with SLE. It is likely that patients with SLE have no decrease in fertility [240, 241].

Over the last decade, an extensive literature has addressed the putative association of antiphospholipid antibodies and infertility. In 1987, El-Roeiy *et al*. [242] reported that 10 of 26 patients undergoing *in vitro* fertilization had aPL antibodies in serum and follicular fluid. Patients with autoantibodies had lower pregnancy rates [242]. One year later the same authors presented evidence of a polyclonal B-cell activation in 26 patients with infertility; half of infertile patients had aPL antibodies and two had lupus anticoagulants [243]. In a different group of 41 infertile women, there was a higher prevalence of antibodies to smooth muscle, nuclear, and phospholipid antigens [244]. Antiphospholipid antibody levels do not change during the course of *in vitro* fertilization; patients undergoing *in vitro* fertilization have a higher prevalence of antiphospholipid antibodies prior to their first procedure [245, 246].

Data implicating the antiphospholipid antibody in infertility and in failure of *in vitro* fertilization and embryo transfer cycles are preliminary. A single clinical study randomized antiphospholipid antibody-positive patients with failed *in vitro* fertilization to aspirin plus heparin or no treatment during the next cycle. Viable pregnancy rates were 49 and 16%, respectively [247].

Patients with the antiphospholipid antibody do not appear to incur serious risks if they undergo ovulation induction and other assisted reproduction techniques. The concern for aPL-positive patients (as opposed to those related to underlying SLE) is an increased risk of thrombosis in the setting of the high estrogen levels achieved. As these levels do not reach those sustained in late pregnancy, risk is likely not increased over that associated with pregnancy itself, the intended outcome of IVF. No thromboses were reported in a series of 10 patients with PAPS who underwent from 1 to 17 cycles of ovarian induction each (a total of 47 cycles). Nine of the 10 patients were treated during their cycles with aspirin and 6 with heparin [248]. The practice of anticoagulant treatment through the early phase of the cycle to reduce thrombosis risk remains unproven but, at least in the case of low-risk aspirin therapy, may be reasonable. Patients with a history of thrombosis and on warfarin prior to IVF should substitute subcutaneous heparin, both before and after time of embryo transfer (holding heparin just before oocyte retrieval).

Postpartum Treatment; Abortion

It is conventional practice to continue aspirin (if used during pregnancy) for at least 1 month *postpartum* to prevent the potential complication of thrombosis. For women who have received heparin up to delivery it is conventional to continue heparin *postpartum* or to change to warfarin and to continue anticoagulation for up to 3 months. If a clotting event has occurred during or following the pregnancy, treatment is continued for a longer period, but absolute points at which anticoagulation might be terminated have not been established.

Patients electing abortion should receive aspirin, 81 to 300 mg/day, for 1 month *postpartum* as a low-risk, low-cost prophylaxis against the theoretical possibility of thromboembolic complications.

Medico-legal Considerations

Physicians treating otherwise well women for recurrent pregnancy loss should keep foremost in their minds the fact that the available treatments engender considerable risk. If pregnancy is not an extremely high priority for the patient, treatment other than aspirin should not be offered, regardless of the number of prior unsuccessful pregnancies or titer of antiphospholipid antibody. Osteoporotic fracture or serious hemorrhage from heparin, osteonecrosis from corticosteroid, or pulmonary edema or hepatitis C from intravenous immunoglobulin are high prices to pay if a child is not intensely desired. If a woman has received such a treat-

ment and has not been forewarned of its complications, and if those complications ensue, her lawyer will certainly remind her that adequate warning is a physician's duty.

Counseling and Other Issues

Because both *oral contraceptive* therapy and antiphospholipid antibody syndrome are associated with a high frequency of vascular occlusive events, it would seem unwise to prescribe oral contraceptives to patients with antiphospholipid antibody. Specific calculations of risks that clearly implicate oral contraceptives in thrombotic events in antiphospholipid antibody syndrome patients have not been defined. Oral contraceptives may increase the risk of venous thromboembolism [249], depending on both the contraceptive "generation" and other patient risk factors. In one large lupus cohort, oral contraceptive use was not associated with venous thromboembolism. An ongoing National Institutes of Health study of oral contraceptives in lupus patients accepts patients with low-to-moderate (<40 GPL units/ml) titer antiphospholipid antibody, suggesting that an answer may be available soon.

Genetic counseling should take cognizance of the fact that the antiphospholipid antibody is often familial [250], including in some cases other family members with SLE, but the degree of penetrance or of disease expression in related family members is not known. In different populations, women with the antiphospholipid antibody have an unusual frequency of HLA-DRw7 [251, 252], HLA-DRw53 [253], HLA-B8, DRw3 [254], or the C4 null allele [255]. There are no known genetic fetal abnormalities associated with the antiphospholipid antibody, and no valid risk calculations regarding the probability that the child will eventually also develop this antibody.

While antiphospholipid antibody is important, many *other causes of recurrent fetal loss* exist [256, 257]. These include abnormalities of chromosomes, sperm, uterus, and cervix, and infection, immune attack on the fetus, and underlying medical illness. Evaluation for recurrent fetal loss generally includes items listed in Table 11 [258].

There are no publications regarding *breast feeding* in women with the antiphospholipid antibody syndrome. Because many live-born infants are premature or growth restricted, breast feeding is often impossible. Those that have been able to breast feed have done so uneventfully. Although maternal antiphospholipid antibody-containing immunoglobulin may be transmitted through breast milk to the newborn, and although low-dose aspirin is also transferred to breast milk, no complications known to be related to breast feeding have been reported. Heparin is not transferred to breast milk and so may be continued if necessary.

TABLE 11 Evaluation for Recurrent Fetal Loss

- General medical and obstetrical history, including chronic illness and diethylstilbesterol exposure
- Complete medical and gynecological examination
- Laboratory evaluation including fasting blood sugar, erythrocyte sedimentation rate, and thyroid function tests
- Blood group, including Rh factor
- Coagulation factors
- VDRL, lupus anticoagulant, anticardiolipin antibodies
- Karyotype of both parents
- Postovulatory progeterone level
- Full endocrine profile
- Pelvic ultrasound
- Hysterosalpingogram
- Laparoscopy

What to Tell Prospective Parents

Most studies associating antiphospholipid antibody with thrombosis or fetal death have begun with the event (thrombosis or prior fetal death) or diagnosis (SLE), identified the antibody, and then either retrospectively or prospectively calculated a frequency of new events in this preselected population. These studies suggest a high risk to all bearers of the antibody. Cross-sectional studies of populations have suggested much lower frequencies of new events in women who have not had a prior adverse event. Therefore, in clinically well women with the antiphospholipid antibody, prophylactic treatment against either thrombosis or fetal death is not indicated, although low-dose aspirin is sometimes used. The occurrence of thrombosis cannot be predicted by changes in antibody titer; the risk of *recurrent* thrombosis in the unanticoagulated patient who is not pregnant is high [259, 260]. In one study of patients referred for obstetrical management, 48% developed a new, vaso-occlusive symptom in an average follow-up of 3.2 years [238]. In another study of women with antiphospholipid antibody and pregnancy loss but no prior thrombosis, 10% of those treated *postpartum* with aspirin and 59% of those untreated had thrombotic events in the subsequent 4.2 years [239]. It is not known whether pregnancy increases the risk for new thrombosis. Discussion of these uncertainties with prospective parents is appropriate.

The likelihood that the patient with primary antiphospholipid antibody syndrome will eventually

develop SLE is low. There are no known risks to the surviving infant beyond those associated with its prematurity.

LUPUS-LIKE DISEASE

Because patients with mixed or undifferentiated connective tissue disease may carry anti-SSA/Ro, anti-SSB/La, or antiphospholipid antibodies, they must be monitored through pregnancy in a manner similar to that appropriate for a patient with SLE or primary antiphospholipid antibody syndrome. Treatment for complications is directed to the organ systems involved, not to the specific diagnosis, and is similar to that used for SLE. Counseling is that relevant to SLE.

Management of pregnancy of patients with secondary Sjögren's syndrome is that of the accompanying connective tissue disease. Treatment of the local manifestations of primary Sjögren's syndrome is not altered by the presence of pregnancy. Most pregnancies in primary Sjögren's syndrome occur before the onset of disease. These patients have an increased risk of fetal loss not attributable to autoantibodies and a risk of neonatal lupus in their offspring as well [261].

It is important to determine whether anti-SSA/Ro or anti-SSB/La antibodies are present in pregnant patients with Sjögren's syndrome and to anticipate the possibility of both cardiac and cutaneous findings of neonatal lupus in infants of those found to be positive (see earlier discussion). There is a possibility that patients with Sjpgren's syndrome are more likely than other anti-SSA/Ro antibody-positive patients to deliver children with neonatal lupus [262]. Counseling should reflect these data.

IN VITRO FERTILIZATION AND RELATED TOPICS

Infertility, Male and Female

The decline of female fertility begins in the middle thirties and becomes marked after age 40. Because the lupus patient has effects of cytotoxic agents, such as cyclophosphamide, and long-term effects of chronic illness, her fertility may decline sooner.

Evaluation for infertility includes consideration of both male and female factors. Testing for the woman includes a day three follicle-stimulating hormone level, thyroid function tests, and cervical cultures. Tests for ovulation include basal body temperature and endometrial biopsy. Low serum progesterone 1 week after ovulation suggests an inadequate luteal phase. A postcoital test gives information regarding quality and quantity of cervical mucus as well as the presence of antisperm antibodies. Tubal function is evaluated with a hysterosalpingogram. Hysteroscopy and laparoscopy may be required [263].

Tests for male factors include semen analysis as well as tests for spermatozoal function, such as the hamster penetration assay and the human zona pellucida assay. Hormone evaluation radiological tests may uncover other causes for infertility [264].

Assisted Reproductive Techniques

A glossary of assisted reproductive techniques is presented in Table 12 [265].

TABLE 12 Glossary of Terms of Assisted Reproduction

Term	Definition
Assisted reproductive technologies (ART)	Infertility treatment procedures involving manipulation of oocytes, spermatozoa, and/or embryos
In vitro fertilization (IVF)	Laboratory culture of aspirated oocytes and spermatozoa followed by transcervical embryo transfer (ET)
Gamete intrafallopian transfer (GIFT)	Direct placement of aspirated oocytes and spermatozoa into fallopian tubes
Controlled ovarian hyperstimulation (COH)	Ovulation induction with monitoring in normal ovulatory women; intent is to induce multiple ovarian follicles
Intrauterine insemination (IUI) Intracervical insemination (ICI) Intratubal insemination (ITI)	Separation of spermatozoa from seminal fluid with suspension in buffer or culture media and insemination into the uterus, cervix, or fallopian tube
Zygote intrafallopian transfer (ZIFT)	Laboratory culture of aspirated oocytes with spermatozoa followed by direct placement of fertilized zygotes or embryos into fallopian tubes
Oocyte donation (OD)	Laboratory culture of aspirated oocytes from a donor woman followed by sperm/oocyte culture and transfer
Intracytoplasmic spermatozoa injection (ICSI)	A micromanipulation technique in which a single sperm is injected into an oocyte. It is used in cases of abnormal spermatazoal function or very low sperm counts

Treatment Regimens Used in Assisted Reproduction

In vitro fertilization, initially developed for patients with tubal disease but now used for a variety of infertility diagnoses, is the most common assisted reproductive technique. The process includes oocyte stimulation, retrieval, *in vitro* fertilization, and embryo placement in the uterus [266].

Preparation of the oocyte donor begins with the administration of gonadotropin-releasing hormone (GnRH) analogues to bring about desensitization of the pituitary gonadotropes to endogenous GnRH. Ovarian stimulation is accomplished by administering human menopausal gonadotropin and ovulation by administering human chorionic gonadotropin (hCG) when many follicles are sufficiently mature. The luteal phase is maintained with low-dose hCG or with exogenous (intramuscular) progesterone. Oocytes are retrieved through transvaginal ultrasound-guided follicular puncture under local anesthesia.

Semen preparation involves separation of motile spermatozoa from seminal fluid, *in vitro* capacitation, addition to cumulus-enclosed oocytes a few hours after their retrieval, and assessment of fertilization. Gamete micromanipulation is used for male factor infertility; the most effective method is intracytoplasmic sperm injection. Fertilized oocytes are reexamined after a further 24-h incubation to confirm that embryonic cleavage has occurred and to assess embryo quality. Embryo placement (usually three per procedure) is accomplished through transcervical transfer to the uterus at the two to four cell stage.

Within 10–12 days of insemination, implantation can be detected by an increase in serum hCG levels. Diagnosis of clinical pregnancy depends on visualization by ultrasound of a gestational sac containing a viable fetus (detectable heart beat) within the uterine cavity.

The pregnancy success rate following *in vitro* fertilization and other assisted reproductive techniques is greater for patients of younger age. For older patients with poor oocyte quality or for patients carrying a maternal genetic defect, oocyte donation is recommended. Use of specific techniques depends on infertility etiology, if identifiable. The description of outcome of assisted reproduction varies. *Biochemical pregnancy* is implantation identified by a transient production of hCG but early loss before sonogram documentation of a fetus. *Clinical pregnancy* progresses to a stage in which a gestational sac and fetal heart motion can be seen. *Ongoing pregnancies* represent currently viable gestations. Success rates may be expressed as pregnancies per transfer or per embryo transferred. There is a greater risk of ectopic pregnancy and of first trimester spontaneous abortions in IVF pregnancies. The most common complication is multiple gestation. Up to 30% of multiple births occur, most being twins [267].

ALTERNATIVES TO ASSISTED REPRODUCTION

Lupus patients sponsor surrogate children, using their own or donated oocytes brought to term in volunteer well women. Lupus patients also adopt children. In the latter case, many patients are severely questioned about their own health. The process varies in different locales. The emotional trauma of enduring such a process may be severe.

Women who have undergone assisted reproduction attempts or who have adopted counsel that the costs are very high and that emotional and physical exhaustion (often including distant travel) is the rule. For women undergoing ovarian hyperstimulation, another burden is added, as the procedure, even in well women, is physiologically exhausting and painful. Lupus exacerbation, however, is rare.

References

1. Moncayo, R., and Moncayo, H. E. (1995). A new endocrinological and immunological syndrome in SLE: Elevation of human chorionic gonadotropin and of antibodies directed against ovary and endometrium antigens. *Lupus* **4**, 39–43.
2. Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al.* (1997). "Williams Obstetrics," 20th Ed., pp. 13–34. Appleton and Lange, Stamford.
3. Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al.* (1997). "Williams Obstetrics," 20th Ed., pp. 607–616. Appleton and Lange, Stamford.
4. Mendonca, L. L., Khamashta, M. A., Nelson-Piercy, C., Hunt, B. J., and Hughes, G. R. (2000). Non-steroidal anti-inflammatory drugs as a possible cause for reversible infertility. *Rheumatology*, **39**, 880–882.
5. Centers of Disease Control and Prevention (1995). Ectopic pregnancy-United States, 1990–1992. *MMWR* **1**, 46.
6. Benirschke, K., and Kim, C. K. (1972). Multiple pregnancy. *N. Engl. J. Med.* **288**, 1276–1281.
7. Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al.* (1997). "Williams Obstetric," 20th Ed., pp. 861–881. Appleton and Lange, Stamford.
8. American College of Obstetricians and Gynecologists (1994). Precis, p. 388.
9. Hollingsworth, D. R., and Kreutner, A. K. (1980). Teenage pregnancy. *N. Engl. J. Med.* **304**, 516–523.

10. Prysak, M., Lorenz, R. P., and Kisley, A. (1995). Pregnancy outcome in nulliparous women 35 years and older. *Obstet. Gynecol.* **85**, 65–70.
11. Cunningham, F. G., Mac Donald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. Q., *et al*. (1997). "Williams Obstetrics," 20th Ed., pp. 533–535. Appleton and Lange, Stamford.
12. Branch, D. W. (1992). Physiologic adaptations of pregnancy. *Am. J. Reprod. Immunol.* **28**, 120–122.
13. Burrows, R. F., and Kelton, J. G. (1988). Incidentally detected thrombocytopenia in healthy mothers and their infants. *N. Engl. J. Med.* **319**, 142–145.
14. Vazifi, N. D., Toohey, K., Powers, D., *et al*. (1986). Activation of intrinsic coagulation pathway in pre-eclampsia. *Am. J. Med.* **80**, 103–107.
15. Lockshin, M. D. (1993). Pregnancy and systemic autoimmune disease. *Semin. Clin. Immunol.* **5**, 5–11.
16. Gleicher, N. (1992). Autoantibodies in normal and abnormal pregnancy. *Am. J. Reprod. Immunol.* **28**, 269–273.
17. El-Roeiy, A., and Gleicher, N. (1988). Definition of normal autoantibody levels in an apparently healthy population. *Obstet. Gynecol.* **72**, 596–602.
18. Kwak, J. Y. H., Gilman-Sachs, A., Beaman, K. D., *et al*. (1992). Autoantibodies in women with primary recurrent spontaneous abortion of unknown etiology. *J. Reprod. lmmunol.* **22**, 15–31.
19. Hill, J. A. (1992). Cytokines considered critical in pregnancy. *Am. J. Reprod. Immunol.* **28**, 123–126.
20. Chaouat, G., Menu, E., Clark, D. A., *et al*. (1990). Control of fetal survival in CBA × DBA/2 mice by lymphokine therapy. *J. Reprod. Fertil.* **89**, 447–458.
21. Brostoff, J., Scadding, G. K., Male, D., *et al*. (eds.) (1992). "Clinical Immunology." Gower, London.
22. Buyon, J. P., Tamerius, J., Ordorica, S., *et al*. (1992). Activation of the alternative complement pathway accompanies disease flares in systemic lupus erythematosus during pregnancy. *Arthritis Rheum.* **36**, 55–61.
23. Levy, R. A., Qamar, T., and Lockshin, M. (1990). Alternative complement pathway in hypocomplementemic/normal C1s-C1 inhibitor complex patients with SLE. *Clin. Exp. Rheumatol.* **8**, 11–15.
24. Holers, V. M., Girardi, G., Mo, L., Guthridge, M., Molina, H., Pierangeli, S. S., Espinola, R., Xiaowei, L. E., Mao, D., Vialpando, C. G., and Salmon, J. E. (2002). Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J. Exp. Med.* **195**, 211–220.
25. Lahita, R. G. (1992). The effects of sex hormones on the immune system in pregnancy. *Am. J. Reprod. Immunol.* **28**, 136–137.
26. Sthoeger, Z. M., Chiorazzi, N., and Lahita, R. G. (1988). Regulation of the immune response by sex hormones. 1. In vivo effects of estradiol and testosterone on pokeweed mitogen induced B-cell differentiation. *J. Immunol.* **141**, 91–98.
27. Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., pp. 693–704. Appleton and Lange, Stamford.
28. Sabatini, S. (1993). Pathophysiology of and therapeutic strategies for hypertension in pregnancy. *Curr. Opin. Nephrol. Hypertens.* **2**, 763–774.
29. Amant, F., Spitz, B., Arnout, J., and Van Assche, F. A. (1997). Hepatic necrosis and haemorrhage in pregnant patients with antiphospholipid antibodies. *Lupus* **6**, 552–555.
30. Ornstein, M., and Rand, J. H. (1994). An association between refractory HELLP syndrome and antiphospholipid syndrome during pregnancy: A report of 2 cases. *J. Rheumatol.* **21**, 1360–1364.
31. Gerbasi, F. R., Bottoms, S., Farag, A., and Mammen, E. (1990). Increased intravascular coagulation associated with pregnancy. *Obstet. Gynecol.* **75**, 385–392.
32. Toglia, M. R., and Weg, J. G. (1996). Venous thromboembolism during pregnancy. *N. Engl. J. Med.* **335**, 107–114.
33. Kittner, S. J., Stern, B. J., Feeser, B. R., Hebil, J. R., Nagey, D. A., Buchholz, D. W., *et al*. (1996). Pregnancy and the risk of stroke. *N. Engl. J. Med.* **335**, 768–774.
34. Bokarewa, M. E., Bremme, K., and Blomback, M. (1996). Arg506-Gln mutation in factor V and risk of thrombosis during pregnancy. *Br. J. Haematol.* **92**, 473–478.
35. Lee, R. V. (1996). Thromboembolic disease and pregnancy: Are all women created equal? *Ann. Intern. Med.* **125**, 1001–1002.
36. Martinelli, I., Taioli, E., Cetin, I., Merinoni, A., Gerosa, S., Villa, M. V., Bozzo, M., and Mannucci, P. M. (2000). Mutations in coagulation factors in women with unexplained late fetal loss. *N. Engl. J. Med.* **343**, 1015–1018.
37. Gris, J. C., Quire, I., Monpeyroux, F., Mercier, E., Ripart-Neveau, S., Tailland, M. L., Hoffet, M., Berlan, J., Daures, J. P., and Mares, P. (1999). Case-control study of the frequency of thrombophilic disorders in couples with late foetal loss and no thrombotic antecedent: The Nimes Obstetricians and Haemotologists Study 5 (NOHA5). *Thrombosis Haemostasis* **81**(6), 891–899.
38. Espana, F., Villa, P., Mira, Y., Grancha, S., Royo, M., Estelles, A., Vaya, A., and Azner, J. (1999). Factor V Leiden and antibodies against phospholipid and protein S in a young women with recurrent thromboses and abortion. *Haematologica* **84**(1), 80–84.
39. Bitsch, M., Johanson, C., Wennevold, A., and Osler, M. (1989). Maternal heart disease: A survey of a decade in a Danish University hospital. *Acta Obstet. Gynecol. Scand.* **68**, 119–127.
40. O'Connell, J. B., Constanzo-Nordin, M. R., Subramanian, R., Robinson, J. A., Wallis, D. E., Scanlon, P. J., *et al*. (1986). Peripartum cardiomyopathy: Clinical, hemodynamic, histologic and prognostic characteristics. *J. Am. Coll. Cardiol.* **8**, 52–58.
41. Thorp, J. R., Jr., Chescheir, N. C., and Fann, B. (1994). Postpartum myocardial infarction in a patient with antiphospholipid syndrome. *Am. J. Perinatol.* **11**, 1–7.
42. Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., pp. 1079–1101. Appleton and Lange, Stamford.

43. American College of Obstetricians and Gynecologists (1994). Diabetes and pregnancy. Technical Bulletin 200, December.
44. Owen, J., Phelan, S. T., Landon, M. P., and Gabbe, S. G. (1995). Gestational diabetes survey. *Am. J. Obstet. Gynecol.* **172**, 615–619.
45. Hanson, U., and Persson, B. (1993). Outcome of pregnancies complicated by type 1 insulin-dependent diabetes in Sweden: Acute pregnancy complications, neonatal mortality and morbidity. *Am. J. Perinatol.* **4**, 330–334.
46. Castle, S. P., Mather-Mondrey, M., Bennion, S., David-Bajar, K., and Huff, C. (1996). Chronic herpes gestationis and antiphospholipid antibody syndrome successfully treated with cyclophosphamide. *J. Am. Acad. Dermatol.* **34**, 333–336.
47. Leslie, K. K., and Reznikov, L. (1996). Intrahepatic cholestasis of pregnancy is associated with abnormal estrogen excretion. *Am. J. Obstet. Gynecol.* **174**, 368–372.
48. Schoor-Lesnick, B., Lebovics, E., Dworkin, B., and Rosenthal, W. S. (1991). Liver diseases unique to pregnancy. *Am. J. Gastroenterol.* **86**, 659–670.
49. Castro, M. A., Goodwin, T. M., Shaw, K. J., Ouzounian, J. G., and McGee, W. G. (1996). Disseminated intravascular coagulation and antithrombin III depression in acute fatty liver of pregnancy. *Am. J. Obstet. Gynecol.* **174**, 211–214.
50. Rasmussen, N., Frolich, A., Hornnes, P. J., and Hegedus, L. (1990). Serum ionized calcium and intact parathyroid hormone levels during pregnancy and postpartum. *Br. J. Obstet. Gynaecol.* **97**, 857–859.
51. Sowers, M. (1996). Pregnancy and lactation as risk factors for subsequent bne loss and osteoporosis. *J. Bone Mineral Res.* **11**, 1052–1060.
52. Rodin, A., Duncan, A., Quertero, H. W. P., *et al*. (1989). Serum concentrations of alkaline phosphatase isoenzymes and osteocalcin in normal pregnancy. *J. Clin. Endocrinol. Metabol.* **68**, 1123–1127.
53. Bertelloni, S., Baroncelli, G. I., Pelletti, A., *et al*. (1994). Parathyroid hormone related protein in healthy pregnant women. *Calcif. Tissue. Int.* **54**, 195–197.
54. Gertner, J., Coustan, D., Kliger, A., *et al*. (1986). Pregnancy as a state of physiologic absorptive hypercalciuria. *Am. J. Med.* **81**, 451–456.
55. Dunne, F., Walters, B., Marshall, T., and Heath, D. A. (1993). Pregnancy-associated osteoporosis. *Clin. Endocrinol.* **39**, 487–490.
56. Kritz-Silverstein, D., Barrett-Connor, E., and Hollenbach, K. A. (1992). Pregnancy and lactation as determinants of bone mineral density in postmenopausal women. *Am. J. Epidemiol.* **136**, 1052–1059.
57. Hoffman, S., Grisso, J. A., Kelsey, J. L., *et al*. (1993). Parity, lactation and hip fracture. *Osteoporosis Int.* **3**, 171–176.
58. Wardlaw, G. M., and Pike, A. M. (1986). The effect of lactation on peak adult shaft and ultradistal forearm bone mass in women. *Am. J. Clin. Nutr.* **44**, 283–286.
59. Tuchman-Duplessis, H., David, G., and Haegel, P. (1975). "Illustrated Human Embryology," Vol. I, pp. 62–84. Springer Verlag, New York.
60. Lipitz, S., Adman, D., Menczer, J., Ben-Baruch, G., and Oelsner, G. (1991). Midtrimester bleeding: Variables which affect the outcome of pregnancy. *Gynecol. Obstet. Invest.* **32**, 24–28.
61. Karegard, M., and Gennser, G. (1986). Incidence and recurrence rate of abruptio placentae in Sweden. *Obstet. Gynecol.* **67**, 523–525.
62. Clark, S. L., Koonings, P. P., and Phelan, J. P. (1985). Placenta previa/accreta and prior cesarian section. *Obstet. Gynecol.* **66**, 89–92.
63. Cunningham, F. G., Mac, Donald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., pp. 746–782. Appleton and Lange, Stamford.
64. Canadian Hydroxychloroquine Study Group (1991). A randomized study of the effect of withdrawing hydroxychloroquine sulfate in systemic lupus erythematosus. *N. Engl. J. Med.* **324**, 150–154.
65. Khamashta, M. A., Cuadrado, M. J., Mujic, F., *et al*. (1995). The management of thrombosis in the antiphospholipid-antibody syndrome. *N. Engl. J. Med.* **332**, 993–997.
66. Liang, M. H., Socher, S. A., Roberts, W. N., *et al*. (1988). Measurement of systemic lupus erythematosus activity in clinical research. *Arthritis Rheum.* **31**, 817–825.
67. Petri, M., Howard, D., and Repke, J. (1991). Frequency of lupus flare in pregnancy: The Hopkins Lupus Pregnancy Center experience. *Arthritis Rheum.* **34**, 1538–1545.
68. Lockshin, M. D., Reinitz, E., Druzin, M. L., *et al*. (1984). Lupus pregnancy. Case-control prospective study demonstrating absence of lupus exacerbation during or after pregnancy. *Am. J. Med.* **77**, 893–898.
69. Ruiz-Irastorza, G., Lima, F., Alves, J., *et al*. (1996). Increased rate of lupus flare during pregnancy and the puerperium: A prospective study of 78 pregnancies. *Br. J. Rheumatol.* **35**, 133–138.
70. Urowitz, M. B., Gladman, D. D., Farewell, V. T., *et al*. (1993). Lupus and pregnancy studies. *Arthritis Rheum.* **36**, 1392–1397.
71. Lockshin, M. D. (1992). Treatment of lupus pregnancy: Can we reach consensus? *Clin. Exp. Rheumatol.* **10**, 429–431.
72. Buyon, J. P., Kalunian, K. C., Ramsey-Goldman, R., Petri, M. A., Ruiz-Irastorza, G., and Khamashta, M. (1999). Assessing disease activity in SLE patients during pregnancy. *Lupus* **8**, 677–684.
73. Ruiz-Irastorza, in press.
74. Alarcon-Segovia, D., and Sanchez-Guerrero, J. (1989). Correction of thrombocytopenia with small dose aspirin in the primary antiphospholipid antibody syndrome. *J. Rheumatol.* **16**, 1359–1361.
75. Martinez-Rueda, J. O., Arce-Salinas, C. A., Kraus, A., *et al*. (1996). Factors associated with fetal losses in severe systemic lupus erythematosus. *Lupus* **5**, 113–119.
76. Packham, D. K., Lam, S. S., Nicholls, K., *et al*. (1992). Lupus nephritis and pregnancy. *Q. J. Med.* **83**, 315–324.
77. Druzin, M. L. (1988). Pregnancy induced hypertension and preeclampsia: The fetus and the neonate. In "Handbook of Hypertension" (P. C. Rubin, ed.), Vol. 10, pp. 267–289. Elsevier, Amsterdam.

78. Buyon, J. P., Cronstein, B. N., Morris, M., *et al*. (1986). Serum complement values (C3 and C4) to differentiate between systemic lupus activity and pre-eclampsia. *Am. J. Med.* **81**, 194–200.
79. Hou, S. (1985). Pregnancy in women with chronic renal disease. *N. Engl. J. Med.* **312**, 836–839.
80. Lockshin, M. D., Qamar, T., Redecha, P., *et al*. (1986). Hypocomplementemia with low C1s-C1 inhibitor complex in systemic lupus erythematosus. *Arthritis Rheum.* **29**, 1467–1472.
81. Parke, A. L. (1988). Antimalarial drugs, systemic lupus erythematosus and pregnancy. *J. Rheumatol.* **15**, 607–610.
82. Lockshin, M. D. (1991). Therapy for systemic lupus erythematosus. *N. Engl. J. Med.* **324**, 189–190.
83. Kuzis, C. S., Druzin, M. L., and Lambert, R. E. (1996). Case report: A patient with severe CNS lupus during pregnancy. *Ann. Med. Intern.* **147**, 274–275.
84. Wolf, R. E., and McBeath, J. G. (1985). Chorea gravidarum in systemic lupus erythematosus. *J. Rheumatol.* **12**, 992–993.
85. Marabani, M., Zoma, A., Hadley, D., *et al*. (1989). Transverse myelitis occurring during pregnancy in a patient with systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**, 160–162.
86. Nossent, H. C., and Swaak, T. J. G. (1990). Systemic lupus erythematosus. VI. Analysis of the interrelationship with pregnancy. *J. Rheumatol.* **17**, 771–776.
87. Beaufils, M., Donsimoni, R., Uzan, S., *et al*. (1985). Prevention of pre-eclampsia by early antiplatelet therapy. *Lancet* **1**, 840–842.
88. Wallenburg, H. C. S., Makovitz, J. W., Dekker, G. A., *et al*. (1986). Low dose aspirin prevents pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae. *Lancet* **1**, 1–3.
89. Dekker, G. A., and Sibai, B. M. (1993). Low-dose aspirin in the prevention of preeclampsia and fetal growth retardation: Rationale, mechanisms, and clinical trials. *Am. J. Obstet. Gynecol.* **168**, 214–227.
90. Schiff, E., and Mashiach, S. (1992). The use of low-dose aspirin in pregnancy. *Am. J. Reprod. Immunol.* **28**, 153–156.
91. Parazzini, F., Benedetto, C., Frusca, T., *et al*. (1993). Low-dose aspirin in prevention and treatment of intrauterine growth retardation and pregnancy-induced hypertension. *Lancet* **341**, 396–400.
92. Himmelstein, D. U. (1983). Aspirin and maternal or neonatal hemostasis. *N. Engl. J. Med.* **308**, 281.
93. Stuart, M. J., Gross, S. J., Eirad, H., *et al*. (1982). Effects of acetylsalicylic-acid ingestion on maternal and neonatal hemostasis. *N. Engl. J. Med.* **307**, 909–912.
94. Moise, K. J., Huhta, J. C., Sharif, D. S., *et al*. (1988). lndomethacin in the treatment of premature labor: Effects on the fetal ductus arteriosus. *N. Engl. J. Med.* **319**, 327–331.
95. Besa, E. C., MacNab, M. W., Solan, A. J., *et al*. (1985). High-dose intravenous IgG in the management of pregnancy in women with idiopathic thrombocytopenic purpura. *Am. J. Hematol.* **18**, 373–379.
96. Fehr, J., Hofmann, V., and Kappeler, U. (1982). Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gamma globulin. *N. Engl. J. Med.* **306**, 1254–1258.
97. Newland, A. C., Boots, M. A., and Patterson, K. G. (1984). Intravenous IgG for autoimmune thrombocytopenia in pregnancy. *N. Engl. J. Med.* **310**, 261–262.
98. Hardwick, R. H., Slade, R. R., Smith, P. A., and Thompson, M. H. (1999). Laparoscopic splenectomy in pregnancy. *J. Laparo. Adv. Surg. Tech*. (*A*) **9**, 439–440.
99. Ray, J., and Sermer, M. (1996). Systemic lupus erythematosus and pulmonary hypertension during pregnancy: Report of a case fatality. *Can. J. Cardiol.* **12**, 753–756.
100. Rubin, L. A., Geran, A., Rose, T. H., *et al*. (1995). A fatal pulmonary complication of lupus in pregnancy. *Arthritis Rheum.* **38**, 710–714.
101. Schoenfeld, A., Bar, Y., Merlob, P., *et al*. (1992). NSAIDS: Maternal and fetal considerations. *Am. J. Reprod. Immunol.* **28**, 141–147.
102. Braga, J., Marques, R., Branco, A., *et al*. (1996). Maternal and perinatal implications of the use of human recombinant erythropoietin. *Acta Obstet. Gynecol. Scand* **75**, 449–453.
103. Kontessis, P. S., Paraskevopoulos, Papageorgiou, I., *et al*. (1995). Successful use of recombinant human erythropoietin in a pregnant woman with lupus nephritis. *Am. J. Kidney Dis.* **26**, 781–784.
104. Schneider, H., and Malek, A. (1995). Lack of permeability of the human placenta for erythropoietin. *J. Perinat. Med.* **23**, 71–76.
105. Levy, M., Buskila, D., Gladman, D. D., *et al*. (1991). Pregnancy outcome following first trimester exposure to chloroquine. *Am. J. Perinatol.* **8**, 174–178.
106. Ostensen, M. (1992). Treatment with immunosuppressive and disease-modifying drugs during pregnancy and lactation. *Am. J. Reprod. Immunol.* **28**, 148–152.
107. Levy, R. A., Vilela, V. S., Cataldo, M. J., Ramos, R. C., Duarte, J. L., Tura, B. R., Albuquerque, E. M., and Jesus, N. R. (2001). Hydroxychloroquine (HCQ) in lupus pregnancy: Double-blind and placebo-controlled study. *Lupus* **10**, 401–404.
108. Cunningham, F. G., Mac Donald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., pp. 943–965. Appleton and Lange, Stamford.
109. Ward, R. M. (1993). Drug therapy of the fetus. *J. Clin. Pharmacol.* **33**, 780–789.
110. Piper, J. M., Baum, C., and Kennedy, D. L. (1987). Prescription drug use before and during pregnancy in a medicaid population. *Am. J. Obstet. Gynecol.* **157**, 148–157.
111. Shepard, T. H. (1986). Human teratogenicity. *Adv. Ped.* **33**, 225–247.
112. Food and Drug Administration Bulletin (1979). Pregnancy categories for prescription drugs. September.
113. Shotan, A., Widerhom, J., Hurst, A., and Eldayam, U. (1994). Risks of angiotensin converting enzyme inhibition during pregnancy: Experimental and clinical evidence, potential mechanisms, and recommendations for use. *Am. J. Med.* **96**, 451–457.

114. Eishi, Y., Hirokawa, K., and Hatakeyama, S. (1983). Long-lasting impairment of immune and endocrine systems of offspring induced by injection of dexamethasone into pregnant mice. *Clin. Immunol. Immunopathol.* **26**, 335–349.
115. Anderson, G. G., Rotchell, Y., and Kaiser, D. G. (1981). Placental transfer of methylprednisolone following maternal intravenous administration. *Am. J. Obstet. Gynecol.* **140**, 699–701.
116. Hensleigh, P. A., Herzenberg, L. A., Lipman, S. H., *et al*. (1983). Transient immunologic effects of betamethasone in human pregnancy after suppression of pre-term labor. *Am. J. Reprod. Immunol.* **4**, 83–87.
117. Rayburn, W. F. (1992). Glucocorticoid therapy for rheumatic disease: Maternal, fetal, and breast-feeding considerations. *Am. J. Reprod. Immunol.* **28**, 138–140.
118. Bermas, B. L., and Hill, J. A. (1995). Effects of immunosuppressive drugs during pregnancy. *Arthritis Rheum.* **38**, 722–732.
119. Meehan, R. T., and Dorsey, K. T. (1987). Pregnancy among patients with systemic lupus erythematosus receiving immunosuppressive therapy. *J. Rheumatol.* **14**, 252–258.
120. Dewitte, D. B., Buick, M. K., Cyran, S. E., *et al*. (1984). Neonatal pancytopenia and severe combined immunodeficiency associated with antenatal administration of azathioprine and prednisone. *J. Pediatr.* **105**, 625–628.
121. Fein, A., Gross, A., Serr, D. M., *et al*. (1983). Effect of lmuran on placental and fetal development in rats. *Isr. J. Med. Sci.* **19**, 73–75.
122. Langevitz, P., Klein, L., Pras, M., *et al*. (1992). The effect of cyclophosphamide pulses on fertility in patients with lupus nephritis. *Am. J. Reprod. Immunol.* **28**, 157–158.
123. Streissguth, A. P., Treder, R. P., Barr, H. M., Shepard, T. H., Bleyer, W. A., Sampson, P. D., and Martin, D. C. (1987). Aspirin and acetaminophen use by pregnant women and subsequent child IQ and attention decrements. *Teratology* **35**, 211–222.
124. Roubenoff, R., Hoyt, J., Petri, M., Hochberg, M. C., and Hellman, D. B. (1988). Effects of anti-inflammatory and immunosuppressive drugs on pregnancy and fertility. *Semin. Arthritis Rheum.* **18**, 88–110.
125. Buchanan, N. M., Toubi, E., Khamashta, M. A., *et al*. (1996). Hydroxychloroquine and lupus pregnancy: Review of a series of 36 cases. *Ann. Rheum. Dis.* **55**, 486–488.
126. Arnout, J., Spitz, B., Wittevrongel, C., *et al*. (1994). High dose intravenous immunoglobulin treatment of a pregnant patient with an antiphospholipid syndrome: Immunological changes associated with a successful outcome. *Thromb. Haemostas.* **71**, 741–747.
127. Spinnatto, J. A., Clark, A. L., Pierangeli, S. S., *et al*. (1995). Intravenous immunoglobulin therapy for the antiphospholipid syndrome. *Am. J. Obstet. Gynecol.* **172**, 690–694.
128. Nakamura, Y., Yoshida, K., Itoh, S., Kanai, Y., Tsuda, H., Hashimoto, H., Mitsuhashi, N., and Kuwabara, Y. (1994). Immunoadsorption plasmapheresis as a treatment for pregnancy complicated by systemic lupus erythematosus with positive antiphospholid antibodies. *Am. J. Reprod. Immunol.* **41**(5), 307–311.
129. Ramon-Wilms, L., Lin-in Tseng, A., Wighardt, S., Einarson, T. R., and Koren, G. (1995). Fetal genital effects of first trimester hormone exposure: A meta-analysis. *Obstet. Gynecol.* **85**, 141–149.
130. Cunningham, F. G., Mac Donald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., pp. 817–820. Appleton and Lange, Stamford.
131. Norton, M. E., Merrill, J., Cooper, B. A. B., Kuller, J. A., and Clyman, R. I. (1993). Neonatal complications after the administration of indomethacin for preterm labor. *N. Engl. J. Med.* **329**, 1602–1607.
132. Petri, M., Ho, A. C., Patel, J., *et al*. (1995). Elevation of maternal alpha-fetoprotein in systemic lupus erythematosus: A controlled study. *J. Rheumatol.* **22**, 1365–1368.
133. Silver, R. M., Draper, M. L., Byrne, J. L. B., *et al*. (1994). Unexplained elevations of maternal serum alpha-fetoprotein in women with antiphospholipid antibodies: A harbinger of fetal death. *Obstet. Gynecol.* **83**, 150–155.
134. Yetman, D. L., Kutteh, W. H., Castorena, R., *et al*. (1997). Prevalence of elevated anticardiolipin antibodies in pregnant women with unexplained elevations of alfafetoprotein. *J. Reprod. Immunol.* **33**, 71–81.
135. Clark, F., Dickinson, J. E., Walters, B. N. J., *et al*. (1995). Elevated midtrimester hCG and maternal lupus anticoagulant. *Prenat. Diag.* **15**, 1035–1040.
136. Friedman, D. M. (1992). Fetal echocardiography in the assessment of lupus pregnancies. *Am. J. Reprod. Immunol.* **28**, 164–167.
137. Friedman, D. M., Zervoudakis, I., and Buyon, J. P. (1998). Perinatal monitoring of fetal well-being in the presence of congenital heart block. *Am. J. Perinatol.* **15**, 669–673.
138. Druzin, M. L., Lockshin, M., Edersheim, T. G., *et al*. (1987). Second-trimester fetal monitoring and preterm delivery in pregnancies with systemic lupus erythematosus and/or circulating anticoagulant. *Am. J. Obstet. Gynecol.* **157**, 1503–1510.
139. Adams, D., Druzin, M. L., Edersheim, T., *et al*. (1992). Antepartum testing-systemic lupus erythematosus and associated serologic abnormalities. *Am. J. Reprod. Immunol.* **28**, 159–164.
140. Weiner, Z., Lorber, M., and Blumenfeld, Z. (1992). Umbilical and uterine artery flow velocity waveforms in pregnant women with SLE treated with aspirin and glucocorticosteroids. *Am. J. Reprod. Immunol.* **28**, 168–171.
141. Kerslake, S., Morton, K. E., Versi, E., *et al*. (1992). Early Doppler studies in lupus pregnancy. *Am. J. Reprod. Immunol.* 172–175.
142. Bennett, F. C., and Scott, D. T. (1997). Long-term perspective on premature infant outcome and contemporary intervention issues. *Semin. Perinatol.* **21**, 190–201.
143. Vohr, B. R., and Msall, M. E. (1997). Neuropsychological and functional outcomes of very low birth weight infants. *Semin. Perinatol.* **21**, 202–220.

144. McAllister, D. L., Kaplan, B. J., Manzi, S., *et al*. (1997). The influence of systemic lupus erythematosus on fetal development: Cognitive, behavior and health trends. *J. Int. Neuropsych. Soc.* **3**, 370–376.
145. Ross, G., Sammaritano, L. R., Nass, R., and Lockshin, M. D. (1999). Learning disabilities in offspring of women with systemic lupus erythematosus. *Arthritis Rheum.* **36**, S87.
146. Salafia, C. M., and Cowchock, F. S. (1997). Placental pathology of antiphospholipid antibodies: A descriptive study. *Am. J. Perinatol.* **14**, 435–441.
147. Levy, R. A., Avvad, E., Oliveira, J. A., and Porto, L. C. (1998). Placental pathology in antiphospholipid syndrome. *Lupus* **87**, s81–s85.
148. Magid, M. S., Kaplan, C., Sammaritano, L. R., Peterson, M., Druzin, M. L., and Lockshin, M. D. (1998). Placental pathology in systemic lupus erythematosus: A prospective study. *Am. J. Obstet. Gynecol.* **179**, 226–234.
149. de Wolf, F., Carreras, L. O., Moerman, P., *et al*. (1982). Decidual vasculopathy and extensive placental infarction in a patient with repeated thromboembolic accidents, recurrent fetal loss and a lupus anticoagulant. *Am. J. Obstet. Gynecol.* **142**, 829–834.
150. Hanley, J. G., Gladman, D. D., Rose, T. H., *et al*. (1988). Lupus pregnancy: A prospective study of placental changes. *Arthritis Rheum.* **31**, 358–366.
151. Labarrere, C. A., Catoggio, L. J., Mullen, E. G., *et al*. (1986). Placental lesion in maternal autoimmune disease. *Am. J. Reprod. Immunol. Microbiol.* **12**, 78–87.
152. Out, H. J., Kooijman, C. D., Bruinse, H. W., *et al*. (1991). Histopathologic findings in placentae from patients with intrauterine fetal death and anti-phospholipid antibodies. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **41**, 179–186.
153. Faulk, W. P., and Labarrere, C. A. (1992). Vascular events in placentae and organ allografts. *Am. J. Reprod. Immunol*. **28**, 176–180.
154. McIntyre, J. A. (1992). Immune recognition at the matemal fetal interface: Overview. *Am. J. Reprod. lmmunol.* **28**, 127–131.
155. Abramowsky, C. R. (1981). Lupus erythematosus. the placenta, and pregnancy: A natural experiment in immunologically mediated reproductive failure. *Prog. Clin. Biol. Res.* **70**, 309–320.
156. Abramowsky, C. R., Vegas, M. E., Swinehart, G., *et al*. (1980). Decidual vasculopathy of the placenta in lupus erythematosus. *N. Engl. J. Med.* **303**, 668–672.
157. Sammaritano, L. R., Gharavi, A. E., Soberano, C., *et al*. (1992). Phospholipid binding of antiphospholipid antibodies and placental anticoagulant protein. *J. Clin. Immunol*. **12**, 27–35.
158. Rand, J. H., Wu, X. X., Andree, H. A. M., *et al*. (1997). Pregnancy loss in the antiphospholipid-antibody syndrome: A possible thrombogenic mechanism. *N. Engl. J. Med.* **337**, 154–160.
159. Cowchock, S. (1997). Autoantibodies and pregnancy loss. *N. Engl. J. Med.* **337**, 197–198.
160. La Rosa, L., Meroni, P. L., Tincani, A., *et al*. (1994). Beta-2-glycoprotein I and placental anticoagulant protein I in placentae from patients with antiphospholipid antibody syndrome. *J. Rheumatol.* **21**, 1684–1693.
161. Bresnihan, B., Oliver, M., Grigor, R. R., *et al*. (1977). Immunological mechanism for spontaneous abortion in systemic lupus erythematosus. *Lancet* **2**, 1205–1207.
162. Grennan, D. M., McCormick, J. N., Wojtacha, D., *et al*. (1978). Immunological studies of the placenta in systemic lupus erythematosus. *Ann. Rheum. Dis.* **37**, 129–134.
163. Cunningham, F. G., Mac Donald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., pp. 535–540. Appleton and Lange, Stamford.
164. Yuen, B. H. (1988). Prolactin in human milk: The influence of nursing and the duration of postpartum lactation. *Am. J. Obstet. Gynecol.* **158**, 583–588.
165. Gardner, D. K. (1992). Drugs in breast milk. *In* "Drug Therapy in Obstetrics and Gynecology." (W. F. Rayburn and F. P. Zuspan, eds.), pp. 312–325. Mosby Yearbook St. Louis.
166. Townsend, R. J., Benedetti, T. J., Erikson, S. H., *et al*. (1984). Excretion of ibuprofen into breast milk. *Am. J. Obstet. Gynecol.* **149**, 184–188.
167. Scott-Conner, C. E., and Schorr, S. J. (1995). The diagnosis and management of breast problems during pregnancy and lactation. *Am. J. Surg.* **170**, 401–405.
168. Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., p. 569. Appleton and Lange, Stamford.
169. Cunningham, F. G., MacDonald, P. C., Gant, N. F., Leveno, K. J., Gilstrap, L. C., Hankins, G. D. V., *et al*. (1997). "Williams Obstetrics," 20th Ed., p. 539. Appleton and Lange, Stamford.
170. Wilson, W. A., Gharavi, A. E., Koike, T., Lockshin, M. D., Branch, D. W., Piette, J.-C., Brey, R., Derksen, R., Harris, E. N., Hughes, G. R. V., Triplett, D. A., and Khamashta, M. A. (1999). International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. *Arthritis Rheum.* **42**, 1309–1311.
171. Lockshin, M. D., Sammaritano, L. S., and Schwartzman, S. S. (2000). Validation of the Sapporo criteria for antiphospholipid antibody syndrome. *Arthritis Rheum.* **43**, 440–443.
172. Kaleli, K., Kaleli, I., Aktan, E., Turan, C., and Aksit, F. (1998). Antiphospholipid antibodies in preeeclamptic women. *Gynecol. Obstetr. Invest.* **45**(2), 81–84.
173. Koskela, P., Vaarala, O., Makitalo, R., Palosuo, T., and Aho, K. (1988). Significance of false positive syphilis reactions and anticardiolipin antibodies in a nationwide series of pregnant women. *J. Rheumatol.* **15**, 70–73.
174. Harris, E. N., and Spinnato, J. A. (1991). Should anticardiolipin tests be performed in otherwise healthy pregnant women? *Am. J. Obstet. Gynecol.* **165**, 1272–1277.
175. Lockwood, C. J., Romero, R., and Feinberg, R. F. (1989). The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. *Am. J. Obstet. Gynecol.* **161**, 369–373.

176. Rix, P., Stentoft, J., Aunsholl, N. A., Dueholm, M., *et al*. (1992). Lupus anticoagulant and anticardiolipin antibodies in an obstetric population. *Acta Obstet. Gynecol. Scand.* **71**, 605–609.
177. Lynch, A., Marlar, R., Murphy, J., *et al*. (1994). Antiphospholipid antibodies in predicting adverse pregnancy outcome: A prospective study. *Ann. Intern. Med.* **120**, 470–475.
178. Infante-Rivard, C., David, M., Gauthier, R., *et al*. (1991). Lupus anticoagulants, anticardiolipin antibodies, and fetal loss: A case-control study. *N. Engl. J. Med.* **325**, 1063–1066.
179. Haddow, J. E., Rote, N. S., Dostal-Johnson, D., *et al*. (1991). Lack of an association between late fetal death and antiphospholipid antibody measurements in the second trimester. *Am. J. Reprod. lmmunol.* **165**, 1308–1312.
180. Parke, A. L., Wilson, D., and Maier, D. (1991). The prevalence of antiphospholipid antibodies in women with recurrent spontaneous abortion, women with successful pregnancies, and women who have never been pregnant. *Arthritis Rheum.* **34**, 1231–1235.
181. Out, H. J., Bruinse, H. W., Christiaens, G. C. M. L., *et al*. (1992). A prospective, controlled multicenter study on the obstetric risks of pregnant women with antiphospholipid antibodies. *Am. J. Obstet. Gynecol.* **167**, 26–32.
182. Petri, M., Golbus, M., Anderson, R., *et al*. (1987). Antinuclear antibody, lupus anticoagulant, and anticardiolipin antibody in women with idiopathic habitual abortion. *Arthritis Rheum.* **30**, 601–606.
183. Out, H. J., Bruinse, H. W., Christiaens, G. C., *et al*. (1991). Prevalence of antiphospholipid antibodies in patients with fetal loss. *Ann. Rheum. Dis.* **50**, 533–537.
184. Ramsey-Goldman, R., Kutzer, J. E., Kuller, L. H., *et al*. (1992). Previous pregnancy outcome is an important determinant of subsequent pregnancy outcome in women with systemic lupus erythematosus. *Am. J. Reprod. lmmunol.* **28**, 195–198.
185. Julkunen, H., Jouhikainen, T., Kaaja, R., *et al*. (1993). Fetal outcome in lupus pregnancy: A retrospective case-control study of 242 pregnancies in 112 patients. *Lupus* **2**, 125–131.
186. Lee, R. M., Emlen, W., Scott, J. R., Branch, D. W., and Silver, R. M. (1999). Anti-β2-glycoprotein I antibodies in women with recurrent spontaneous abortion, unexplained fetal death, and antiphospholipid syndrome. *Am. J. Obstet. Gynecol.* **181**, 642–648.
187. Franklin, R. D., Hollier, N., and Kutteh, W. H. (2000). Beta-2 glycoprotein 1 as a marker of antiphospholipid syndrome in women with recurrent pregnancy loss. *Fertil. Steril.* **73**(3), 531–535.
188. Branch, D. W., Silver, R., Pierangeli, S., van Leeuwen, I., and Harris, E. N. (1997). Antiphospholipid antibodies other than lupus anticoagulant and anticardiolipin antibodies in women with recurrent pregnancy loss, fertile controls, and antiphospholipid syndrome. *Obstetr. Gynecol.* **89**(4), 549–555.
189. Pierro, E., Cirino, G., Bucci, M. R., Lazzarin, N., Andreani, C. L., Mancuso, S., Lanzone, A., and Navarra, A. (1999). Antiphospholipid antibodies inhibit prostaglandin release by decidual cells of early pregnancy: Possible involvement of extracellular secretory phospholipase A2. *Fertil. Steril.* **71**(2), 342–346.
190. Di Simone, N., Meroni, P. L., del Papa, N., Raschi, E., Caliandro, D., de Carolis, S., Khamashta, M. A., Atsumi, T., Hughes, G. R. V., Balestrieri, G., Tincani, A., Casali, P., and Caruso (2000). Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered β_2-glycoprotein I. *Arthritis Rheum.* **43**, 140–150.
191. Lynch, A., Silver, R., and Emlen, W. (1997). Antiphospholipid antibodies in healthy pregnant women. *Rheum. Dis. Clin. North Am.* **23**, 55–70.
192. Houwert-de Jong, M. H., Tennijtelen, A., Eskes, T. K., *et al*. (1989). The natural course of habitual abortion. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **33**, 221–228.
193. Vlaanderen, W., and Treffers, P. E. (1987). Prognosis of subsequent pregnancies after recurrent spontaneous abortion in first trimester. *Br. Med. J.* **295**, 92–93.
194. Parazzini, F., Acaia, B., Ricciardiello, O., *et al*. (1988). Short-term reproductive prognosis when no cause can be found for recurrent miscarriage. *Br. J. Obstet. Gynecol.* **95**, 654–658.
195. Cauchi, M. N., Pepperell, R., Kloss, M., *et al*. (1991). Predictors of pregnancy success in repeated miscarriage. *Am. J. Reprod. Immunol.* **26**, 72–75.
196. Knudsen, U. B., Hansen, V., Juul, S., *et al*. (1991). Prognosis of a new pregnancy following previous spontaneous abortions. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **39**, 31–36.
197. Lima, F., Khamashta, M. A., Buchanan, N. M., *et al*. (1996). A study of sixty pregnancies in patients with antiphospholipid syndrome. *Clin. Exp. Rheumatol.* **14**, 131–136.
198. Le Thi Huong, D., Wechsler, B., Edelman, P., *et al*. (1993). Postpartum cerebral infarction associated with aspirin withdrawal in the antiphospholipid syndrome. *J. Rheumatol.* **20**, 1229–1232.
199. Airoldi, M. L., Eid, O., Tosetto, C., *et al*. (1996). Case report: Postpartum dilated cardiomyopathy in antiphospholipid positive woman. *Lupus* **5**, 247–250.
200. Thorp, J. M., Jr., Chescheir, N. C., and Fann, B. (1994). Postpartum myocardial infarction in a patient with antiphospholipid syndrome. *Am. J. Perinatol.* **11**, 1–3.
201. Le Thi Huong, D., Wechsler, B., Edelman, P., Fournie, A., Le Tallec, Y., Piette, J. C., and Godeau, P. (1993). Postpartum cerebral infarction associated with aspirin withdrawal in the antiphospholipid syndrome. *J. Rheumatol.* **20**, 1229–1232.
202. Kang, A. H., and Graves, C. R. (1999). Libman-Sacks endocarditis in a pregnant woman with acute respiratory distress syndrome. *Obstet. Gynecol.* **93**, 819–821.
203. Wislowska, M. (1999). Successful treatment of catastrophic antiphospholipid syndrome in a pregnant woman. *Clin. Exp. Rheum.* **17**, 261.

204. Branch, D. W., Andres, R., Digre, K. B., *et al*. (1989). The association of antiphospholipid antibodies with severe preeclampsia. *Obstet. Gynecol.* **73**, 541–545.
205. Ilbery, M., Jones, A. R., and Sampson, J. (1995). Lupus anticoagulant and HELLP syndrome complicated by placental abruption, hepatic, dermal and adrenal infarction. *Austr. N.Z. J. Obstet. Gynecol.* **35**, 215–217.
206. Alsulyman, O. M., Castro, M. A., Zuckerman, E., *et al*. (1996). Preeclampsia and liver infarction in early pregnancy associated with the antiphospholipid syndrome. *Obstet. Gynecol.* **88**, 644–646.
207. Kinoshita, K. (1993). Hepatic infarction during pregnancy complicated by antiphospholipid syndrome. *Am. J. Obstet. Gynecol.* **169**, 199–202.
208. Millan-mon, A., Porto, J. L., Novo, C., *et al*. (1993). Case report: Hepatic infarction in a pregnant patient with the "primary" antiphospholipid syndrome. *Lupus* **2**, 275–279.
209. Kon, S. P., Kwan, J. T., and Raftery, M. J. (1995). Reversible renal failure due to antiphospholipid antibody syndrome, preeclampsia and renal thrombotic microangiopathy. *Clin. Nephrol.* **44**, 271–273.
210. Mor, F., Beigel, Y., Inbal, A., Goren, M., and Wysenbeek, A. J. (1989). Hepatic infarction in a patient with the lupus anticoagulant. *Arthritis Rheum.* **32**, 491–495.
211. Navarro, F., Dona-Naranjo, M. A., and Villanueva, I. (1997). Neonatal antiphospholipid syndrome. *J. Rheum.* **24**, 1240–1241.
212. Pollard, J. K., Scott, J. R., and Branch, D. W. (1992). Outcome of children born to women treated during pregnancy for the antiphospholipid syndrome. *Obstet. Gynecol.* **80**, 365–368.
213. Birkenfeld, A., Mukaida, T., Minichiello, L., *et al*. (1994). Incidence of autoimmune antibodies in failed embryo transfer cycles. *Am. J. Reprod. Immunol.* **31**, 65–68.
214. Gleicher, N., Pratt, D., and Dudkiewicz, A. B. (1992). Autoantibodies in *in vitro* fertilization patients. *Fertil. Steril.* **58**, 863.
215. Sthoeger, Z. M., Mozes, E., and Tartakovsky, B. (1993). Anti-cardiolipin antibodies induce pregnancy failure by impairing embryonic implantation. *Proc. Natl. Acad. Sci. USA* **90**, 6464–6467.
216. Kutteh, W. H. (1996). Antiphospholipid antibody-associated recurrent pregnancy loss: Treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. *Am. J. Obstet. Gynecol.* **174**, 1584–1589.
217. Kutteh, W. H., and Ermel, L. D. (1996). A clinical trial for the treatment of antiphospholipid antibody-associated recurrent pregnancy loss with lower dose heparin and aspirin. *Am. J. Reprod. Immunol.* **35**, 402–407.
218. Rai, R., Cohen, H., Dave, M., and Regan, L. (1997). Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *Br. Med. J.* **314**, 253–257.
219. Nelson-Piercy, C. (1997). Heparin-induced osteoporosis in pregnancy. *Lupus* 500–504.
220. Barbour, L. A., Kick, S. D., Steiner, J. F., *et al*. (1994). A prospective study of heparin induced osteoporosis in pregnancy using bone densitometry. *Am. J. Obstet. Gynecol.* **170**, 862–869.
221. Dahlman, T. C., Sjoberg, H. E., and Ringertz, H. (1994). Bone mineral density during long-term prophylaxis with heparin in pregnancy. *Am. J. Obstet. Gynecol.* **170**, 1315–1320.
222. Dulitzki, M., Pauzner, R., Langevitz, P., *et al*. (1996). Low-molecular weight heparin during pregnancy and delivery: Preliminary experience with 41 pregnancies. *Obstet. Gynecol.* **87**, 380–383.
223. Inbar, O., Blank, M., Faden, D., *et al*. (1993). Prevention of fetal loss in experimental antiphospholipid syndrome by low-molecular weight heparin. *Am. J. Obstet. Gynecol.* **169**, 423–426.
224. Many, A., Pauzner, R., Carp, H., *et al*. (1992). Treatment of patients with antiphospholipid antibodies during pregnancy. *Am. J. Reprod. Immunol.* 216–218.
225. Sherer, Y., Levy, Y., and Shoenfeld, Y. (2000). Intravenous immunoglobulin therapy of antiphospholipid syndrome. *Rheumatology (Oxford)* **39**(4), 421–426.
226. Branch, D. W., Peaceman, A. M., Druzin, M., Silver, R. K., El-Sayed, Y., Silver, R. M., Esplin, M. S., Spinnato, and Harger, J. (2000). A multicenter placebo-controlled study of intravenous immunoglobulin treatment of antiphospholipid syndrome during pregnancy: The pregnancy loss study group. *Am. J. Obstet. Gynecol.* **182**, 122–127.
227. Blank, M., Shoenfeld, Y., Cabilly, S., Heldman, Y., Fridkin, M., and Katchalski-Katzir, E. (1999). Prevention of experimental antiphospholipid syndrome and endothelial activation by synthetic peptides. *Proc. Natl. Acad. Sci. USA* **96**(9), 5164–5168.
228. Lubbe, W. F., Palmer, S. J., Butler, W. S., *et al*. (1983). Fetal survival after prednisone suppression of' maternal lupus anticoagulant. *Lancet* **1**, 1361–1363.
229. Silveira, L. H., Hubble, C. L., Jara, L. J., *et al*. (1992). Prevention of anticardiolipin antibody-related pregnancy losses with prednisone and aspirin. *Am. J. Med.* 403–411.
230. Cowchock, F. S., Reece, E. A., Balaban, D., *et al*. (1992). Repeated fetal losses associated with antiphospholipid antibodies: A collaborative randomized trial comparing prednisone to low dose heparin treatment. *Am. J. Obstet. Gynecol.* **166**, 1318–1323.
231. Branch, D. W., Silver, R. M., Blackwell, J. L., *et al*. (1992). Outcome of treated pregnancies with antiphospholipid syndrome: An update of the Utah experience. *Obstet. Gynecol.* **80**, 614–620.
232. Spinnato, J. A., Clark, A. L., Pierangeli, S. S., *et al*. (1995). Intravenous immunoglobulin therapy for the antiphospholipid syndrome. *Am. J. Obstet. Gynecol.* **172**, 690–694.
233. Kaaja, R., Julkunen, H., Ammala, P., *et al*. (1993). Intravenous immunoglobulin treatment of pregnant patients with recurrent pregnancy losses associated with antiphospholipid antibodies. *Acta Obstet. Gynecol. Scand.* **72**, 63–66.
234. Scott, J. R., Branch, D. W., Kochenour, N. K., *et al*. (1988). Intravenous immunoglobulin treatment of pregnant

patients with recurrent pregnancy loss caused by antiphospholipid antibodies and Rh immunization. *Am. J. Obstet. Gynecol.* **159**, 1055–1056.
235. Parke, A. (1992). The role of IVIG in the management of patient with antiphospholipid antibodies and recurrent pregnancy losses. *Clin. Rev. Allergy* **10**, 105–118.
236. Shoenfeld, Y. (1992). Induction of experimental primary and secondary antiphospholipid syndromes in naive mice. *Am. J. Reprod. Immunol.* **28**, 219–221.
237. Fishman, P., Falach-Vaknine, E., Zigelman, R., *et al*. (1993). Prevention of fetal loss in experimental antiphospholipid syndrome by in vivo administration of recombinant interleukin-3. *J. Clin. Invest.* **91**, 1834–1837.
238. Silver, R. M., Draper, M. L., Scott, J. R., Lyon, J. L., Reading, J., and Branch, D. W. (1994). Clinical consequences of antiphospholipid antibodies: An historic cohort study. *Obstet. Gynecol.* **83**, 372–377.
239. Erkan, D., Merrill, J. T., Yazici, Y., Sammaritano, L., Buyon, J. P., and Lockshin, M. D. (2001). High thrombosis rate after fetal loss in antiphospholipid syndrome: Effective prophylaxis with aspirin. *Arthritis Rheum.* **44**, 1466–1467.
240. Friedman, E., and Rutherford, J. (1956). Pregnancy in lupus erythematosus. *Obstet. Gynecol.* **8**, 601–610.
241. Fraga, A., Mintz, G., Orozco, J., and Orozco, J. H. (1974). Sterility and fertility rates, fetal wastage and maternal morbidity in systemic lupus erythematosus. *J. Rheumatol.* **1**, 293–298.
242. El-Roeiy, A., Gleicher, N., Friberg, J., *et al*. (1987). Correlation between peripheral blood and follicular fluid autoantibodies and impact on *in vitro* fertilization. *Obstet. Gynecol.* **70**, 163–170.
243. Gleicher, N., El-Roeiy, A., Confino, E., and Friberg, J. (1989). Reproductive failure because of autoantibodies: Unexplained infertility and pregnancy wastage. *Am. J. Obstet. Gynecol.* **160**, 1376–1384.
244. Taylor, P. V., Campbell, J. M., and Scott, J. S. (1989). Presence of autoantibodies in women with unexplained infertility. *Am. J. Obstet. Gynecol.* **161**, 377–389.
245. Fisch, B., Rikover, Y., Shohat, L., *et al*. (1991). The relationship between *in vitro* fertilization and naturally occurring antibodies; evidence for increased production of antiphospholipid antibodies. *Fertil. Steril.* **56**, 718–274.
246. Fisch, B., Fried, S., Manor, Y., *et al*. (1995). Increased antiphospholipid antibody activity in in-vitro fertilization patients is not treatment dependent but rather an inherent characteristic of the infertile state. *Am. J. Reprod. Immunol.* **34**, 370–374.
247. Sher, G., Feinman, M., Zouves, C., *et al*. (1994). High fecundity rates following *in vitro* fertilization and embryo transfer in antiphospholipid antibody seropositive women treated with heparin and aspirin. *Hum. Reprod.* **9**, 2278–2283.
248. Guballa, N., Sammaritano, L., Schwartzman, S., Buyon, J., and Lockshin, M. (2000). Ovulation induction and *in vitro* fertilization in systemic lupus erythematosus and antiphospholipid syndrome. *Arthritis Rheum.* **43**, 550–556.
249. Petri, M., and Robinson, C. (1997). Review: Oral contraceptives and systemic lupus erythematosus. *Arthritis Rheum.* **40**, 797–803.
250. Dagenais, P., Urowitz, M. B., Gladman, D. D., *et al*. (1992). A family study of the antiphospholipid syndrome associated with other autoimmune diseases. *J. Rheumatol.* **19**, 1393–1396.
251. Savi, M., Ferraccioli, G. F., Neri, T. M., *et al*. (1988). HLA-DR antigens and anticardiolipin antibodies in northern Italian systemic lupus erythematosus patients. *Arthritis Rheum.* **31**, 1568–1570.
252. Trabace, S., Nicotra, M., Cappellacci, S., *et al*. (1991). HLA-DR and DQ antigens and anticardiolipin antibodies in women with recurrent spontaneous abortions. *Am. J. Reprod. Immunol.* **26**, 147–149.
253. Hartung, K., Coldewey, R., Corvetia, A., *et al*. (1992). MHC gene products and anticardiolipin antibodies in systemic lupus erythematosus: Results of a multicenter study. *Autoimmunity* **13**, 95–99.
254. Colucci, A. T., Di Lorenzo, G., lngrassia, A., *et al*. (1992). Blood antiphospholipid antibody levels are influenced by age, sex and HLA-B8,DR3 phenotype. *Exp. Clin. Immunogenet.* **9**, 72–79.
255. Wilson, W. A., Perez, M. C., Michalski, J. P., *et al*. (1988). Cardiolipin antibodies and null alleles of C4 in black Americans with systemic lupus erythematosus. *J. Rheumatol.* **15**, 1768–1772.
256. Stephanson, M. D. (1996). Frequency of factors associated with habitual abortion in 197 couples. *Fertil. Steril.* **66**, 24–29.
257. Coulam, C. B., Clark, D. A., Beer, A. E., *et al*. (1997). Current clinical options for diagnosis and treatment of recurrent spontaneous abortion. *Am. J. Reprod. Immunol.* **38**, 57–74.
258. Mantingh, A., and Houwert-de Jong, M. H. (1990). Clinical management of recurrent abortion. *In* "Early Pregnancy Failure" (H. J. Huisjes and T. Lind, eds.), pp. 224–230. Churchill Livingstone, New York.
259. Derksen, R. H. W. M., de Groot, P. H. G., Kater, L., *et al*. (1993). Patients with antiphospholipid antibodies and venous thrombosis should receive lifelong anticoagulant treatment. *Ann. Rheum. Dis.* 689–969.
260. Rosove, M. H., and Brewer, P. M. (1992). Antiphospholipid thrombosis: Clinical course after the first thrombotic event in 70 patients. *Ann. Intern. Med.* **117**, 303–308.
261. Julkunen, H., Kaaja, R., Kurki, P., *et al*. (1995). Fetal outcome in women with primary Sjögren's syndrome: A retrospective case-control study. *Clin. Exp. Rheum.* **13**, 65–671.
262. Herrerman, G., Bétous, F., Batisse, P. H., *et al*. (1982). Blocs auriculo-ventriculaires détectés in utero chez 2 enfants dont la mère a un syndrome de Sjögren. *Nouv Presse Med.* **11**, 657–660.
263. Glass, R. H. (1995). Female infertility. *In* "Infertility: Evaluation and Treatment" (W. R. Keye, Jr., R. J. Chang, R. W. Rebar, and M. R. Soules, eds.), pp. 57–61. Saunders, Philadelphia.

264. McClure, R. D. (1995). Male infertility. *In* "Infertility: Evaluation and Treatment" (W. R. Keye, Jr., R. J. Chang, R. W. Rebar, and M. R. Soules, eds.), pp. 57–61. Saunders, Philadelphia.
265. Soules, M. R. (1995). Glossary of terminology for assisted reproductive technologies and early embryonic development. *In* "Infertility: Evaluation and Treatment" (W. R. Keye, Jr., R. J. Chang, R. W. Rebar, and M. R. Soules, eds.), pp. 731–735. Saunders, Philadelphia.
266. Von Steirteghem, A., Liebaers, I., and Devroey, P. (1996). Assisted reproduction. *In* "Scientific Essentials of Reproductive Medicine" (S. G. Hillier, H. C. Kitchener, and J. P. Neilson, eds.), pp. 230–241. Saunders, London.
267. Davis, O. K., and Rosenwaks, Z. (1995). *In vitro* fertilization. *In* "Infertility: Evaluation and Treatment" (W. R. Keye, Jr., R. J. Chang, R. W. Rebar, and M. R. Soules, eds.), pp. 759–771. Saunders, Philadelphia.

23

EPIDEMIOLOGY OF SYSTEMIC LUPUS ERYTHEMATOSUS

Dafna D. Gladman

Epidemiology is the study of the frequency, distribution, and determinants of disease in populations. Epidemiological studies of systemic lupus erythematosus (SLE) have focused on the following areas: (1) development and validation of criteria for disease classification; (2) estimation of morbidity and mortality rates in different populations; (3) estimation of prognosis and survivorship of patients with SLE; and (4) determination of etiologic factors, both genetic and environmental. Major findings regarding disease classification, morbidity and mortality rates, prognosis and survivorship, and etiologic factors are reviewed in this chapter.

CLASSIFICATION CRITERIA

A proper case definition for the disease is required for both the conduct and the interpretation of epidemiologic studies [1–4]. In 1971, the American Rheumatism Association (now American College of Rheumatology, ACR) published preliminary criteria for the classification of SLE [5]. Until recently, the 1982 revised criteria were used for case definition [6]; the authors of that revision noted that criteria "should be used mainly for the purpose of classifying patients in reports relating to clinical, serologic, cellular, or pathogenetic studies of SLE." The 1982 dataset has been reanalyzed by Edworthy and colleagues, [7] using the method of recursive partitioning to generate a classification tree in an effort to "identify simpler and more explicit rules to classify patients with SLE." The resultant simple classification tree requires knowledge of only two variables: immunologic disorder and malar rash. Based on the criteria dataset (Table 1), the sensitivity, specificity, and accuracy of the 1982 revised criteria and the classification tree were 96 and 92%, respectively. Criteria were updated by the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology in 1997 [8] (Table 2). The updated version of criteria has yet to be tested.

It should be noted that the methods used for classifying patients with SLE were not designed for diagnostic purposes and lack the sensitivity for recognizing milder cases. Although this is a virtue for purposes of analytic epidemiological studies of etiologic features to ensure homogeneity of the case population, it may alternatively be a limitation in descriptive studies of morbidity and observational studies of prognosis, as subjects with a multisystem disease consistent with SLE may not fulfill the criteria [9]. Use of the 1982 revised criteria enabled the recognition of cases with latent lupus [10] and incomplete lupus [11], terms used to describe groups of patients with one or more symptoms or signs of SLE who fail to fulfill the 1982 revised criteria. Although these patients fulfill some criteria for SLE, it is important that they are excluded from epidemiologic studies of SLE because only a fifth to a third of such patients may go on to develop full-blown disease [10, 12]. The inclusion of these patients in studies of prognosis may lead to an erroneous description of better prognosis for patients with SLE.

TABLE 1 1982 Revised Criteria for SLE[a]

	Sensitivity	Specificity	Accuracy
Malar rash	57	98	76
Discoid rash	18	99	57
Photosensitivity	43	96	68
Oral ulcers	27	96	60
Nonerosive arthritis	86	37	63
Pleuritis or pericarditis	56	86	70
Renal disorder	51	94	71
Seizure or psychotic	20	08	57
Hematologic disorder	59	89	73
Immunologic disorder	85	93	88
Positive antinuclear antibody	99	49	77
Four of criteria items	96	96	96
Classification tree	92	92	92

[a] Data are from Tan *et al.* [6] and Edworthy *et al.* [7]. For definition of items, see Table 2.

MORBIDITY DATA

Prevalence

The overall prevalence of SLE has varied from 12 to 50.8 cases per 100,000 persons (Table 3) [13–33]. The individual studies vary over time and place and used different methods of case ascertainment. Thus, while studies conducted in Rochester, Minnesota [16], and San Francisco, California [14], utilized both inpatient and outpatient records for case identification and published criteria for case validation, there were differences in the sampling frame: residents of Rochester and members of Kaiser Foundation Health Plan, respectively, and in the racial composition of the populations, 81 and 99% Caucasian, respectively. The sex- and race-specific prevalence estimates for Caucasian males and Caucasian females, however, are comparable as 95% confidence intervals for these ratios overlap; estimates for the overall prevalence in Caucasians were 44 and 40 per 100,000, respectively. Variability in prevalence rates

TABLE 2 1997 Update of the 1982 American College of Rheumatology Classification Criteria for SLE

Item	Definition
Malar rash	Fixed erythema, flat or raised, over the malar eminences, sparing the nasolabial folds
Discoid rash	Erythematosus-raised patches with adherent keratotic scaling and follicular plugging: atrophic scarring may occur in older lesions
Photosensitivity	Skin rash as a result of unusual reaction to sunlight by patient history of physician observation
Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by a physician
Nonerosive arthritis	Involving two or more peripheral joints, characterized by tenderness, swelling, or effusion
Pleuritis or pericarditis	a. Pleuritis—convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion b. Pericarditis—documented by ECG or rub or evidence of pericardial effusion
Renal disorder	a. Persistent proteinuria greater than 0.5 g per day or greater than 3+ if quantitative not performed b. Cellular casts—may be red cell, hemoglobin, granular, tubular, or mixed
Seizures or psychosis	a. Seizures—in the absence of offending drugs or known metabolic derangement, e.g. uremia, ketoacidosis, or electrolyte imbalance b. Psychosis—in the absence of offending drugs or known metabolic derangement, e.g., uremia, ketoacidosis, or electrolyte imbalance
Hematologic disorder	a. Hemolytic anemia with reticulocytosis b. Leukopenia—less than 4000/mm^3 on two occasions c. Lymphopenia—less than 1500/mm^3 on two occasions d. Thrombocytopenia—less than 100,000/mm^3 in the absence of offending drugs
Immunologic disorder	a. Anti-DNA: antibody to native DNA in abnormal titer b. Anti-Sm: presence of antibody to Sm nuclear antigen c. Positive finding of antiphospholipid antibodies based on (1) an abnormal serum level of IgG or IgM anticardiolipin antibodies, (2) a positive test for lupus anticoagulant using a standard method, or (3) a false-positive test for at least 6 months and confirmed by *Treponema pallidum* immobilization or fluorescent treponaml antibody absorption test.
Positive antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time in the absence of drug

[a] From Hochberg [8].

TABLE 3 Prevalence Rates in Published Series

Author	Year	Location	Source of patients	Prevalence
Siegel and Lee [13]	1965	New York		14.6
Fessel [14]	1973	San Francisco	Community based	50.8
Helve [15]	1978	Finland	National Board of Health Registry	28.0
Michet *et al*. [16]	1980	Rochester, Minnesota	Rochester epidemiology program project	40.0
Meddings and Grennan [17]	1980	New Zealand	Hospital records	15.0
Nived *et al*. [17]	1982	Sweden	Hospital/community	39.0
Hochberg [19]	1982	England/Wales	Morbidity statistics from general practice	12.0
Nakae *et al*. [20]	1984	Japan		21.0
Maskarinec and Katz [25]	1989	Hawaii	Medical facility and support groups	41.8
Samanta *et al*. [21]	1989	Leichester, UK	Multiple	26.1
Nossent [22]	1989	Curaçao	Clinic, hospital, death certificate	47.0
Hopkinson *et al*. [23]	1990	Nottingham, UK	Multiple	24.0
Johnson *et al*. [26]	1991	Birmingham, UK	Multiple	27.7
Gourley *et al*. [24]	1993	Northern Ireland	Multiple	25.4
Peschken and Esdaile [31]	1996	Manitoba, Canada	Multiple	22.1

among studies may be due to different methods of case ascertainment, which have utilized general practice diagnostic registries [19], hospital discharge records [15, 17, 20], and outpatient clinic records or combinations thereof [18]. Several studies from the United Kingdom utilized multiple sources to trace patients, including contacting primary care and specialist physicians, a rheumatology database, a renal unit database, immunology and pathology laboratory reports and databases, and hospital activity records (including both inpatients and outpatients and local branches of the Lupus Society [21, 23, 24, 26]).

A study from Denmark used central population registry, outpatient registry, physicians' documentation, and laboratory registry information [32]. In comparing studies using similar methodologies for case identification and validation, the prevalence of SLE is almost identical. Thus Michet *et al*. [16], Nived *et al*. [18], and Nossent [33] used hospital records to ascertain their cases and arrived at a prevalence rate of 40, 39, and 49.5, respectively, whereas three British groups and a Danish group who used several sources to ascertain cases also arrived at similar prevalence rates ranging from 21.7 to 26.1 [21, 23, 24, 26, 32]. The lowest rate at 12/100,000 was determined through use of general practice diagnostic registries [19]. A study based on self-reported, physician-diagnosed SLE suggests that the prevalence of SLE in the United States may have been underestimated and that a more realistic rate is that of 124/100,000 [27]. Indeed the most recent study from Rochester, Minnesota, estimated an age- and sex-adjusted prevalence rate on January 1, 1992, to be 1:1000 [28]. The prevalence in Norwegian women aged more than 30 is 1:1000 [33]. A study from London suggests a similar prevalence rate among Afro-Caribbean and West-African blacks [34].

Incidence

The average annual incidence of SLE in the continental United States has been estimated in several studies; incidence rates vary from 2.0 to 7.6 cases per 100,000 persons per year [13, 14, 16, 18, 22, 28, 29, 32; Table 4]. As noted previously, several reasons may help explain differences between studies. The differences may be attributed to changes in diagnostic classification, as the ACR criteria have been modified from 1971 to 1982, and to the use of different methods for case ascertainment. Of particular interest are differences in incidence reported for the same population utilizing the identical medical record retrieval system in the studies from Rochester, Minnesota; Michet *et al*. [16] attributed the differences to changes in diagnostic classification. The most recent study from the same center, using the 1982 ACR classification criteria, supports that notion [28]. Studies that utilized community-based outpatient medical records [14] documented a high incidence of SLE. In the Swedish study, Nived *et al*. [18] used a system for case identification that employed review of not only inpatient and outpatient computerized

TABLE 4 Incidence Rates in Published Series

Author	Year	Location	Source of patients	Incidence[a]
Siegel and Lee [13]	1965	New York		2.0
Fessel [14]	1973	San Francisco	Community based	7.6
Hochberg [27]	1977	Baltimore, Maryland	Hospital discharge	4.6
Michet *et al*. [16]	1979	Rochester, Minnesota	Rochester epidemiology program project	2.2
Nived *et al*. [18]	1982	Sweden	Hospital/community	4.6
Nossent [22]	1989	Curaçao	Clinic, hospital, death certificate	4.6
Hopkinson *et al*. [23]	1990	Nottingham, UK	Multiple	4.4
Gudmundsson and Steinsson [36]	1990	Iceland	Hospital/community	5.8
McCarty *et al*. [29]	1990	Pennsylvania	University/community	2.8
Johnson *et al*. [26]	1991	Birmingham, UK	Multiple	3.8
Uramato *et al*. [28]	1992	Rochester, Minnesota	Rochester epidemiology program project	5.8
Voss *et al*. [32]	1994	County of Funen, Denmark	Multiple	3.6
Nossent [33]	1996	Arctic region of Norway	Multiple	2.7

[a] Per 100,000.

registers, but also outpatient clinic files. These authors identified 15 cases of SLE for an incidence of 4.8 cases per 100,000 per year, which has remained constant over the 1981–1991 decade [35]. A similar annual incidence rate (2.4 and 2.7) was observed over two consecutive 9-year periods between 1978 and 1996 in the arctic region of Norway [33]. Moreover, some studies may include both definite and probable cases of SLE. McCarty *et al*. [29] demonstrated that while utilizing only one source of data collection may underestimate the true incidence rate (2.4 vs 2.8), the inclusion of probable cases increases the incidence rate from 2.4 to 3.4 per 100,000. The Danish county-based cohort study composed primarily of Caucasians documented an increased incidence from 1/100,000 to 3.6/100,000 during the 1980–1994 period [32].

Thus while it has been pointed out that a single source of case ascertainment may be insufficient to document the true incidence of SLE, care must be taken to achieve true ascertainment of cases so as not to overestimate the rate by including probable cases. As pointed out earlier, probable cases, which may be "latent" or "incomplete" lupus, may not evolve into full-blown SLE.

Effects of Age, Gender, and Race on Morbidity Rates

Another reason to explain the variability in prevalence and incidence rates in the published studies may be the effect of age, gender, and race on these rates. Overall prevalence and incidence rates are higher in females compared to males and are higher in American Blacks, Afro-Caribbeans, Asians, and Native North American Indians than in Caucasian populations. Among Caucasian females, age-specific incidence rates have been estimated in four studies and showed maximum rates per 100,000 per year of 3.8 in the 15- to 44-year age group [13], 6.3 in the 25- to 44-year age group [16], 7.0 in the 35- to 54-year age group [29], and 15.9 in the 45- to 64-year age group. Similar observations for 1-year prevalence rates were made in Leichester, United Kingdom [21], whereas in Nottingham, United Kingdom [22], the peak incidence for Caucasians was in the 50- to 59-year age group. The highest incidence among the Norwegian group was in the 30- to 49-year age group [33]. Median ages at diagnosis for Caucasian females in the first three studies were 39, 39, and 42 years, respectively. Age-specific incidence rates in Caucasian males are difficult to interpret because of small numbers of studied cases, but peak rates of 4.5 and 0.9 per 100,000 per year in the age group 65 years and older have been reported in the New York City [13] and Rochester [16] studies, respectively. In Iceland, the overall age standardized rate was 5.8 for women and 0.8 for males [36]. Almost identical numbers were derived from the study in the arctic region of Norway. Both the Baltimore population study [29] and the Leichester, United Kingdom study [21] noted that SLE develops later in Caucasian males than in Caucasian females.

Age-specific incidence rates in Black females were greatest in the 15- to 44-year age group in New York City [13] and in the 25- to 34-year age group in Baltimore [30], with Birmingham, United Kingdom [26] exceeding 20 per 100,000 per year; age-specific rates in Black males can only be estimated reliably from the

Baltimore study and reached a peak in the 45- to 64-year age group of 5 per 100,000 per year.

Clinical studies have consistently demonstrated a female predominance approaching 90% of SLE cases. This excess is especially noteworthy within the 15- to 64-year age group wherein ratios of age- and sex-specific incidence rates show a 6- to 10-fold female excess in Caucasians and Blacks. No such excess was noted in the age groups 14 years and below and 65 years and above in New York City [13], Rochester [16], or Sweden [18]. A 4-fold excess incidence rate in females aged 65 years and above was found among Baltimore Caucasians but not Blacks [30]. Similar observations were recorded in the Swedish cohort, where the incidence rate was notably higher for women age 65–74 (14.1/100,000) compared to men of the same age (3.2/100,000). These age-related differences in the ratio of sex-specific incidence rates have been thought to be related to hormonal changes occurring during puberty and the child-bearing years. Among the North American Indians of Manitoba the age-adjusted prevalence rate was 42.3/100,000, and the highest rate was noted among the 35- to 54-year age group [31].

Reasons for the excess morbidity from SLE in American Blacks are unknown but may be related to differences in exposure to environmental factors rather than differences in genetic predisposition [30].

Conflicting data exist regarding the excess prevalence of SLE among Asians compared with Caucasians [37]. In Hawaii, the prevalence of SLE was estimated at 41.8/100,000. It was 1.3 times higher in Japanese, 1.5 higher in Filipinos, 2.4 higher in Chinese, but lower in Hawaiians [25]. In Britain, the rate for Chinese was much higher than that of Caucasian or Asians, the latter including individuals from Indian and Pakistan [23, 26]. In the San Francisco study, the prevalence of SLE was not apparently increased among Chinese compared with Caucasians [15]. Data from China, based on population surveys, suggest a prevalence of SLE of 40–70 per 100,000 [38, 39]. A survey in Taiwan identified only one case of SLE among 1836 residents and no cases among 2000 female students [40]. Thus population-based data in three countries fail to support an excess prevalence of SLE among Chinese. Prevalence data from Japan also fail to support the observations in Hawaii of an excess prevalence in Japanese [21]. These discrepancies may also be related to case definitions and to the use of different sets of criteria for the classification of SLE.

An excess incidence and prevalence of SLE among North American Indians compared with Caucasians was suggested in two U.S. studies and one Canadian study [31, 41, 42]. This excess was isolated to only 3 of 75 American Indian tribes and a single Pacific Northwest Indian population, the Nootka, and was also noted among the Manitoba Indians. In the province of Manitoba, Canada, both the crude annual incidence (0–7.4/100,000 vs 0–2.3/100,000) and the prevalence of SLE (33.4/100,000 vs 20.6/100,000) were higher among North American Indians than the rest of the population. These observations could represent chance findings; however, in-breeding and/or environmental factors may explain the clustering. Further studies of Native American Indian populations could identify additional clusters with excess morbidity from SLE in an effort to test hypotheses regarding risk factors for SLE.

TABLE 5 Mortality from SLE by Gender and Race Group in the United States[a]

Author	Years	WM	WF	BM	BF
Cobb [43]	1959–1961	1.1	4.0	1.8	10.6
Siegel and Lee [13]	1956–1963	1.6	6.6	4.4	20.0
Kaslow and Masi [44]	1968–1972	1.5	5.2	2.2	14.8
Gordon *et al.* [45]	1972–1976	1.2	4.5	1.9	13.1
Lopez-Acuna *et al.* [46][b]	1968–1978	1.8	6.0	3.0	17.6

[a] Rates per 1 million persons per year. WM, white males; WF, white females; BM, black males; BF, black females.

[b] Data include deaths attributed to both discoid and systemic lupus.

MORTALITY DATA

Mortality attributed to SLE in the continental United States has been estimated from community base [13] as well as national [43–46] data (Table 5). Lopez-Acuna and colleagues [46] identified all deaths attributed to both discoid and systemic lupus erythematosus from National Center for Health Statistics (NCHS) data tapes for the period 1968–1978. A total of 11,156 deaths were identified: 2568 (23.0%) were attributed to discoid lupus and 8588 (77.0%) to SLE. There were no differences in this distribution by sex, race, region, or year. There were a total of 6452 deaths in Caucasian females, 2573 in Black females, 1760 in Caucasian males, and 371 in Black males, with average annual age-adjusted mortality rates of 6.0, 17.6, 1.8, and 3.0 per million person-years, respectively (Table 5). Age-specific average annual mortality rates showed a unimodal distribution for all sex and race groups, with maximum rates occurring in the 45- to 54-year age group in Blacks and in the 65- to 74-year age group in Caucasians (Fig. 1).

Kaslow [47] analyzed a subset of these mortality records and examined deaths attributed to SLE alone

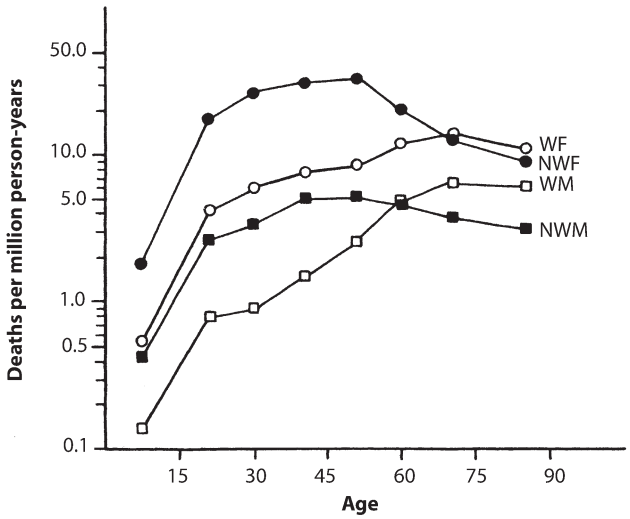


FIGURE 1 Average annual age-specific mortality rates attributed to SLE by sex and race group in the United States, 1968–1989. WM, white males; WF, white females; NWM, nonwhite males; NWF, nonwhite females.

from 1968 through 1976 in 12 states that have 88% of the U.S. residents of Asian descent. Mortality rates were threefold greater among Blacks and twofold greater among Asians compared with Caucasians: 8.4, 6.8, and 2.8 per million person-years, respectively. The age- and sex-adjusted mortality rates for Chinese, Japanese, and Filipinos were 7.5, 6.8, and 5.1 per million persons-years, respectively. Age- and sex-adjusted race-specific mortality rates for Hawaii were greater among Filipinos and the combined Asian group than for the U.S. mainland population, confirming previous observations [48]. A further study from Hawaii demonstrated that mortality were three times higher in non-Caucasians than Caucasians in 1985 to 1989 [25]. It is unclear whether the differences in mortality rates between Asians and Caucasians mirror true differences in incidence rates as is seen with American Blacks (see earlier discussion).

Siegel and Lee [13] noted greater mortality and morbidity from SLE among Puerto Ricans in New York City than among Caucasians. Lopez-Acuna and colleagues [46] analyzed mortality from SLE in Puerto Rico from 1970 through 1977 as well as a subset of the NCHS dataset for five southwestern states: Arizona, California, Colorado, New Mexico, and Texas. A total of 92 deaths from SLE occurred in Puerto Rico; the average annual age-adjusted mortality rates of 7.5 and 2.0 deaths per million person-years in females and males, respectively, were not significantly different from those noted among U.S. Caucasians over the same time period. A correlation between the proportion of Spanish-heritage population and county-specific mortality rates from SLE was noted for females but not males in the five states; the implications of this finding may reflect both ethnic/racial and socioeconomic factors.

Nationwide mortality data regarding SLE have been reported from Finland [15] and from England and Wales [48]; average annual mortality rates were 4.7 and 2.5 per million person-years, respectively. Patterns of age-specific mortality rates in both countries were similar to those in Caucasians; the fourfold greater age-adjusted mortality among English females compared with males is similar to that seen in the United States. Similar results were obtained in a study of the arctic region of Norway, with a crude annual mortality rate of 4.6 per million. A study from Canada comparing the mortality among patients with SLE to the general population demonstrated an overall mortality risk (presented as a standardized mortality ratio, SMR) of 4.9. The SMR was higher for patients under 24 years old than for those 55 or older (14.9 vs 2.83, respectively). A similar SMR (4.6) was described for a Danish multicenter study where the overall mortality rate was 2.9% per year [50]. While the SMR for men and women did not differ, there was an effect of age. The SMR was higher for patients with early onset disease compared to those with late onset disease (57 vs 2.1), supporting the Toronto experience. However, the Norwegian study revealed an overall SMR of 2.2 with rates increasing with age from 1.7 for the 30- to 49-year age group to 3.9 in the over 50 age group [33]. Among the 1000 patients included in the "Euro-lupus project" there was a 4.5% mortality in the first 5 years [51].A multicenter study from Argentina revealed that 12% of 366 patients died over a 6-year study. SMR are not available [52].

Temporal trends in mortality rates have been examined in both the United States [45, 46] and England and Wales [48]. In the United States there was a significant decline in age-adjusted annual mortality rates between 1968 and 1978 for all sex and race groups (Fig. 2). A significant temporal decline in age-adjusted annual SLE mortality rates was also observed among females in England and Wales from 1974 to 1983 but not among males, probably because of small numbers of deaths [48]. A similar decline in mortality rates was observed in the Toronto cohort, where the estimated risk for death was compared for patients entered into the cohort between 1970 and 1977, 1978 and 1986, and 1987 and 1994 [53]. SMRs declined from 10.1 in the first group to 3.3 in the last group. The same decline was observed for the first two groups followed over the next time period. The decline in mortality rates observed in these developed countries is probably due to improved survival in patients with SLE as reflected by (1) a temporal increase in mean age at death from SLE in the United States between 1968 and 1978 (Fig. 3) and (2) 10-year

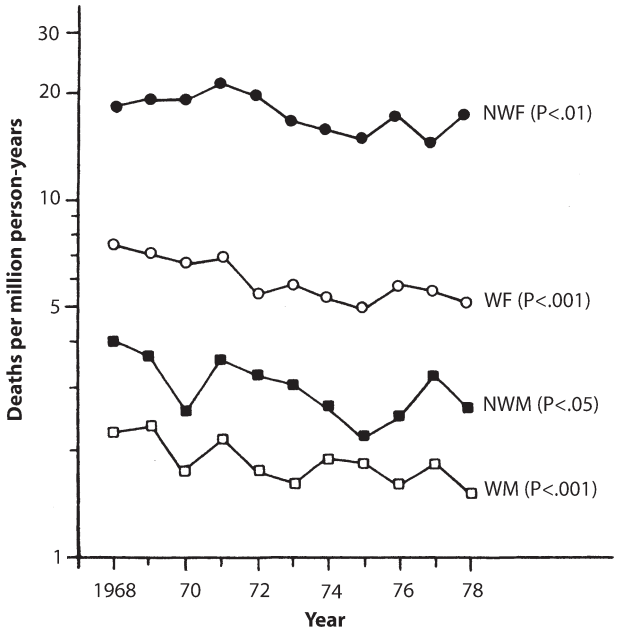


FIGURE 2 Trends in age-adjusted mortality rates from SLE by sex and race group in the United States, 1968–1978. WM, white males; WF, white females; NWM, nonwhite males; NWF, nonwhite females.

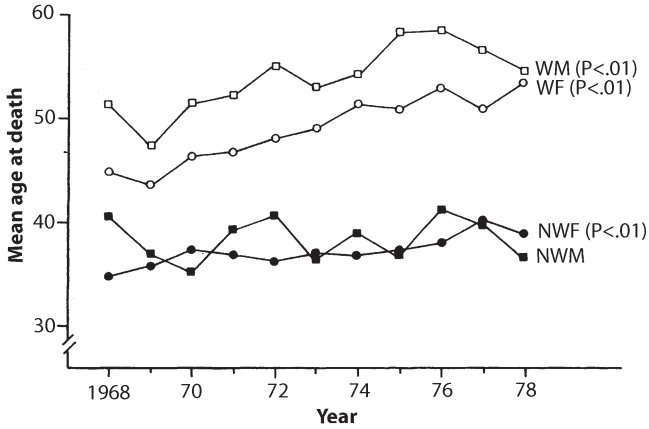


FIGURE 3 Trends in mean age at death from SLE by sex and race group in the United States, 1968–1978. WM, white males; WF, white females; NWM, nonwhite males; NWF, nonwhite females.

cumulative survival rates approaching or exceeding 90% in recent studies [54].

PROGNOSIS AND SURVIVORSHIP

Longitudinal observational studies of patients with SLE to estimate prognosis with respect to survival have been conducted for the past four decades; the first such study, conducted by Merrell and Shulman [55], found a 51% cumulative survival rate at 4 years after diagnosis (Table 6). The survival of patients with SLE has improved remarkably from that study to a 90% 10-year survival in the 1990s, and the 20-year survival figures approach 70% (Table 6) [33, 49–80]. This improved survival is typical of the Western world, whereas in developing countries such as India [71], and among Black Caribbean patients [74], poorer survival has been recorded. Among the Manitoba population, Native Indians fared less well than Caucasians, but they still fared better than patients in the developing countries [31].

Factors Associated with Survival Outcomes

Demographic Factors

A number of features not related to the SLE process itself may be predictive for mortality in patients with SLE, including gender, age at onset, race, and socioeconomic status. Table 7 summarizes the prognostic factors associated with mortality identified in published series. The effect of gender on mortality has been controversial, but the majority of studies suggest that there is no gender effect. However, a Spanish study of 306 patients identified male sex to be associated with worse survival [52]. The role of age at onset of SLE in predicting mortality has also been disputed. Several studies suggested that a higher age at presentation was a risk factor for death [61, 67, 72, 73], but a study comparing adult- and childhood-onset SLE patient populations did not reveal any differences in the 1- to 5-year mortality figures [77]. The latter study, however, did not provide the relationship between the age at onset in the adult-onset patients and mortality. Wallace *et al.* [60] found that an earlier age at onset was detrimental to survival. However, a retrospective study from Taiwan compared survival in patients presenting over age 65, with patients whose SLE started between age 50 and 64 and those in whom disease onset was before age 50 [81]. Survival rates for patients with SLE onset over 65 were 55% at 5 and 10 years compared to 88 and 82%, respectively, for patients with disease onset less than 50. Patients with disease onset between age 50 and 64 demonstrated 5- and 10-year survival rates of 66 and 54%, respectively [81]. Studies that used an age cutoff at 50 or 55 were more likely to find that older age at the diagnosis of SLE was predictive for earlier mortality. Rood *et al.* [82] found than the relative risk of mortality of patients whose onset of SLE occurred between age 17 and 46 was 4.2 times higher than patients with onset of disease before age 14 or after age 50. Similar results were described in a single center study from Hungary [83]. Race also

TABLE 6 Survival Rates in Published Series (1955–2001)

Author	Year	Center	Years			
			5	10	15	20
Merrel and Shulman [55]	1955	Baltimore	50	—	—	—
Kellum and Hasericke [56]	1964	Cleveland	69	54	—	—
Urman and Rothfield [59]	1968	New York	70	63	—	—
Estes and Christian [57]	1971	New York	77	60	50	—
Urowitz *et al.* [58]	1974	Toronto	75	63	53	—
Urman and Rothfield [59]	1976	Farmington	93	84	—	—
Wallace *et al.* [60]	1981	Los Angeles	88	79	74	—
Ginzler *et al.* [61]	1982	Multicenter	86	76	—	—
Studenski *et al.* [63]	1984	North Carolina	90	—	—	—
Jonsson *et al.* [65]	1985	Sweden	97	—	—	—
Malaviya *et al.* [62]	1986	India	68	50	—	—
Stafford-Brady *et al.* [64]	1988	Toronto	84	75	64	—
Swaak *et al.* [66]	1989	Holland	92	87	—	—
Reveille *et al.* [67]	1990	Alabama	89	83	79	—
Gripenberg and Helve [68]	1991	Finland	—	91	81	—
Pistiner *et al.* [69]	1991	Los Angeles	97	93	83	—
Seleznick and Fries [70]	1991	Stanford	88	64	—	—
Kumar *et al.* [71]	1992	India	68	50	—	—
Ward *et al.* [72]	1993	Durham	82	71	63	—
Abu-Shakra *et al.* [73]	1993	Toronto	93	85	79	68
Nossent [74]	1993	Curaçao	56	—	—	—
Murali *et al.* [78]	1993	India	77	60	—	—
Blanco *et al.* [79]	1993	Spain	90	85	80	—
Ståhl-Hallengen *et al.* [35]	1994	Sweden	93	83	—	—
Tucker *et al.* [77]	1995	London	93	85	79	—
Peschken and Esdaile [31]	1996	Manitoba Caucasian	98	96	90	—
		Manitoba Indians	94	80	75	—
Jacobsen *et al.* [50]	1999	Denmark	91	76	64	53
Mok *et al.* [88]	2000	Southern China	93	—	—	—
Alarcon *et al.* [80]	2001	U.S. multicenter	86	70	—	—
Nossent [33]	2001	Norway	92	75	—	—
Bellomio *et al.* [52]	2001	Argentina	91	85	—	—

appears to play a role in both the development of lupus and its prognosis, although it has been difficult to separate off the effects of race from socioeconomic status, particularly with reference to differences between Caucasians and Black patients in the United States. Ginzler and colleagues [61] noted a worse prognosis for Blacks compared with Caucasians in the multicenter lupus survival study. When they entered the study, Blacks also had more severe disease than Caucasians, as reflected in a higher mean number of ARA criteria, mean serum creatinine level, lower mean hematocrit, and greater frequency of central nervous system involvement. Furthermore, Blacks were more likely to be recipients of public rather than private medical insurance. When these possible confounding variables were considered in a multivariate model, Black race was no longer a significant predictor of decreased survival. Studenski and colleagues [63] studied the survival of 411 patients with SLE seen at the Duke University Medical Center between 1969 and 1984. Nonwhite race and public funding of medical insurance were both significantly associated with decreased survival, even after adjustment in a multivariate model. Reveille and colleagues [67] studied survival among 389 patients with SLE seen

TALBE 7 Factors Associated with Mortality in Large Series[a]

Author	Year	Time	Age	Race	Sex	SES	Renal	CNS	BP	Plat	DA
Estes and Christian [57]	1955	A	–	–	–	?	+	+	?	?	?
Wallace *et al*. [60]	1981	D	+	–	–	?	+	–	–	–	?
Reveille *et al*. [67]	1990	D	+	+	–	–	+	?	+	+	?
Pistiner *et al*. [69]	1991	D	–	–	+	–	+	?	?	+	?
Ward *et al*. [72]	1993	A	+	–	–	+	?	?	?	?	?
Abu-Shakra *et al*. [73]	1993	A	+	–	–	–	+	–	–	+	+*
Massardo *et al*. [75]	1993	A	–	–	–	–	+	–	–	+	+*
Blanco *et al*. [79]	1993	A	–	–	+	?	+	+	?	?	?
Jacobsen *et al*. [50]	1999	A	+	NA	+	?	–	+	?	?	?
Peschken and Esdaile [31]	2001	A	–	+	–	?	–	–	–	–	+

[a] A, any time prior to death; D, at diagnosis; *, at study entry; ?, undetermined; SES, socioeconomic status; BP, hypertension; Plat, thrombocytopenia; DA, disease activity.

at the University of Alabama at Birmingham between 1975 and 1984. They also found that Black patients had a worse prognosis than Caucasians, even after adjustment for the source of funding of medical insurance in multivariate analysis. Of interest was the finding of a higher frequency of renal involvement in the Black compared with Caucasian patients; Black patients are recognized as a group to have more severe disease than Caucasian patients. Poverty was an independent predictor of mortality in the LUMINA study [80].

SLE Factors

Major organ involvement, particularly central nervous system and renal disease, has been associated with poor prognosis. Only three studies have identified central nervous system disease as a risk factor in any of the studies to date [57, 66, 84]. However, the presence of renal involvement carried a poor prognosis for lupus patients in most studies [52, 57, 60, 61, 66–71, 73, 84]. The Mayo cohort of lupus nephritis patients [85] demonstrated lower than expected survival rates at 5, 10, and 20 years, with rates of 80, 69, and 53%, respectively. Moreover, elevated serum creatinine, reduced creatinine clearance, and progressive World Health Organization (WHO) class were associated with decreased survival. Similarly, patients with lupus nephritis from Los Angeles who underwent renal transplantation had a 5-year survival rate of only 85.9%, a rate much lower than the >95% rates reported for lupus cohorts [86]. Thus, patients with severe nephritis clearly have reduced survival.

Lung involvement, including pleuritis, pulmonary infiltrate not related to infection or heart failure, pulmonary hemorrhage, and pulmonary hypertension, emerged as a predictive factor for mortality in the analysis performed by Abu-Shakra *et al*. [73]. Lung damage was also identified as a risk for mortality in a British study, which investigated the validity of a damage index [87]. Mok *et al*. [88] found that the main risk factors of death included heart involvement, hyperlipidemia, and renal damage. They also found that treatment with steroids, antimalarial agents, and cyclophosphamide improved survival. Similar risk factors for mortality were identified in a multicenter cohort from Argentina [52]

Using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index, they found that damage in the pulmonary system predicted death within 10 years of diagnosis. Early damage as reflected by the initial SLICC/ACR damage index is associated with a higher rate of mortality [89].

Overall Disease Activity as a Predictor for Mortality in SLE

The assessment of disease activity in SLE has become easier with the development and validation of a number of instruments over the past several years [90]. Overall disease activity has been shown to be a prognostic factor for mortality in a number of studies [79, 91–97]. Disease activity, measured by the systemic lupus erythematosus disease activity index (SLEDAI), at the time of presentation to the lupus clinic, was a predictor for mortality in the Toronto cohort [73]. Overall SLEDAI predicted short-term mortality within 6 months of a visit [97]. Disease activity at last visit emerged as a predictive factor for death in a mutlivariate analysis performed by Peschken and Esdaile [31].

SLEDAI-2K, a recent modification of SLEDAI, was also found to be predictive of mortality [98]. Although a high SLEDAI score (>10) was not a predictor among Black Caribbean patients, a high weighted SLEDAI score, depicting disease activity over the course of disease, was associated with decreased survival in both univariate and multivariate analyses [99]. Disease activity over time measured by the average mean SLEDAI (AMS) was a prognostic factor for death in the Toronto cohort [100].

It is of interest that socioeconomic factors, such as higher education and private insurance, were associated with less disease activity at diagnosis in a sample of 99 subjects enrolled in a multicenter study of modifiable risk factors for SLE in the United States [101]. As noted, because the degree of disease activity has been found to predict mortality, these factors may be relevant. Indeed, the question arises as to whether contribution of the socioeconomic status and disease activity to mortality are independent from each other. The LUMINA study suggests that both disease activity (measured by the systemic lupus activity measure—SLAM) and poverty are independent risk factors for mortality [80].

Reasons for Improved Survival

The improved survival in patients with SLE may have resulted from advances in medical therapy in general (e.g., improved antibiotics, antihypertensive agents, and the availability of renal dialysis and transplantation), as well as a more judicious use of lupus-specific therapy. Other explanations proposed for improved survival include earlier diagnosis and inclusion of milder cases in more recent studies. These latter theories are not supported by the pattern of the disease seen over two decades [53, 76]. Moreover, it is clear that the improvement in survival is above and beyond the improvement in survival of the general population [53].

Causes of Death

Despite the improved survival for patients with SLE over the past several decades, patients with SLE are three times more likely to die earlier than the general population. The majority of fatalities among patients with SLE are attributable to active disease, especially renal and central nervous system involvement and infections [32, 49, 56, 58, 61, 71, 74, 75, 79, 102]. The infections may be bacterial and involve multiple organisms, often occurring in the setting of active SLE, or opportunistic, occurring in patients on high-dose steroids and cytotoxic therapy [49, 103]. Comorbid conditions and other complications of therapy account for the remainder of deaths. During the past decade, increasing attention has been directed toward a preventable cause of mortality; steroid-associated coronary artery disease [49, 53, 104, 105]. This has been particularly noticeable among patients who have lived for more than 5 years. Studies suggest that premature atherosclerosis is common among patients with SLE [105–111]. Thus efforts in patient management should be directed toward not only keeping corticosteroid doses as low as possible, with the use of hydroxychloroquine and other steroid-sparing agents, but also preventing and aggressively treating known coronary artery disease risk factors, including hypercholesterolemia, obesity, hypertension, and diabetes, and counseling against smoking and a sedentary lifestyle [110]. Routine preventive medical interventions, including those just mentioned, as well as vaccination for influenza and pneumococcal infection [112], should be promulgated for all patients with SLE to improve survival. Abu-Shakra *et al*. [113] demonstrated that vaccinations do not aggravate disease activity in patients with SLE.

Morbidity Outcome

With improving survival among patients with SLE, attention over the past decade has been directed increasingly toward outcome measures other than survival. Gladman and Urowitz [114] reviewed disease-related morbidity outcomes, including renal failure, atherosclerosis, coronary artery disease, avascular necrosis of bone, and cognitive neuropsychological dysfunction. Petri *et al*. [115] used important renal disease, the number of hospitalizations, and neurologic diseases as morbidity outcomes in their lupus cohort. These outcomes were not related to race or socioeconomic status but were related to noncompliance with medications and to type of medical insurance. Indeed patients were hospitalized not only for active disease, but more commonly for complications of the disease [116]. The Systemic Lupus International Collaborating Clinics developed the SLICC/ACR damage index for clinical epidemiologic studies [117]. This index includes items in 12 organ systems and describes accumulated damage since the onset of SLE without attribution. The index has been proven to be reliable [118] and valid [87, 119]. Similarly, measurement of disease activity using validated quantitative indices has been recommended [120], and representative indices have been developed for use in clinical research [121]. Several of these indices have been shown to be comparable [122–124]. The use of disease activity indices has been shown to be helpful in a clinic setting as well [125]. In addition to the description of disease activity and damage in patients

with SLE, several investigators have studied physical disability and psychosocial adjustment as health status outcomes in patients with SLE [126–129]. Fatigue has been recognized as a major problem for patients with SLE, and it does not appear to be simply related to disease activity [130, 131]. Petri [132] showed that the morbidity of SLE is influenced by race in the Hopkins lupus cohort. It has been suggested that three aspects of the disease are necessary to describe prognosis in SLE, namely disease activity, accumulated damage, and health status/quality of life [133].

ETIOLOGIC FACTORS

Epidemiologic studies of etiologic factors in SLE have focused on three broad areas: (1) endocrine–metabolic factors (2) environmental factors, and (3) genetic factors.

Endocrine–Metabolic Factors

The strongest risk factor for the development of SLE is female gender. In a review of five series of juvenile-onset SLE and seven series of adult-onset SLE totaling 317 and 1177 cases, respectively, Masi and Kaslow [134] showed that the sex ratio at age of onset or diagnosis rises with puberty from 2:1 to approximately 6:1, peaks in young adulthood at 8:1, and then declines with female menopause in the sixth decade. The authors thought that these data indicated that study of sex-related factors offered a clue to the pathogenesis of SLE. Studies in the (NZB × NZW)F_1 hybrid mouse, a murine model of SLE, support a role for female hormones in the modulation of autoantibody production and development of renal disease and death [135]. Indeed, Lahita and colleagues [136–141] and others [142, 143] have reported abnormalities in the metabolism of estrogens and androgens in both males and females with SLE. Data also indicate that increased estrogen levels might initiate autoimmune diseases in many women and men. Estrogen hydroxylation is increased in both men and women with autoimmune diseases such as lupus [144].

Grimes and colleagues [145] studied 109 newly hospital-diagnosed cases and an equal number of controls discharged from Emory University Hospital between 1973 and 1982; cases differed significantly in age distribution and race distribution from controls. The investigators found no association of age at menarche, parity, history of infertility, fetal wastage, or oral contraceptive usage with SLE. Hysterectomy appeared to be protective, with a crude odds ratio of 0.55; however, after adjustment for age, the odds ratio was 0.73, with 95% confidence intervals (CI) of 0.4 and 1.5. Simultaneous adjustment by age and race was not performed. A history of endometriosis was more common in women with SLE, but the odds ratio did not significantly differ from unity. Sanchez-Guerrero *et al*. [146] used a prospective cohort to study the role of the use of oral contraceptives in the development of SLE and found that past use of oral contraceptives was associated with a slightly increased risk of developing SLE [relative risk of 1.9 (95% CI 1.1–3.3)]. The same authors examined the relation between postmenopausal hormone use and development of systemic lupus erythematosus in a prospective cohort study (Nurses' Health Study) [147]. With never users of postmenopausal hormones as the reference group, age-adjusted relative risks for systemic lupus erythematosus ($n = 45$ women) were 2.1 (95% CI, 1.1 to 4.0) for ever users, 2.5 (CI, 1.2 to 5.0) for current users, and 1.8 (CI, 0.8 to 4.1) for past users. A proportional increase in the risk for systemic lupus erythematosus was observed that was related to the duration of use of postmenopausal hormones (test for trend, $P = 0.011$). Thus they concluded that postmenopausal hormone therapy is associated with an increased risk for developing systemic lupus erythematosus. However, hormonal therapy does not have a deleterious effect on existing SLE [148].

Environmental Factors

Historically, SLE has been considered to have a viral etiology [149, 150]; however, despite several decades of investigations, no firm documentation of a definite viral etiology has been identified. More recent studies have focused on human retroviruses, especially human T lymphotropic virus, type I (HTLV-I); however, the majority of studies have failed to demonstrate an association between antibodies to HTLV-I and SLE [151–154]. Nonetheless, investigations employing molecular–biologic techniques to identify viral proteins, including reverse transcriptase, continue. All but 1 of 196 patients with ANA-positive lupus had been exposed to EBV, whereas 22 of 392 controls had not [155]. Moreover, SLE patients had a higher titer of anti-EBV-VCA antibodies compared with unaffected controls.

The presence of a transmissible agent, presumed viral, was hypothesized to explain the concurrence of human and canine SLE in the same household [156]. An epidemiologic study comparing households that included dogs that had lupus to households including healthy dogs, matched by veterinarian, failed to find any excess cases of SLE or asymptomatic, seropositive subjects with anti-DNA antibodies among household contacts of index dogs [157].

The most likely noninfectious environmental factors to have an etiologic role in SLE are chemicals. The syndrome of drug-induced lupus, reported most commonly with hydralazine, procainamide, and isoniazid, provides a model to study the possible effects of other chemicals, especially aromatic amines [158]. In a case–control study of 44 subjects with connective tissue disease, including 23 with SLE, a further 9 with unclassified connective tissue disease, and 88 age-, sex-, and race-matched controls, Freni-Titulaer and colleagues [159] determined exposure to occupational and environmental factors, including medications associated with drug-induced lupus and chemicals with structural similarities to these medications. Results of matched univariate analyses demonstrated significant associations among use of hair dyes, use of hair permanent solutions, and use of hair spray with connective tissue disease; in the multivariate analysis, the association of use of hair dyes with connective tissue disease remained significant, with an odds ratio of 7.1 (95% confidence interval: 1.9, 26.9). Some hair dyes contain aromatic amines, which are absorbed through the scalp and then metabolized through acetylation. However, these results were not confirmed by a large prospective study [160].

Genetic Factors

The application of genetic epidemiology to SLE has generated strong evidence of a hereditary predisposition to this disorder. Familial aggregation of SLE has been demonstrated in two studies [161, 162]. Hochberg [161] studied the occurrence of SLE among first-degree relatives of 77 patients with SLE and age-, sex-, and race-matched controls without a history of rheumatic disease. Eight (10.4%) of the SLE probands had one or more first-degree relatives with SLE compared with only one (1.3%) of the controls [relative risk (RR) = 8, $P = 0.03$]. SLE occurred in 9 (1.67%) of 541 first-degree relatives of SLE probands but in only one (0.18%) of 540 first-degree relatives of controls (RR = 9, $P = 0.01$). Of the 9 affected first-degree relatives of SLE probands, 7 were female and 2 male, whereas the only affected control first-degree relative was female; thus the prevalence of SLE in female first-degree relatives was 2.65% vs 0.40% (RR = 6.8, $P = 0.04$) Lawrence *et al*. [162] studied 41 consecutive patients with SLE, identified from hospital registers, who had 147 available first-degree relatives, aged 15 years and older, of whom 128 were fully evaluated with examinations and serologic studies. Control relatives were selected from family surveys of probands with osteoarthritis, psoriasis, and colitis. Definite SLE was found in 5 (3.9%) of first-degree relatives of SLE probands compared with only 1 (0.8%) of 128 matched first-degree relatives of controls ($P = 0.001$).

Twin studies have demonstrated a greater concordance rate among monozygotic (MZ) than dizygotic (DZ) twin pairs, providing further support for a genetic contribution to the mechanism of familial aggregation of SLE [163–165]. Block *et al*. [163] studied 12 twin pairs and reviewed data on 17 additional published pairs. Concordance of clinical SLE occurred in 4 (57%) of the 7 monozygotic pairs and none of the 3 dizygotic pairs. Of the 12 MZ pairs in the literature, concordance for SLE was documented in 7 (58%); thus concordance for SLE was present in 11 (58%) of 19 total monozygous pairs. The authors interpreted these results as strong evidence for a genetic contribution to SLE; however, they also acknowledged a role for nongenetic factors in the expression of the illness. Arnett and Shulman [164] noted a striking concordance of clinical and laboratory features of SLE in monozygotic twin pairs compared with nontwin sib pairs. These observations also support a genetic influence on disease expression in SLE. A subsequent study based on self-reported diagnoses in persons listed in a nationwide, chronic disease twin registry found a lower rate of concordance in monozygous twin pairs [165]. Of 107 twin pairs meeting the American College of Rheumatology 1982 revised criteria for the diagnosis of SLE, 24% of 45 MZ pairs and 2% of 62 DZ pairs were concordant.

Two separate analyses support a polygenic mode of inheritance of SLE. Using Block's data on concordance of SLE in monozygotic twin pairs, Winchester and Nunez-Roldan [166] calculated that a multigenic hypothesis with either three or four dominant alleles best explained the familial inheritance of SLE. Lawrence *et al*. [162] determined that a pattern of polygenic inheritance with only moderate heritability best fit their family data. Advances in quantitative methods of segregation analysis, however, have allowed the study of single gene effects in other conditions thought to have a polygenic mode of inheritance.

Segregation or pedigree analysis has been applied to SLE to determine the mode of mendelian inheritance in multicase families. Arnett and colleagues [167] studied 19 multicase SLE families with 232 relatives of whom 24 had SLE, 27 had other autoimmune diseases, and 47 others had serologic abnormalities, including high-titer antinuclear antibodies, antibody to single-stranded DNA, or a false-positive test for syphilis. They postulated a single, genetically determined "autoimmune" trait and applied segregation analysis to family data. Results were consistent with a mendelian dominant inheritance pattern with a gene frequency of 0.06 and a 91% penetrance. The expression of autoimmunity

was modified by gender and age at time of study, but not by HLA-DR phenotype. Further studies by this group estimated the population frequency of the "autoimmune" gene as 0.10, with penetrance of 92% in females and 49% in males [168]. Linkage of this autoimmune gene to other genetic markers was also studied by these investigators; they failed to demonstrate linkage with any HLA phenotype, immunoglobulin allotype, or 21 other polymorphic genetic markers [167, 168].

The genetics of SLE is complex. There are likely many genes involved. While the major histocompatibility complex (MHC) likely plays a role, the exact nature of the contribution of the MHC to the etiopathogensis of SLE is unclear. Moreover, a number of other genes may play a role in this disease, including complement genes, genes related to inflammation, hormones, and genes on the X chromosome [169].

Several investigations into familial SLE have demonstrated a 5–10% familial aggregation [170–172]. Half of the multiplex families were of sib-pair type [170]. Familial SLE did not differ from its sporadic form [169, 171]. Genome scans have implicated a number of genes, including genes on chromosome 6, chromosome 1, and chromosome 4, as well as other chromosomal regions [173–178]. Several of these regions were described in more than one genome scan, whereas others were described in a single investigation. Further studies are clearly necessary before the exact nature of the genetic contribution to SLE is identified.

SUMMARY

Descriptive epidemiologic studies of SLE have been conducted worldwide; the most extensive data are available from Scandinavia, especially Sweden, and the United States. In the United States, Blacks have threefold higher incidence, prevalence, and mortality rates than Caucasians; the reasons for this, however, remain unknown. Observational epidemiologic studies have demonstrated an increasingly favorable prognosis for patients with SLE, allowing the identification of potentially preventable causes of death and a better understanding of long-term morbidity and impact on overall health status. Analytic and genetic epidemiologic studies suggest a multifactorial etiology of SLE; results support a polygenic mode of inheritance, including important roles for an autosomal-dominant autoimmune gene and female sex hormones. A viral etiology remains attractive, particularly in view of recent observations regarding the Epstein–Barr virus. Other environmental factors such as chemical exposures may be important as "triggers" of disease in susceptible hosts.

References

1. Masi, A. T. (1984). Clinical epidemiologic perspective of systemic lupus erythematosus. *In* "Epidemiology of the Rheumatic Diseases" (R. C. Lawrence and L. E. Shulman, eds.), p. 145. Gower, New York.
2. Nived, O., and Sturfelt, G. (1987). Epidemiology of systemic lupus erythematosus *Monogr. Allergy* **21**, 197–204.
3. Fessel, W. J. (1988). Epidemiology of systemic lupus erythematosus. *Rheum. Dis. Clin. North. Am.* **14**, 15–23.
4. Hochberg, M. C. (1990). Systemic lupus erythematosus. *Rheum. Dis. Clin. North. Am.* **16**, 671–639.
5. Cohen, A. S., Reynolds, W. E., Franklin, E. C., Kulka, J. P., Ropes, M. W., Shulman, L. E., and Wallace, S. I. (1971). Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21**, 643–648.
6. Tan, E. N., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N., Green Schaller, J., Talal, N., and Winchester, R. J. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* **25**, 1271–1277.
7. Edworthy, S. M., Zatarain, E., McShane, D. J., and Block, D. A. (1988). Analysis of the 1982 ARA lupus criteria data set by recursive partitioning methodology: New insights into the relative merit of individual criteria. *J. Rheumatol.* **15**, 1493–1498.
8. Hochberg, M. C. (1997). Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **40**, 1725.
9. Smith, E. L., and Shmerling, R. H. (1999). The American College of Rheumatology criteria for the classification of systemic lupus erythematosus: Strengths, weaknesses, and opportunities for improvement. *Lupus* **8**, 586–595.
10. Ganczarczyk, L., Urowitz, M. B., and Gladman, D. D. (1989). Latent lupus. *J. Rheumatol.* **16**, 475–478.
11. Greer, J. M., and Panush, R. S. (1989). Incomplete lupus ervthematosus. *Arch. Intern. Med.* **149**, 2473–2476.
12. Swaak, A. J., van den Brink, H. G., Smeenk, R. J., Manger, K., Kalden, J. R., Tosi, S., Domljan, Z., Rozman, B., Logar, D., Pokorny, G., Kovacs, L., Kovacs, A., Vlachoyiannopoulos, P. G., Moutsopoulos, H. M., Chwalinska-Sadowska, H., Kiss, E., Cikes, N., Anic, B., Schneider, M., Fischer, R., Bombardieri, S., Mosca, M., Graninger, W., and Smolen, J. S. (2001). Incomplete lupus erythematosus: Results of a multicentre study under the supervision of the EULAR Standing Committee on International Clinical Studies Including Therapeutic Trials (ESCISIT). *Rheumatology* **40**, 89–94.
13. Siegel, M., and Lee, S. L. (1973). The epidemiology of svstemic lupus erythematosus. *Semin. Arthritis. Rheum.* **3**, 1–54.
14. Fessel, W. J. (1974). Systemic lupus ervthematosus in the community: Incidence, prevalence, outcome, and first symptoms; the high prevalence in Black women. *Arch. Intern. Med.* **134**, 1027–1035.
15. Helve, T. (1985). Prevalence and mortality rates of systemic lupus ervthematosus and causes of death in SLE patients in Finland. *Scand. J. Rheumatol.* **14**, 43–46.

16. Michet, C. J., McKenna, C. H., Elveback, L. R., Kaslow, R. A., and Kurland, L. T. (1985). Epidemiology of systemic lupus erythematosus and other connective tissue disease in Rochester, Minnesota, 1950 through 1979. *Mayo Clin. Proc.* **60**, 105–113.
17. Meddings, J., and Grennan, D. M. (1980). The prevalence of svstemic lupus ervthematosus (SLE) in Dunedin. *N.Z. Med. J.* **91**, 205–206.
18. Nived, O., Sturfelt, G., and Wollheim, F. (1985). Systemic lupus erythematosus in an adult population in Southern Sweden: Incidence, prevalence and validity of ARA revised classification criteria. *Br. J. Rheumatol.* **24**, 147–154.
19. Hochberg, M. C. (1987). Prevalence of systemic lupus erythematosus in England and Wales, 1981–2. *Ann. Rheum. Dis.* **46**, 664–666.
20. Nakae, K., Furusawa, F., Kasukawa, R., *et al*. (1987). A nationwide epidemiological survey on diffuse collagen diseases: Estimation of prevalence rate in Japan. *In* "Mixed Connective Tissue Disease and Anti-Nuclear Antibodies" (R. Kasukawa and G. C. Sharp, eds.), p. 9. Elsevier Science, Amsterdam.
21. Samanta, A., Roy, S., Peehally, J., and Symmons, D. P. M. (1992). The prevalence of diagnosed systemic lupus erythematosus in whites and Indian Asian immigrants in Leicester City, UK. *Br. J. Rheumatol.* **31**, 679–682.
22. Nossent, J. C. (1992). Systemic lupus erythematosus on the Caribbean island of Curacao: An epidemiological investigation. *Ann. Rheum. Dis.* **51**, 1197–1201.
23. Hopkinson, N. D., Doherty, M., and Powell, R. J. (1994). Clinical features and race-specific incidence/prevalence rates of systemic lupus erythematosus in a geographically complete cohort of patients. *Ann. Rheum. Dis.* **53**, 675–680.
24. Gourley, I. S., Patterson, C. C., and Bell, A. L. (1997). The prevalence of systemic lupus erythematosus in Northern Ireland. *Lupus* **6**, 399–403.
25. Maskarinec, G., and Katz, A. R. (1995). Prevalence of systemic lupus erythematosus in Hawaii: Is there a difference between ethnic groups?. *Hawaii Med. J.* **54**, 406–409.
26. Johnson, A. E., Gordon, C., Palmer, R. G., and Bacon, P. A. (1995). The prevalence and incidence of systemic lupus erythematosus in Birmingham, England: Relationship to ethnicity and country of birth. *Arthritis Rheum.* **38**, 551–558.
27. Hochberg, M,C., Perlmutter, S. L., Medsger, T. A., Steen,V., Weisman, M. H., White, B., and Wigley, F. M. (1995). Prevalence of self-reported physician-diagnosed systemic lupus erythematosus in the USA. *Lupus* **4**, 454–456.
28. Uramoto, K. M., Michet, C. J., Jr., Thumboo, J., Sunku, J., O'Fallon, W. M., and Gabriel, S. E. (1999). Trends in the incidence and mortality of systemic lupus erythematosus. *Arthritis Rheum.* **42**, 46–50.
29. McCarty, D. J., Manzi, S., Medsger, T. A., Jr, Ramsey-Goldman, R., LaPorte, R. E., and Kwoh, C. K. (1995). Incidence of systemic lupus erythematosus: Race and gender differences. *Arthritis Rheum.* **38**, 1260–1270.
30. Hochberg, M. C. (1985). The incidence of systemic lupus erythematosus in Baltimore, Maryland, 1970–1977. *Arthritis Rheum.* **28**, 80–86.
31. Peschken, C. A., and Esdaile, J. M. (2000). Systemic lupus erythematosus in North American Indians: A population based study. *J. Rheumatol.* **27**, 1884–1891.
32. Voss, A., Green, A., and Junker, P. (1998). Systemic lupus erythematosus in Denmark: Clinical and epidemiological characterization of a county-based cohort. *Scand. J. Rheumatol.* **27**, 98–105.
33. Nossent, H. C. (2001). Systemic lupus erythematosus in the Arctic region of Norway. *J. Rheumatol.* **28**, 539–546.
34. Molokhia, M., McKeigue, P. M., Cuadrado, M., and Hughes, G. (2001). Systemic lupus erythematosus in migrants from west Africa compared with Aftro-Caribbean people in the UK. *Lancet* **357**, 1414–1415.
35. Ståhl-Hallengegre, C., Jönsen, A., Nived, O., and Stuffelt, G. (2000). Incidence studies of systemic lupus erythematosus in southern Sweden: Increasing age, decreasing frequency of renal manifestations and good prognosis. *J. Rheumatol.* **27**, 685–691.
36. Gudmundsson, S., and Steinsson, K. (1990). Systemic lupus erythematosus in Iceland 1975 through 1984: A nationwide epidemiological study in an unselected population. *J. Rheumatol.* **17**, 1162–1167.
37. Hart, H. H., Grigor, R. R., and Caughley, D. E. (1983). Ethnic difference in the prevalence of systemic lupus erythematosus. *Ann. Rheum. Dis.* **42**, 529–532.
38. Nai-Zheng, Z. (1989). Epidemiology of systemic lupus erythematosus (SLE) in China. *In* "Proceedings of the Second International Conference on Svstemic Lupus Erythematosus, Singapore, 1989," p. 29. Professional Postgraduate Services, KK, Tokyo.
39. Hochberg, M. C. (1989). Racial differences in the descriptive and clinical epidemiology of systemic lupus erythematosus in the United States. *In* "Proceedings of the Second International Conference on Svstemic Lupus Ervthematosus, Singapore, 1989," p. 32. Professional Postgraduate Services, KK, Tokyo.
40. Chou, C.-T., Lee, F.-T., and Schumacher, H. R. (1986). Modification of a screening technique to evaluate systemic lupus erythematosus in a Chinese population in Taiwan. *J. Rheumatol.* **13**, 806–809.
41. Morton, R. O., Gershwin, M. E., Brady, C., and Steinberg, A. D. (1976). The incidence of svstemic lupus erythematosus in North American Indians. *J. Rheumatol.* **3**, 186–190.
42. Atkins, C., Rueffel, L., Roddy, J. Platts, M., Robinson, H., and Ward, R. (1988). Rheumatic disease in the Nuu-Chah-Nulth Native Indians of the Pacific Northwest. *J. Rheumatol.* **15**, 684–690.
43. Cobb, S. (1971). "The Frequency of the Rheumatic Diseases," p. 99. Harvard Univ. Press, Cambridge.
44. Kaslow, R. A., and Masi, A. T. (1978). Age, sex, and race effects on mortality from systemic lupus erythematosus in the United States. *Arthritis Rheum.* **21**, 473–479.

45. Gordon, M. F., Stolley, P. D., and Schinnar, R. (1981). Trends in recent systemic lupus erythematosus mortality rates. *Arthritis Rheum.* **24**, 762–769.
46. Lopez-Acuna, D., Hochberg, M. C., and Gittelsohn, A. M. (1982). Mortality from discoid and systemic lupus erythematosus in the United States, 1968–1978. *Arthritis Rheum.* **25**(Suppl.), S80.
47. Kaslow, R. A. (1982). High rate of death caused by systemic lupus erythematosus among U.S. residents of Asian descent. *Arthritis Rheum.* **25**, 414–418.
48. Hochberg, M. C. (1987). Mortality from systemic lupus erythematosus in England and Wales, 1973–1984. *Br. J. Rheumatol.* **26**, 437–441.
49. Abu-Shakra, M., Urowitz, M. B., Gladman, D. D., and Gough, J. (1995). Mortality studies in systemic lupus erythematosus: Results from a single centre. I. Causes of death. *J. Rheumatol.* **22**, 1259–1264.
50. Jacobsen, S., Petersen, J., Ulman, S., Junker, P., Voss, A., Rasmussen, J. M., Tarp, U., Poulsen, L. H., van Overseem Hansen, G., Skaarup, N., Hansen, T. M., Podenphant, J., and Halberg, P. (1999). Mortality and causes of death of 513 Danish patients with systemic lupus erythematosus. *Scand. J. Rheumatol.* **28**, 75–80.
51. Cervera, R., Khamashta, M. A., Font, J., Sevastiani, G. D., Gil, A., Lavilla, P., Aydintug, A. O., Jedryka-Goral, A., de Ramon, E., Fernandez-Nebro, A., Galeazzi, J., Haga, H. J., Mathieu, A., Houssiau, F., Ruiz-Irastorza, G., Ingelmo, M., and Hughes, G,R. (1999). Morbidity and mortality in systemic lupus erythematosus during a 5-year period: A multicenter prospective study of 1000 patients. *Medicine* **78**, 167–175.
52. Bellomio, V., Spindler, A., Lucero, E., Berman, A., Santana, M., Moreno, C., Hidalgo, R. P., Paira, S., Graf, C., Maldonado Cocco, J. A., Citera, G., Arriola, M. S., Gomez, G., Barreira, J. C., Messina, O., Asnal, C., Carrillo, D., Gervilla, A., Garcia, L., Mascolo, M., De la Sota, M. D., Rosso, G., Somma, L. F., Sosa, R. F., Rillo, O., Caracciolo, J. A., Lancioni, G., and Gomez, A. (2000). Systemic lupus erythematosus: Mortality and survival in Argentina. A multicenter study. *Lupus* **9**, 377–381.
53. Urowitz, M. B., Abu-Shakra,M., Gladman, D. D., Gough, J., and Farewell, V. T. (1997). Improved survival in SLE. *J. Rheumatol.* **24**, 1061–1065.
54. Gladman, D. D. (1996). Prognosis and treatment of systemic lupus erythematosus. *Curr. Opin. Rheumatol.* **8**, 430–437.
55. Merrell, M., and Shulman, L. E. (1955). Determination of prognosis in chronic disease, illustrated by systemic lupus erythematosus. *J. Chronic. Dis.* **1**, 12–32.
56. Kellum, R. E., and Hasericke, J. R. (1964). Systemic lupus erythematosus, a statistical evaluation of mortality based on a consecutive series of 299 patients. *Arch. Intern. Med.* **113**, 200–207.
57. Estes, D., and Christian, C. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85–95.
58. Urowitz, M. B., Bookman, A. A. M., Koehler, B. E., Gordon, D. A., Smythe, H. A., and Ogryzlo, M. A. (1976). The bimodal mortality pattern of systemic lupus erythematosus. *Am. J. Med.* **60**, 221–225.
59. Urman, J. D., and Rothfield, N. F. (1977). Corticosteroid treatment in systemic lupus erythematosus: Survival studies. *JAMA* **238**, 2272–2276.
60. Wallace, D. J., Podell, T., Klinenberg, J. R., Forouzesh, S., and Dubois, E. L. (1981). Systemic lupus erythematosus: Survival patterns. *JAMA* **245**, 934–938.
61. Ginzler, E. M., Diamond, H. S., Weiner, M., Schlesinger, M., Fries, J. F., Wasner, C., Medsger, T. A., Jr, Ziegler, G., Klippel, J. H., Hadler, N. M., Albert, D. A., Hess, E. V., Spencer-Green, G., Grayzel, A., Worth, D., Hahn, B. H., and Barnett, E. V. (1982). A multicenter study of outcome in systemic lupus erythematosus. I. Entry variables as predictors of prognosis. *Arthritis Rheum.* **25**, 601–611.
62. Malaviya, A. N., Misral, R., Banerjee, S., Kumar, A., Tiwari, S. C., Bhuyan, U. N., Malhotra, K. K., and Guleria, J. S. (1986). Systemic lupus erythematosus in North Indian Asians: A prospective analysis of clinical and immunological features. *Rheumatol. Int.* **6**, 97–101.
63. Studenski, S., Allen, N. B., Caldwell, D. S., Rice, J. R., and Polisson, R. P. (1987). Survival in systemic lupus erythematosus: A multivariate analysis of demographic factors. *Arthritis Rheum.* **30**, 1326–1332.
64. Stafford-Brady, F., Urowitz, M. B., and Gladman, D. D. (1988). Lupus retinopathy: Patterns, associations and prognosis. *Arthritis Rheum.* **31**, 1105–1110.
65. Jonsson, H., Nived, O., and Sturfeld, G. (1989). Outcome in systemic lupus erythematosus: A prospective study of patients from a defined population. *Medicine* **68**, 141–150.
66. Swaak, A. J. G., Nossent, J., Bronsveld, W., Van Rooyden, A., Nieuwenhuys, E. J., Theuns, L., and Smeenk, R. J. (1989). Systemic lupus erythematosus. I. Outcome and survival: Dutch experience with 110 patients studied prospectively. *Ann. Rheum. Dis.* **48**, 447–454.
67. Reveille, J. D., Bartolucci, A., and Alarcón, G. S. (1990). Prognosis in systemic lupus erythematosus: Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. *Arthritis Rheum.* **33**, 37–48.
68. Gripenberg, M., and Helve, T. (1991). Outcome of systemic lupus erythematosus: A study of 66 patients over 7 years with special reference to the predictive value of anti-DNA antibody determination. *Scand. J. Rheumatol.* **20**, 104–109.
69. Pistiner, M., Wallace, D. J., Nessim, S., Metzger, A.L, and Klineberg, J. R. (1991). Lupus erythematosus in the 1980s: A survey of 570 patients. *Semin. Arthritis Rheum.* **21**, 55–64.
70. Seleznick, M. J., and Fries, J. F. (1991). Variables associated with decreased survival in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **21**, 73–80.
71. Kumar, A., Malaviya, A. N., Singh, R. R., Singh, Y. N., Adya, C. M., and Kakkar, R. (1992). Survival in patients with systemic lupus erythematosus in India. *Rheumatol. Int.* **12**, 107–109.
72. Ward, M. M., Pyun, E., and Studenski, S. (1995). Long-term survival in systemic lupus erythematosus: Patient

characteristics associated with poorer outcomes. *Arthritis Rheum.* **38**, 274–283.
73. Abu-Shakra, M., Urowitz, M. B., Gladman, D. D., and Gough, J. (1995). Mortality studies in systemic lupus erythematosus: Results from a single centre. II. Predictor variables for mortality. *J. Rheumatol.* **22**, 1265–1270.
74. Nossent, J. C. (1993). Course and prognosis of systemic lupus erythematosus disease activity index in black Caribbean patients. *Semin. Arthritis Rheum.* **23**, 16–21.
75. Massardo, L., Martinez, M. E., Jacobelli, S., Villarroel, L., Rosenberg, H., and Rivero, S. (1993). Survival of Chilean patients with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **24**, 1–11.
76. Swaak, A. J. G., Nieuwenhuis, E. J., and Smeenk, R. J. T. (1992). Changes in clinical features of patients with systemic lupus erythematosus followed prospectively over 2 decades. *Rheumatol. Int.* **12**, 71–75.
77. Tucker, L. R., Menon, S., Schaller, J. G., and Isenberg, D. A. (1995). Adult- and childhood-onset systemic lupus erythematosus: A comparison of onset, clinical features, serology, and outcome. *Br. J. Rheumatol.* **44**, 866–872.
78. Murali, R., Jeyaseelan, L., Rajaratnam, S, John, L., and Ganesh, A. (1997). Systemic lupus erythematosus in Indian patients: Prognosis, survival and life expectancy. *Natl. Med. J. India* **10**, 159–164.
79. Blanco, F. J., Gomez-Reino, J. J., de la Mata, J., Corrales, A., Rodriguez-Valverde, V., Rosas, J. C., Gomez de la Camara, A., and Pascual, E. (1998). Survival analysis of 306 Euorpean Spanish patients with systemic lupus erythematosus. *Lupus* **7**, 159–163.
80. Alarcon, G. S., McGwin, G., Jr., Bastian, H. M., Roseman, J., Lisse, J., Fessler, B. J., Friedman, A. W., and Reveille, J. D. (2001). Systemic lupus erythematosus in three ethnic groups. VII. Predictors of early mortality in the LUMINA cohort. *Arthritis Rheum.* **45**, 191–202.
81. Pu, S. J., Luo, S. F., Wu, Y. J. J., Cheng, H. S., and Ho, H. H. (2000). The clinical features and prognosis of lupus with disease onset at age 65 or older. *Lupus* **9**, 96–100.
82. Rood, M. J., van der Velder, E. A., Ten Cate, R., Breedveld, F. C., and Huizinga, T. W. J. (1998). Femal sex hormones at the onset of systemic lupus erythematosus affect survival. *Br. J. Rheumatol.* **37**, 1008–1010.
83. Kiss, E., Regecsy, N., and Szegedi, G. (1999). Systemic lupus erythematosus survival in Hungary: Results from a single centre. *Clin. Exp. Rheumatol.* **17**, 171–177.
84. Ward, M. M., Pyun, E., and Studenski, S. (1996). Mortality risks associated with specific clinical manifestaions of systemic lupus erythematosus. *Arch. Intern. Med.* **156**, 1337–1344.
85. Donadio, J. V., Hart, G. M., Bergstralh, E. J., and Holley, K. E. (1995). Prognostic determinants in lupus nephritis: A long-term clinicopathological study. *Lupus* **4**, 109–115.
86. el-Shahawy, M. A., Aswad, S., Mendez, R. G., Bangsil, R., Mendez, R., and Massry, S. G. (1995). Renal transplantation in systemic lupus erythematosus: A single-center experience with sixty-four cases. *Am. J. Nephrol.* **15**, 123–128.
87. Stoll, T., Seifert, B., and Isenberg, D. A. (1996). SLICC/ACR damage index is valid, and renal and pulmonary organ scores are predictors of severe outcome in patients with systemic lupus erythematosus. *Br. J. Rheumatol.* **35**, 248–254.
88. Mok, C. C., Lee, K. W., Ho, C. T., Lau, C. S., and Wong, R. W. (2000). A prospective study of survival and prognostic indicators of systemic lupus erythematosus in a southern Chinese population. *Rheumatology* **39**, 399–406.
89. Rahman, P., Gladman, D. D., Urowitz, M. B., Hallett, D., and Tam, L. S. (2001). Early damage as measured by the SLICC/ACR damage index is a predictor of mortality in systemic lupus erythematosus. *Lupus* **10**, 93–96.
90. Hay, E. M. (1995). Systemic lupus erythematosus. *Baillière's Clin. Rheumatol.* **9**, 437–470.
91. Iliopoulos, A. G., and Tsokos GC. (1996). Immunopathogenesis and spectrum of infections in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **26**, 318–336.
92. McLaughlin, J. R., Bombardier, C., Farewell, V. T., Gladman, D. D., and Urowitz, M. B. (1994). Kidney biopsy in systemic lupus erythematosus. III. Survival analysis controlling for clinical and laboratory variables. *Arthritis Rheum.* **37**, 559–567.
93. Esdaile, J. M., Abrahamowicz, M., MacKenzie, T., Hayslett, J. P., and Kashgarian, M. (1994). The time-dependence of long-term prediction in lupus nephritis. *Arthritis Rheum.* **37**, 359–368.
94. Goulet, J. R., Mackenzie, T., Levinton, C., Hayslett, J. P., Ciampi, A., and Esdaile, J. M. (1993). The longterm prognosis of lupus nephritis: The impact of disease activity. *J. Rheumatol.* **20**, 59–65.
95. Cohen, M. G., and Li, E. K. (1992). Mortality in systemic lupus erythematosus: Active disease is the most important factor. *Aust. N. Z. J. Med.* **22**, 5–8.
96. Drenkard, C., Villa, A. R., Alarcon-Segovia, D., and Perez-Vazquez, M. E. (1994). Influence of the antiphospholipid syndrome in the survival of patients with systemic lupus erythematosus. *J. Rheumatol.* **21**, 1067–1072.
97. Cook, R. J., Gladman, D. D., Pericak, D., and Urowitz, M. B. (2000). Prediction of short-term mortality in SLE with time-dependent measures of disease activity. *J. Rheumatol.* **27**, 1892–1895.
98. Gladman, D. D., Ibañez, D., and Urowitz, M. B. (2002). SLEDAI 2000. *J. Rheumatol.* **29**, 288–291.
99. Nossent, J. C. (1998). SLICC/ACR damage index in Afro-Carribean patients with SLE: Changes in and relationship to disease activity, corticosteroid therapy and prognosis. *J. Rheumatol.* **25**, 654–659.
100. Urowitz, M. B., Ibañez, D., and Gladman, D. D. (2001). Disease activity over time predicts outcome in SLE. *Lupus* **10**(Suppl. 1), S96.
101. Karlson, E. W., Daltroy, L. H., Lew, R. A., Wright, E. A., Partridge, A. J., Roberts, W. N., Stern, S. H., Stratton, K. V., Wacholtz, M. C., Grosflam, J. M., and Liang, M. (1995). The independence and stability of socioeconomic predictors of morbidity in systemic lupus erythematosus. *Arthritis Rheum.* **38**, 267–273.
102. Rosner, S., Ginzler, E. M., Diamond, H. S. Weiner, M., Schlesinger, M., Fries, J. F., Wasner, C., Medsger, T. A., Jr., Ziegler, G., Klippel, J. H., Hadler, N. M., Albert, D. A.,

Hess, E. V., Spencer-Green, G., Grayzel, A., Worth, D., Hahn, B. H., and Barnett, E. V. (1982). A multicenter study of outcome in systemic lupus erythematosus. II. Causes of death. *Arthritis Rheum.* **25**, 612–617.
103. Watanabe Duffy, K. N., Duffy, C. M., and Gladman, D. D. (1991). Infection and disease activity in SLE: A review of hospitalized patients. *J. Rheumatol.* **18**, 1180–1184.
104. Rubin, L. A., Urowitz, M. B., and Gladman, D. D. (1985). Mortality in systemic lupus erythematosus: The bimodal pattern revisited. *Q. J. Med.* **55**, 87–98.
105. Urowitz, M. B., and Gladman, D. D. (1980). Late mortality in SLE: The price we pay for control. *J. Rheumatol.* **7**, 412–416.
106. Petri, M., Spence, D., Bone, L. R., and Hochberg, M. C. (1992). Coronary artery risk factors in the Johns Hopkins lupus cohort: Prevalence, recognition by patients and preventive practices. *Medicine* **71**, 291–302.
107. Manzi, S., Meilahn, E. N., Rairie, J. E., Conte, C. G., Medsger, T. A., Jr., Jansen-McWilliams, L., D'Agostino, R. B., and Kuller, L. H. (1997). Age-specific incidence rates of myocardial infacrtion and angina in women with systemic lupus erythematosus: Comparison with the Framingham study. *Am. J. Epidmiol.* **145**, 408–415.
108. Bruce, I. N., Burns, R. J., Gladman, D. D., and Urowitz, M. B. (2000). Single photon emission computed tomography (SPECT) dual isotope myocardial perfusion imaging (DIMPI) in women with SLE. I. Prevalence and distribution of abnormalities. *J. Rheumatol.* **27**, 2372–2377.
109. Manzi, S., Selzer, F., Sutton-Tyrrell, K., Fitzgerald, S.G, Rairie, J. E., Tracy, R. P., and Kuller, L. H. (1999). Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum.* **42**, 51–60.
110. Petri, M., Perez-Gutthann, S., Spence, D., and Hochberg, M. C. (1992). Risk factors for coronary artery disease in patients with systemic lupus erythematosus. *Am. J. Med.* **93**, 513–519.
111. Bruce, I. N., Gladman, D. D., and Urowitz, M. B. (2000). Premature atherosclerosis in SLE. *Rheum. Dis. Clin. North Am.* **26**, 257–278.
112. Arnold, C. M., and Hochberg, M. C. (1989). Development and implementation of an immunization program for patients with rheumatoid arthritis. *Arthritis Care Res.* **2**, 162–164.
113. Abu-Shakra, M., Zalmanson, S., Neumann, L., Flusser, D., Sukenik, S., and Buskila, D. (2000). Influenza virus vaccination of patients with systemic lupus erythematosus: Effects on disease activity. *J. Rheumatol.* **27**, 1681–1685.
114. Gladman, D. D., and Urowitz, M. B. (1987). Morbidity in systemic lupus erythematosus. *J. Rheumatol.* **14**(Suppl. 13), 223–226.
115. Petri, M., Perez-Gutthann, S., Longenecker, J. C., and Hochberg, M. C. (1991). Morbidity of systemic lupus erythematosus: Role of race and socieconomic status. *Am. J. Med.* **91**, 345–353.
116. Petri, M., and Genovese, M. (1992). Incidence of and risk factors for hospitalizations in systemic lupus erythematosus: A prospective study of the Hopkins lupus cohort. *J. Rheumatol.* **19**, 1559–1565,
117. Gladman, D., Ginzler, E., Goldsmith, C., Fortin, P., Liang, M., Urowitz, M., Bacon, P., Bombardieri, S., Hanly, J., Hay, E., Isenberg, D., Jones, J., Nived, O., Petri, M., Richter, M., Sanchez-Guerrero, J., Snaith, M., Sturfelt, G., and Symmons, D. (1996). The development and initial validation of the SLICC/ACR damage index for SLE. *Arthritis Rheum.* **39**, 363–369.
118. Gladman, D. D., Urowitz, M. B., Goldsmith, C. H., Fortin, P., Ginzler, E., Gordon, C., Hanly, J., Isenberg, D., Kalunian, K., Nived, O., Petri, P., Sanchez-Guerrero, J., Snaith, M., and Sturfelt, G. (1997). The reliability of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index in patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 809–813.
119. Gladman, D. D., and Urowitz, M. B. (1999). The SLICC/ACR damage index: Progress report and experience in the field. *Lupus* **8**, 632–637.
120. Liang, M. H., Socher, S. A., Roberts, W. N., and Esdaile, J. M. (1988). Measurement of systemic lupus ervthematosus activity in clinical research. *Arthritis Rheum.* **31**, 817–825.
121. Urowitz, M. B., and Gladman, D. D. (1998). Assessment of disease activity and damage in SLE. *Baillière's Clin. Rheumatol.* **12**, 405–413.
122. Liang, M. H., Socher, S. A., Larson, M. G., and Schur, P. H. (1989). Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. *Arthritis Rheum.* **32**, 1107–1116.
123. Gladman, D. D., Goldsmith, C. H., Urowitz, M. B., Bacon, P., Bombardier, C., Isenberg, D., Kalunian, K., Liang, M., Maddison, P., Nived, O., Richter, M., Snaith, M., Symmons, D., and Zoma, A. (1992). Cross-cultural validation of three disease activity indices in systemic lupus erythematosus (SLE). *J. Rheumatol.* **19**, 608–611.
124. Gladman, D. D., Goldsmith, C. H., Urowitz, M. B., Bacon, P., Bombardier, C., Isenberg, D., Kalunian, K., Liang, M., Maddison, P., Nived, O., Petri, M., Richter, M., Snaith, M., Symmons, D., and Zoma, A. (1994). Sensitivity to change of 3 systemic lupus erythematosus disease activity indices: International validation. *J. Rheumatol.* **21**, 1468–1471.
125. Petri, M., Hellmann, D. B., and Hochberg, M. C. (1992). Validity and reliability of lupus activity measures in the routine clinic setting. *J. Rheumatol.* **19**, 53–59.
126. Engle, E. W., Callahan, L. F., Pincus, T., and Hochberg, M. C. (1990). Learned helplessness in systemic lupus erythematosus: Analysis using the rheumatology attitudes index. *Arthritis Rheum.* **33**, 281–286.
127. Hochberg, M. C., and Sutton, J. D. (1988). Physical disability and psychosocial dysfunction in systemic lupus erythematosus. *J. Rheumatol.* **15**, 959–964.
128. Gladman, D., Urowitz, M., Ong, A., and Gough, J. (1996). A comparison of five health status instruments in patients with systemic lupus erythematosus (SLE). *Lupus* **5**, 190–195.

129. Stoll, T., Gordon, C., Seifert, B., Richardson, K., Malik, J., Bacon, P. A., and Isenberg, D. A. (1997). Consistency and validity of patient administered assessment of quality of life by the MOS SF-36; its association with disease activity and damage in patients with systemic lupus erythematosus. *J. Rheumatol.* **24**, 1608–1614.
130. Wang, B. W. E., Gladman, D. D., and Urowitz, M. B. (1998). Fatigue in lupus is not correlated with disease activity. *J. Rheumatol.* **25**, 892–895.
131. Bruce, I. N., Mak, V., Hallett, D. C., Gladman, D. D., and Urowitz, M. B. (1999). Factors associated with fatigue in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **58**, 379–381.
132. Petri, M. (1998). The effect of race on incidence and clinical course in systemic lupus erythematosus: The Hopkins lupus cohort. *J. Am. Med. Womens Assoc.* **53**, 9–12.
133. Gladman, D. D., Urowitz, M., Fortin, P., Isenberg, D., Goldsmith, C., Gordon, C., and Petri, M. (1996). Workshop report: Systemic Lupus Erythematosus International Collaborating Clinics(SLICC) Conference on Assessment of Lupus Flare and Quality of Life Measures in SLE. *J. Rheumatol.* **23**, 1953–1955.
134. Masi, A. T., and Kaslow, R. A. (1978). Sex effects in systemic lupus erythematosus: A clue to pathogenesis. *Arthritis Rheum.* **21**, 480–484.
135. Roubinian, J. R., Talal, N., Greenspan, J. S., Goodman, J. R., and Siiteri, P. K. (1978). Effect of castration and sex hormone treatment on survival, antinucleic acid antibodies, and glomerulonephritis in NZB/NZW F mice. *J. Exp. Med.* **147**, 1568–1583.
136. Lahita, R. G. (1985). Sex steroids and the rheumatic diseases. *Arthritis Rheum.* **28**, 121–126.
137. Lahita, R. G., Bradlow, H. L., Fishman, J., and Kunkel, H. G. (1982). Estrogen metabolism in systemic lupus erythematosus: Patients and family members. *Arthritis Rheum.* **25**, 843–846.
138. Lahita, R. G., Bradlow, H. L., Ginzler, E. Pang, S., and New, M. (1987). Low plasma androgens in women with svstemic lupus erythematosus. *Arthritis Rheum.* **30**, 241–248.
139. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1979). Alterations of estrogen metabolism in svstemic lupus ervthematosus. *Arthritis Rheum.* **22**, 1195–1198.
140. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1981). Increased 16 alpha-hydroxylation of estradiol in systemic lupus erythematosus. *J. Clin. Endocrinol. Metab.* **53**, 174–178.
141. Lahita, R. G., Kunkel, H. G., and Bradlow, H. L. (1983). Increased oxidation of testosterone in systemic lupus ervthematosus. *Arthritis Rheum.* **26**, 1517–1521.
142. Jungers, P., Nahoul, K., Pelissier, C., Dougados, M., Tron, F., and Bach, J. F. (1982). Low plasma androgens in women with active or quiescent svstemic lupus ervthematosus. *Arthritis Rheum.* **25**, 454–457.
143. Miller, M. H., Urowitz, M. B., Gladman, D. D., and Killinger, D. W. (1983). Systemic lupus erythematosus in males. *Medicine* **62**, 327–334.
144. Lahita, R. G. (1996). The connective tissue diseases and the overall influence of gender. *Int. J. Fertil. Menopausal Stud.* **41**, 156–165.
145. Grimes, D. A., LeBolt, S. A., Grimes, K. R., and Wingo, P. A. (1985). Systemic lupus erythematosus and reproductive function: A case-control studv. *Am. J. Obstet. Gynecol.* **153**, 179–186.
146. Sanchez-Guerrero, J., Karlson, E. W., Liang, M. H., Hunter, D. J., Speizer, F. E., and Colditz, G. A. (1997). Past use of oral contraceptives and the risk of developing systemic lupus erythematosus. *Arthritis Rheum.* **40**, 804–808.
147. Sanchez-Guerrero, J., Liang, M. H., Karlson, E. W., Hunter, D. J., and Colditz, G. A. (1995). Postmenopausal estrogen therapy and the risk for developing systemic lupus erythematosus. *Ann. Intern. Med.* **122**, 430–433.
148. Sanchez-Guerrero J, Villegas, A., Mendoza-Fuentes, A., Romero-Diaz, J., Moreno-Coutino, G., and Cravioto, M. C. (2001). Disease activity during the premenopausal and postmenopausal periods in women with systemic lupus erythematosus. *Am. J. Med.* **111**, 464–468.
149. Phillips, P. E. (1981). The potential role of microbial agents in the pathogenesis of systemic lupus erythematosus. *J. Rheumatol.* **8**, 344–347.
150. Pincus, T. (1982). Studies regarding a possible function for viruses in the pathogenesis of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 847–856.
151. Boumpas, D. T., Popovic, M., Mann, D. L. Balow, J. E., and Tsokos, G. C. (1986). Type C retroviruses of the human T cell leukemia family are not evident in patients with systemic lupus erythematosus. *Arthritis Rheum.* **29**, 185–188.
152. Koike, T., Kagami, M., Takabayshi, K. Maruyama, N., Tomioka, H., and Yoshida, S. (1985). Antibodies to human T cell leukemia virus are absent in patients with systemic lupus erythematosus. *Arthritis Rheum.* **28**, 481–484.
153. Murphy, E. L., Jr, DeCeulaer, K., Williams, W., Clark, J. W., Saxinger, C., Gibbs, W. N., and Blattner, W. A. (1988). Lack of relation between human T-lymphotropic virus type I infection and systemic lupus erythematosus in Jamaica, West Indies. *J. Acquir. Immune Defic. Syndr.* **1**, 18–22.
154. McDougal, J. S., Kennedy, M. S., Kalyanaraman, V. S., and McDuffie, F. C. (1985). Failure to demonstrate (cross reacting) antibodies to human T lymphotropic viruses in patients with rheumatic diseases. *Arthritis Rheum.* **28**, 1170–1174.
155. James, J. A., Neas, B. R., Moser, K. L., Hall, T., Bruner, G. R., Sestak, A. L. *et al*. (2001). Systemic lupus erythematosus in adults is associated with previous Epstein-Barr virus exposure. *Arthritis Rheum.* **44**, 1122–1126.
156. Beaucher, W. N., Garman, R. H., and Condemi, J. J. (1977). Familial lupus erythematosus: Antibodies to DNA in household dogs. *N. Engl. J. Med.* **296**, 982–984.
157. Reinertsen, J. L., Kaslow, R. A., Klippel, J. H., Hurvitz, A. I., Lewis, R. M., Rothfield, N. F., Zvaifler, N. J., Steinberg, A. D., and Decker, J. L. (1980). An epidemiologic study

of households exposed to canine systemic lupus erythematosus. *Arthritis Rheum.* **23**, 564–568.
158. Hess, E. (1988). Drug-related lupus. *N. Engl. J. Med.* **318**, 1460–1462.
159. Freni-Titulaer, L. W. J., Kelley, D. B., Grow, A. G., McKinley, T. W., Arnett, F. C., and Hochberg, M. C. (1989). Connective tissue disease in southeastern Georgia: A case-control study of etiologic factors. *Am. J. Epidemiol.* **130**, 404–409.
160. Sanchez-Guerrero, J., Karlson, E. W., Colditz, G. A., Hunter, D. J., Speizer, F. E., and Liang, M. H. (1996). Hair dye use and the risk of developing systemic lupus erythematosus. *Arthritis Rheum.* **39**, 657–662.
161. Hochberg, M. C. (1987). The application of genetic epidemiology to systemic lupus erythematosus. *J. Rheumatol.* **14**, 867–869.
162. Lawrence, J. S., Martins, L., and Drake, G. (1987). A family survey of lupus erythematosus. 1. Heritability. *J. Rheumatol.* **14**, 913–921.
163. Block, S. R., Winfield, J. B., Lockshin, M. C., D'Angelo, W. A., and Christian, C. L. (1975). Studies of twins with systemic lupus erythematosus: A review of the literature and presentation of 12 additional sets. *Am. J. Med.* **59**, 533–552.
164. Arnett, F. C., and Shulman, L. E. (1976). Studies in familial systemic lupus erythematosus. *Medicine* **55**, 313–322.
165. Deapen, D., Escalante, A., Weinrib, L., Horwitz, D., Bachman, B., Roy-Burman, P., Walker, A., and Mack, T. M. (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 311–318.
166. Winchester, R. J., and Nunez-Rolden, A. (1982). Some genetic aspects of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 833–837.
167. Arnett, F. C., Reveille, J. D., Wilson, R. W., Provost, T. T., and Bias, W. B. (1984). Systemic lupus erythematosus: Current state of the genetic hypothesis. *Semin. Arthritis Rheum.* **14**, 24–35.
168. Bias, W. B., Reveille, J. D., Beaty, T. H., Meyers, D. A., and Arnett, F. C. (1987). Evidence that autoimmunity in man is a mendelian dominant trait. *Am. J. Hum. Genet.* **39**, 584–602.
169. Sullivan, K. E. (2000). Genetics of systemic lupus erythematosus: Clinical implications. *Rheum. Dis. Clin. North Am.* **26**, 229–256.
170. Koskenmies, S., Widen, E., Kere, J., and Julkunen, H. (2001). Familial systemic lupus erythematosus in Finland. *J. Rheumatol.* **28**, 758–760.
171. Giles, I., and Isenberg, D. (2001). Lupus in the family: Analysis of a cohort followed from 1978 to 1999. *Lupus* **10**, 38–44.
172. Michel, M., Johanet, C., Meyer, O., Frances, C., Wittke, F., Michel, C., Arfi, S., Tournier-Lasserve, E., and Piette, J. C. (2001). Familial lupus erythematosus: Clinical and immunologic features of 125 multiplex families. *Medicine* **80**, 153–158.
173. Tsao, B. P., Cantor, R. M., Kalunian, K. C., Chen, C. J., Badsha, H., Singh, R., Wallace, D. J., Kitridou, R. C., Chen, S. L., Shen, N., Song, Y. W., Isenberg, D. A., Yu, C. L., Hahn, B. H., and Rotter, J. I. (1997). Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J. Clin. Invest.* **99**, 725–731.
174. Moser, K., Neas, B. R., Bruner, G., Salmon, J., and Harley, J. B. (1998). Scanning of the genome for human SLE genes design and candidate regions. *Lupus* 7(Suppl. 1), 13.
175. Moser, K. L., Neas, B. R., Salmon, J. E., Yu, H., Gray-McGuire, C., Asundi, N., Bruner, G. R., Fox,J., Kelly, J., Henshall, S., Bacino, D., Dietz, M., Hogue, R., Koelsch, G., Nightingale, L., Shaver, T., Abdou, N. I., Albert, D. A., Carson, C., Petri, M, Treadwell, E. L., James, J. A., and Harley, J. B. (1998). Genome scan of human systemic lupus erythematosus: Evidence for linkage on chromosome 1q in African-American pedigrees. *Proc. Na. Acad. Sci. USA* **95**, 14869–14874.
176. Johanneson, B., Steinsson, K., Lindqvist, A. K., Kristjansdottir, H., Grondal, G., Sandino, S., Tjernstrom, F., Sturfelt, G., Granados-Arriola, J., Alcocer-Varela, J., Lundberg, I., Jonasson, I., Truedsson, L., Svenungsson, E., Klareskog, L., Alarcon-Segovia, D., Gyllensten, U. B., and Alarcon-Riquelme, M. E. (1999). A comparison of genome-scans performed in multicase families with systemic lupus erythematosus from different population groups. *J. Autoimmun.* **13**, 137–141.
177. Shai, R.,Quismorio, F. P., Jr., Li, L., Kwon, O. J., Morrison, J., Wallace, D. J., Neuwelt, C. M., Brautbar, C., Gauderman, W. J., and Jacob, C. O. (1999). Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum. Mol. Genet.* **8**, 639–644.
178. Gaffney, P. M., Ortmann, W. A., Selby, S. A., Shark, K. B., Ockenden, T. C., Rohlf, K. E., Walgrave, N. L., Boyum, W. P., Malmgren, M. L., Miller, M. E., Kearns, G. M., Messner, R. P., King, R. A., Rich, S. S., and Behrens, T. W. (2000). Genome screening in human systemic lupus erythematosus: Results from a second Minnesota cohort and combined analyses of 187 sib-pair families. *Am. J. Hum. Genet.* **66**, 547–556.

24

OVERLAP SYNDROMES

Mixed Connective Tissue Disease and Sjögren's Syndrome

Robert W. Hoffman

CONCEPT OF OVERLAP SYNDROME

Over recent decades we have developed increased recognition of the distinctive nature of seemingly similar connective tissue diseases (CTDs). Many distinct entities are now recognized that were first unified under the broad category of "collagen disease" in the seminal work of Klemperer *et al*. [1]. Most recently, important advances in the characterization of autoantibodies and immunogenetics of CTD have helped to substantially increase our understanding of these diseases. Despite these advances, controversy and confusion remain over the classification of many rheumatic diseases. Classification criteria have been proposed for both mixed connective tissue disease (MCTD) [2–4] and Sjögren's syndrome [5–7], but there are no uniformly accepted classification criteria for either. Furthermore, there remain patients with rheumatic diseases who fail to meet any standardized classification criteria. These patients are often designated as having "undifferentiated connective tissue disease" (UCTD). Over time these patients may evolve to meet existing classification criteria for one CTD, such as systemic lupus erythematosus (SLE), MCTD, or Sjögren's syndrome, or they may remain unclassifiable, and therefore remain labeled UCTD. Finally, additional groups of patients meet the classification criteria for more than one disease entity, such as SLE and rheumatoid arthritis (RA) or SLE and systemic sclerosis (SSc). These patients are also considered part of the spectrum of the overlap syndrome.

Immunogenetic markers and autoantibodies have proven to be useful in disease classification. Investigators have combined the use of both with success. As shown in Table 1, many selective immunogenetic associations have been reported with the presence of specific autoantibodies. Population-based immunogenetic studies have shown association of the presence of antinuclear ribonucleoprotein (RNP) antibodies with human leukocyte antigen (HLA)-DR4 [8, 9]. We have demonstrated that the presence of autoantibodies against one specific polypeptide of the RNP complex, U1-70-kDa, is independently associated with select genotypes of HLA-DR4 and HLA-DR2 [10, 11]. Structural analysis of HLA genes demonstrated that this association could be mapped to a shared sequence in the highly polymorphic antigen-binding segment of the HLA-DRB1 gene [11]. This HLA epitope was shared among all of the disease-associated HLA-DRB1 alleles. When the clinical features of these patients (selected based on the presence of the anti-U1-70-kDa antibody) were analyzed, the results come full circle by finding that these patients exhibited clinical features characteristic of MCTD [10].

In Sjögren's syndrome, studies on anti-Ro/SSA and anti-La/SSB autoantibodies have demonstrated strong association between these autoantibodies and the presence of specific HLA genes. The presence of anti-Ro/SSA and anti-La/SSB autoantibodies was associated with both HLA-DR and HLA-DQ alleles [12–15]. The highest anti-Ro/SSA antibody levels occur in patients heterozygous for the presence of two HLA-DQ

TABLE 1 Production of Specific Autoantibodies Is Associated with Distinctive HLA Phenotypes or Genotypes[a]

Disease	Autoantibody specific	Immunogenetic association
MCTD	RNP[b]	HLA-DR4
SLE	DNA	HLA-DR2, DQw1
CTD	RNP	HLA-DR4
CTD	RNP/Sm	HLA-DR4
CTD	U1-70 kDa[c]	HLA-DR4/DRB1*0401, *0404, *0407, *0408 HLA-DR2/DRB1*1501
Sjögren's syndrome	Ro/SSA	HLA-DQw1/DQw2, HLA-DR3, HLA-DR2
SSc	Centromere Scl-70/topoisomerase I	HLA-DR1, DR4(w13), DRw8; HLA-DR5 (w11) HLA-DR5(w11)/DRB1*1104; HLA-DPB1*0201, *0601, *1001, *1301
Polymyositis	Jo-1/transfer RNA synthetase	HLA-DR3

[a] CTD, connective tissue disease; HLA, human leukocyte antigen; MCTD, mixed connective tissue disease; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; Sm, Smih antigen; SSc, systemic sclerosis. Data from Hoffman and Sharp [120].
[b] Nuclear RNP or U1 RNP.
[c] A polypeptide of the U1 RNP complex.

alleles: HLA-DQw1 and HLA-DQw2 [13]. Similar immunogenetic associations have been reported for a variety of autoantibodies in other rheumatic diseases, as shown in Table 1. These studies, combining characterization of patients by autoantibodies and immunogenetics, provide a valuable new framework for disease classification.

Despite advances in defining new autoantibodies and characterizing the immunogenetics of CTDs, inherent problems remain in classifying such diseases with pleomorphic clinical manifestations and unknown etiology. We have been reminded, with the discovery of Lyme disease, that a disease caused by a single etiologic agent can result in many different clinical manifestations. In the case of Lyme disease, symptoms can range from arthritis to skin rashes to carditis to central nervous system disease. Approaching the problem of disease classification from the opposite point of view can be equally difficult. A single clinical syndrome, such as polyarthritis, can have many different etiologies. The results of disease classification studies are also influenced by additional factors, such as ascertainment bias in data collection and the background genetics of the population being studied. Until the etiology or etiologies of SLE, MCTD, Sjögren's syndrome and the overlap syndromes are proven, classification will remain problematic and open to dispute. Classification does, however, provide a valuable framework for research, treatment, and prognosis. To that end, the fact that MCTD and Sjögren's syndrome can be classified by clinical, serologic, and, more recently, distinctive immunogenetic characteristics supports the concept that such classification serves useful purposes.

MIXED CONNECTIVE TISSUE DISEASE

Historical Overview

The initial observations on MCTD were made by Sharp *et al.* [16] in a rheumatic disease clinic at Stanford University during 1961–1969. In this clinic, a complement fixation test for antinuclear autoantibodies (ANA) was used in monitoring patients' response to therapy. In patients with typical SLE, their ANA titers declined and usually became negative during clinical remission. In contrast, some interesting patients were noted to have extraordinarily high titers of ANA that persisted during periods of both active disease and clinical remission. The clinical characteristics of these patients did not fit any of the traditional rheumatic disease classifications but combined features of SLE, polymyositis, and SSc and therefore were referred to as MCTD.

In order to further elucidate the autoantibody pattern in these MCTD patients, a very sensitive passive hemagglutination (PHA) test was developed that revealed that their sera had extremely high titers (often >1:1,000,000) of antibodies to an extractable nuclear antigen (ENA) described previously by Sharp *et al.* [17]. Using the PHA test, enzyme digestion studies further revealed that these antibodies were directed against an RNase- and trypsin-sensitive component of the crude ENA. Northway and Tan [18] performed fluorescent ANA (FANA) analyses on these MCTD sera and found that they produced speckled patterns at high titers that were eliminated by RNase treatment of the tissue

sections. Thus, the circulating antibody in MCTD appeared to have a specificity for a nuclear RNP (nRNP) antigen. In comparison, PHA testing revealed that 50% of the sera of typical SLE patients in this clinic also contained antibodies to ENA that were usually present at a lower titer and frequently were directed against an RNase-resistant component of ENA [17]. Antibodies to the Smith (Sm) antigen and native DNA, known serologic markers for SLE, were seldom detected in these MCTD patients. Because of the similar clinical and serologic features in this group of patients, it was proposed that MCTD might represent a distinct rheumatic disease syndrome [16].

In the early 1970s, reports from several laboratories indicated that Ouchterlony double immunodiffusion can also be used to detect autoantibodies reacting with ENA by the PHA method [19–21]. Thus it became clear that the crude antigenic complex referred to as ENA contains at least two immunologically distinct components: the RNase- and trypsin-sensitive RNP antigen and the RNase- and trypsin-resistant Sm antigen. The Ouchterlony method showing lines of identity with immunoprecipitation bands produced by well-characterized reference sera provides a very precise identification of the specificity of RNP and Sm antibodies; however, PHA with titers before and after RNase digestion of ENA can be useful because of its great sensitivity (100,000-fold more sensitive that the Ouchterlony and 100- to 1000-fold more sensitive than the FANA) [21]. Thus, the FANA may not be adequate as a screening test very early in the course of MCTD when antibody levels may be lower [22].

Following the initial report of MCTD, studies in several centers agreed that the serologic pattern of high titers of RNP antibodies in the absence of Sm antibodies is usually associated with MCTD and is much less common in SLE, is infrequent in SSc, and is almost never seen in other rheumatic diseases [19, 21, 23, 24]. In contrast, Sm antibodies, usually in association with lower titers of RNP antibodies, are seen most commonly in SLE [21, 23].

Clinical Features

General Features

In recent years, the fact that patients are coming to medical attention earlier and being observed longitudinally has led to a greater appreciation of the gradual evolution of MCTD over time. Early in the course of the disease, patients are likely to have only Raynaud's phenomenon, fatigue, swollen hands, and myalgias and arthralgias, findings that do not fulfill any of the CTD classification criteria (sometimes referred to as UCTD).

TABLE 2 Transitions in MCTD in Longitudinal Study

Disease classification	No. classified at initial medical evaluation	No. classified at latest medical evaluation
MCTD	14	31
UCTD	7	3
SLE	6	0
Adult or juvenile RA	4	0
SSc	2	0
Polymyositis	1	0

This mild syndrome may persist for years in some, but others develop additional manifestations, leading to a diagnosis of MCTD [24–29]. Thus, retrospective reviews of medical records and cross-sectional studies may not provide an accurate assessment of this evolving CTD. In the initial prospective longitudinal study at the University of Missouri–Columbia, 60% of 34 patients with high anti-RNP antibody titers on entering the study had more limited clinical manifestations and were diagnosed by their physicians as UCTD, SLE, RA, SSc, or polymyositis (Table 2) [28]. However, over a period of years, 91% had developed clinical features typical of MCTD, whereas only 9% remained undifferentiated. In another prospective long-term study in Sweden [27], 17 of 23 patients with high titers of RNP antibodies had developed sufficient clinical manifestations to fulfill criteria for MCTD at the end of the study, whereas 17 of 23 had not fulfilled any CTD criteria on initial evaluation. Less frequently MCTD may have an acute onset with high fever, inflammatory myositis, serositis, arthritis, aseptic meningitis, or digital gangrene, and the typical overlapping disease is fully expressed when the patients first come to medical attention [16, 24, 28–30].

In a national epidemiologic survey in Japan, MCTD had a prevalence of 2.7 per 100,000 compared with 20.9 for SLE, 5.7 for scleroderma, and 4.9 for polymyositis-dermatomyositis [31]. The female-to-male ratio of MCTD was 16:1.

In our clinic, which is likely to be biased toward referral of MCTD patients, MCTD occurs less frequently than SLE but occurs as often as SSc and more frequently than polymyositis. MCTD typically presents when patients are between 20 and 30 years of age and the majority of these patients are female [28, 29]. There is a report in the literature of MCTD occurring in siblings but in our experiences it is very uncommon in families (unpublished results) [32].

The most common clinical features of MCTD in our longitudinal studies [28, 29] were Raynaud's phenome-

TABLE 3 Characteristics of Patients with MCTD

Characteristic	Number of patients	(%)
Clinical		
Raynaud's phenomenon	31	91
Polyarthritis	29	85
Swollen hands or sclerodactyly	29	85
Pulmonary disease	29	85
Inflammatory myositis	27	79
Esophageal hypomotility	25	74
Lymphadenopathy	17	50
Alopecia	13	41
Pleuritis	12	35
Malar rash	10	29
Pulmonary hypertension	10	29
Renal disease	9	26
Cardiac disease	9	26
Anemia	8	24
Leukopenia	7	21
Diffuse scleroderma	7	21
Sjögren's syndrome	4	12
Trigeminal neuropathy	2	6
Laboratory		
Positive FANA	34	100
Positive anti-U1 RNP	34	100
Positive rheumatoid agglutinins	20	59
Hypergammaglobulinemia	18	53
Hypocomplementemia	11	32

non, polyarthritis, swollen hands or sclerodactyly, pulmonary disease, inflammatory myositis, and abnormal esophageal motility (Table 3). Less frequent findings included lymphadenopathy, alopecia, malar rash, pleuritis, pulmonary hypertension, cardiac disease, renal disease, anemia, and leukopenia. The very high frequency of pulmonary disease detected in the study by Sullivan *et al*. [28] had not been noted in our initial report of MCTD, perhaps because the fact that significant pulmonary dysfunction can occur even in asymptomatic patients was not appreciated at that time. The occurrence of pulmonary hypertension as the most frequent disease-associated cause for death was documented in a longitudinal study of clinical outcomes in MCTD [29].

At the 1986 International Symposium on MCTD and Antinuclear Antibodies, three sets of diagnostic criteria for classification of MCTD were proposed (Table 4) [2–4]. Major criteria included Raynaud's phenomenon, synovitis, swollen hands or sclerodactyly, myositis, pulmonary disease, and high-titer anti-RNP antibody. Preliminary evaluations of these criteria have shown a very high sensitivity and specificity [33–35]. It should be noted, however, that advances describing more precise autoantibody (anti-U1-RNP-70-kDa polypeptide and anti-U1 RNA) and immunogenetic specificities that are linked extremely closely with MCTD (see later) were not fully elucidated when these diagnostic criteria were proposed. More recently, a strong linkage has been demonstrated among Raynaud's phenomenon, synovitis, swollen hands or sclerodactyly, myositis, and esophageal dysfunction with anti-U1-70-kDa polypeptide serologic reactivity [10, 11, 29].

Occasionally, patients with MCTD transiently develop an illness while maintaining their serum anti-RNP antibodies that resembles typical SLE with the appearance of antibodies to DNA and/or Sm, higher levels of circulating immune complexes, lupus-like skin lesions, and glomerulonephritis. These findings were present in 4 MCTD patients who had defective reticuloendothelial system (RES) clearance of immune complexes but not in 14 MCTD patients with normal RES clearance and lower levels of circulating immune complexes [36]. Possibly normal RES function in most MCTD patients permits effective clearance of immune complexes and precludes serious renal disease. In the follow-up study of the originally reported MCTD patients, only 1 patient had developed membranous glomerulonephritis [37]. In a longitudinal study, only 18% had clinical evidence of renal disease and no patients developed renal failure [28]. In a subsequent study with follow-up as long as 25 years, 11% of MCTD patients had renal disease [29]. In the Swedish prospective study of MCTD, only 1 patient had glomerulonephritis, in association with a rising anti-DNA titer and a falling anti-RNP titer [27].

In contrast to SLE, where renal and central nervous system disease are the most frequent serious disease complications, pulmonary hypertension associated with proliferative vasculopathy is the most frequent cause of disease-related deaths in MCTD [28, 29, 38–40]. In a longitudinal study, 38% of MCTD patients had pulmonary hypertension [29]. It appears that MCTD patients with high anti-RNP titers and patients with limited SSc (or CREST) with anticentromere antibodies are at a greater risk for developing pulmonary hypertension and proliferative vascular lesions with minimal pulmonary fibrosis than patients with classical SLE, SSc, and polymyositis [28, 29, 40–44]. Pulmonary hypertension in diffuse SSc is usually associated with severe interstitial pulmonary fibrosis [43]. In a study of SLE patients who developed pulmonary hypertension [45], anticardiolipin antibodies were present in 68% and anti-RNP antibodies in 21%. It is not clear, however, what percentage of the patients classified as RNP-positive "SLE" would also have met classification criteria for MCTD in this study. Clearly, there appears to be linkage between anti-RNP and anticardiolipin antibodies and an increased risk for developing pulmonary hypertension [29].

TABLE 4 Diagnostic Criteria for MCTD[a]

Criteria defined by Sharp [4]		Criteria defined by Kasukawa *et al*. [3]	Criteria defined by Alarcón-Segovia and Villarreal [2]
A. Major criteria 1. Myositis, severe 2. Pulmonary involvement with one or more of a. DLCO < 70% normal b. Pulmonary hypertension c. Proliferative vascular 3. Raynaud's phenomenon or esophageal hypomotility 4. Swollen hands observed or sclerodactyly 5. Highest observed anti-ENA (PHA) ≥ 1 10,000 and anti-U1 RNP +, anti-Sm –	B. Minor criteria 1. Alopecia 2. Leukopenia 3. Anemia 4. Pleuritis 5. Pericarditis 6. Arthritis 7. Trigeminal neuropathy 8. Malar rash 9. Thrombocytopenia 10. Myositis, mild 11. History of swollen hands	I. Common symptoms A. Raynaud's phenomenon B. Swollen fingers or hands II. Anti-U1 RNP III. Mixed findings A. SLE-like findings 1. Polyarthritis 2. Lymphadenopathy 3. Facial erythema 4. Pericarditis or pleuritis 5. Leukopenia or Thrombocytopenia B. SSc-like findings 1. Sclerodactyly 2. Pulmonary fibrosis, restrictive change of lung, or reduced DLCO 3. Hypomotility or dilation or esophagus C. PM-like findings 1. Muscle weakness 2. Increased serum Creatine kinase 3. Myogenic pattern in EMG	1. Serological + anti-U1 RNP, PHA titer ≥ eq 1:1600 2. Clinical a. Edema of the hands b. Synovitis c. Myositis d. Raynaud's phenomenon e. Acrosclerosis
Requirements for diagnosis of MCTD			
Definite Four major criteria filled Anti-U1 RNP ≥ 1:4000 by PHA	Probable 1. Three major criteria filled 2. Two major criteria from 1, 2, and 3 and two minor criteria with anti-U1 RNP ≥ 1:1000 (PHA)	1. Positive in either one or two common symptoms 2. Positive anti-U1 RNP 3. Positive in one or more findings in two or three disease categories of A, B, and C	1. Serological 2. At least three clinical findings 3. The association of edema of the hand Raynaud's phenomenon, and acrosclerosis requires the addition of at least one of the other two clinical criteria

[a] DLCO, diffusing capacity for carbon monoxide; ENA, extractable nuclear antigen; EMG, electromyogram; PHA, passive hemagglutination; PM, polymyositis; RNP, ribonucleoprotein; SLE, systemic lupus erythematosus; SSc, systemic sclerosis. From Sharp, [4], Kasukawa *et al*. [3], and Alarcón-Segovia and Villareal [2], with permission.

The follow-up report of the original 25 MCTD patients [37] has been cited as showing evidence of a transition of MCTD to classical SSc. In subsequent more recent studies of larger groups of MCTD patients, diffuse sclerosis developed in combination with other disease features in 19% of the MCTD patients [29]. In the prospective longitudinal study of Lundberg and Hedfors [27] of 32 patients with RNP antibodies, none of them fulfilled criteria for SSc. Thus, it appears that MCTD infrequently spontaneously evolves into diffuse SSc. Interestingly, work has suggested that patients with antibodies against the oxidatively modified form of the U1-70-kDa polypeptide are more likely to have Raynaud's phenomenon and scleroderma-spectrum illness, whereas patients with antibodies against the apoptotically cleaved form of the U1-70-kDa polypeptide are more likely to have lupus skin disease and a lupus-like clinical spectrum of disease [46].

MCTD can be distinguished from polymyositis and other polymyositis-scleroderma overlap syndromes by clinical and serologic features. The diagnostic criteria of Bohan and Peter were used to classify myositis patients as idiopathic myositis ($n = 10$), myositis associated with another CTD ($n = 13$), and dematomyositis ($n = 6$) in a longitudinal, 10-year study from Sweden [47]. Seven patients who had anti-RNP antibodies all were in the category of myositis associated with other CTD features and fulfilled criteria for MCTD [33]. In these patients, myositis appeared during the course of the disease and was rarely the initial manifestation. The other six patients with myositis associated with other CTD features all had a myositis-scleroderma overlap and were

negative for anti-RNP antibodies. PM-1 (PM-Scl) and Jo-1 autoantibodies are markers for polymyositis and a polymyositis-scleroderma overlap syndrome that may resemble MCTD [48, 49]. However, these patients are almost always negative for anti-RNP antibodies and do not manifest other SLE-like features of MCTD.

Specific Manifestations

Mucocutaneous Abnormalities

Raynaud's phenomenon is the most frequent finding in MCTD and may precede other disease manifestations by months or years [16, 19, 21, 24, 25, 29, 50]. Swollen hands and fingers leading to sausage-shaped digits (Fig. 1) are seen in over two-thirds of MCTD patients [16, 19, 21, 24, 25, 50]. Ischemic necrosis or ulcerations of the fingertips are much less common than in SSc [16, 27, 29]. Initial observations that early edematous sclerodermatous changes resolve completely in some patients [16] and observations in a more recent longitudinal study that diffuse scleroderma was transient and sclerodactyly fluctuated in its severity in MCTD [27] contrast with the usual progressive course of the disease in SSc.

About one-third of patients have lupus-like malar rashes and chronic discoid lesions [16, 21, 24, 25, 28, 29, 50]. Other findings include "squared" telangiectasia over the hands and face, periungual telangiectasia, calcinosis cutis, erythema over the knuckles, and violaceous discoloration of the eyelids resembling dermatomyositis, nonscarring alopecia, and buccal and orogenital ulceration. Skin biopsies have shown edema and increased dermal collagen [16]. Direct immunofluorescent examinations of skin biopsies in MCTD patients have shown deposits of immunoglobulins (Ig) at the dermal–epidermal junction [16, 25] and speckled intranuclear immunofluorescence of epidermal cells.

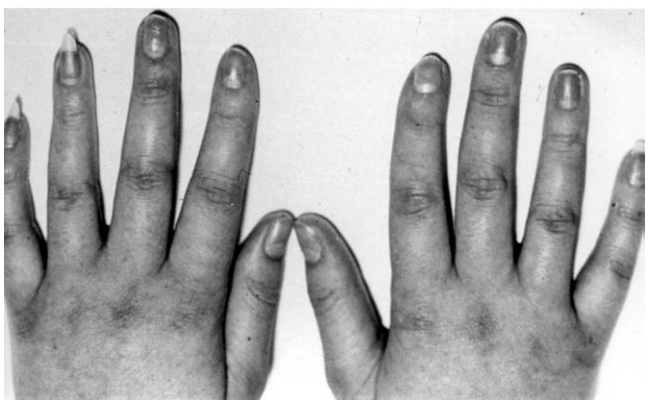


FIGURE 1 Hands of a patient with MCTD demonstrating the tapered or sausage appearance of the fingers and discoloration over the metacarpal phalangeal, proximal interphalangeal, and distal interphalangeal joints.

Joints

Most MCTD patients early in their disease experience joint stiffness and polyarthralgias, and three-quarters of them develop objective arthritis [16, 21, 24, 55]. Typically the arthritis is nondeforming, but it may occasionally resemble RA with ulnar deviation, boutonniere, and swan neck deformities [16, 28, 29, 51–55]. In some studies, rheumatoid-like changes were present in 30–35%, but in contrast to RA, joint involvement was usually limited to the hands and wrists. Although erosive disease is not common, radiographs may show small, marginal erosions, and even a more destructive arthritis including rib erosions has been reported [51–55]. Subcutaneous rheumatoid-like nodules are noted infrequently [16]. Hand deformities in MCTD may occur and result from a flexor tenosynovitis [56]. Rheumatoid factor is frequently present in MCTD (see later).

Muscles

Inflammatory myositis with proximal muscle weakness with or without muscle tenderness is sometimes a severe clinical manifestation of MCTD [16, 21, 28, 29, 57]. Elevated serum levels of creatine kinase and aldolase, myopathic electromyograms (EMGs) and biopsy findings of muscle fiber degeneration, and inflammatory infiltrates resembling polymyositis may be seen. Muscle enzyme levels and EMGs may sometimes be abnormal early in the course of the disease when muscle weakness is minimal. In such patients, histochemical and immunofluorescent analyses of muscle biopsies may reveal perifascicular atrophy, type I fiber predominance, and immunoglobulin deposition within normal-appearing muscle fibers, around or on the sarcoplasmic membrane, or within the perimysial connective tissue [57]. A longitudinal study comparing myositis in MCTD patients positive for anti-RNP antibodies with idiopathic polymyositis and dermatomyositis in patients negative for anti-RNP antibodies noted that the MCTD group had less severe histopathologic changes in their muscle biopsies and more rapid improvement of muscle strength on lower doses of corticosteroids [47]. In a longitudinal study, 51% of patients cumulatively had muscle involvement [29].

Gastrointestinal Tract

Any area of the gastrointestinal tract may be affected by MCTD, although the esophagus is by far the most common location. In a study of 61 patients with MCTD, the first 34 being part of a prospective longitudinal study that included systemic and periodic manometric and

radiographic evaluation of the esophagus, lower esophageal sphincter (LES) pressure and amplitude of peristaltic pressures in the distal esophagus were significantly less than in normal controls [58]. Of the MCTD patients undergoing manometry, 17% had aperistalsis and 43% had low-amplitude peristalsis of the distal esophagus. Of particular note, among patients with abnormal peristalsis, 24% had no esophageal symptoms and 33% had normal cine esophagrams. Heartburn and dysphagia were the most common gastrointestinal symptoms in this study. A statistically significant improvement in LES pressure was observed in 10 MCTD patients who received corticosteroid therapy (mean duration 67 weeks, average dose 25 mg/day) for severe multisymptom involvement. Mean upper esophageal sphincter (UES) pressures were also significantly lower in these MCTD than in normal controls; abnormal UES function may be less frequent in SSc and may represent a distinguishing feature of MCTD [58]. In an MCTD patient whose disease exacerbation was characterized solely by marked esophageal dysfunction and recurrent aspiration pneumonia, corticosteroid therapy was associated with improvement in UES and LES function and resolution of aspiration pneumonia episodes [58]. In another study comparing esophageal motility in SLE and MCTD, 91% of 17 patients with MCTD and only 20% of 14 patients with SLE had manometric abnormalities [59]. Use of proton pump inhibitors has shown that these are very effective for esophageal symptoms in MCTD.

Other much less frequent gastrointestinal manifestations in MCTD have included intestinal hypomotility, dilatation, malabsorption, sclerosis, serositis, small bowel and colon perforations due to vasculitis, pseudodiverticula, pneumatosis cystoides intestinalis, secretory diarrhea, mesenteric vasculitis with bowel hemorrhage, chronic active hepatitis, Budd–Chiari syndrome, acute pancreatitis, and pancreatic pseudocyst [24, 28, 29, 53, 60–62].

Lungs

Pulmonary involvement was not appreciated in the initial report on MCTD [16], probably because of the fact that pulmonary dysfunction is usually asymptomatic in the early phases of MCTD and may be initially undetected unless sought by testing, such as by pulmonary function tests (PFTs) [28, 29]. It has now become apparent that pulmonary involvement is common in MCTD and may result in seriously compromised function, leading to exertional dyspnea and, in some cases, pulmonary hypertension, particularly in the later stages of the disease [28, 29 42, 63, 64]. In a prospective longitudinal study of 47 patients with MCTD [29], 66% had evidence of pulmonary disease, whereas in an earlier study of 34 patients [28], 85% had pulmonary disease. The most frequent clinical findings included dyspnea in 58%, bibasilar rales in 42%, and pleuritic chest pain in 40%. However, pulmonary involvement was also detected in 8 of 11 patients (73%) who had no pulmonary symptoms. Pulmonary function tests were the most sensitive indicators of pulmonary disease, particularly the single-breath diffusing capacity for carbon monoxide (DLCO) that was abnormal (22–69% of predicted) in 72%. In addition, small irregular opacities involving predominantly the bases and middle regions of the lungs were noted in 30% of chest roentgenograms. Following treatment of their multisystem disease with corticosteroids and/or cyclophosphamide, over half of those with abnormal PFTs who were serially studied had improvement in their PFTs and 45% had improvement of dyspnea on exertion for a measured distance. Diaphragmatic dysfunction and pulmonary hemorrhage have occasionally been reported in MCTD [65–68].

Right heart catheterization was performed in 15 of 34 patients in one longitudinal study of MCTD from the University of Missouri [28]; 10 had increased pulmonary artery pressure (31/12–88/39, mean 59/26 mm Hg) and elevated pulmonary vascular resistance (2.2–12.2, mean 6.0 units), but pulmonary wedge pressure was abnormal in only 1 patient. The high frequency in our center may reflect a biased referral of more complicated patients. Severe capillary loop changes similar to those seen on nailfold capillaroscopy in SSc and a progressive decline in DLCO may identify MCTD patients who are at a greater risk for developing pulmonary hypertension [28]; only 1 of 10 patients with pulmonary hypertension had a normal DLCO. In contrast to diffuse SSc in which pulmonary hypertension is usually associated with interstitial pulmonary fibrosis, in MCTD pulmonary hypertension is usually associated with proliferative vascular abnormalities with marked narrowing of the lumen of small- and medium-sized pulmonary arteries and arterioles, but without significant interstitial fibrosis [28, 29, 40–44] (Fig. 2). The presence of antiphospholipid or anticardiolipin antibodies has been found to be associated with pulmonary hypertension in some studies [29], although not in others [69]. Death from pulmonary hypertension may have biased the selection against an inclusion of patients who will develop pulmonary hypertension in some studies [69]. Early pulmonary hypertension in MCTD may be treated successfully, and pulmonary hypertension is the most common disease-associated cause of death in MCTD. Thus, it is important to identify and treat early pulmonary disease and pulmonary hypertension in MCTD before major dysfunction develops. It appears that the presence of antiphospholipid or anticardiolipin anti-

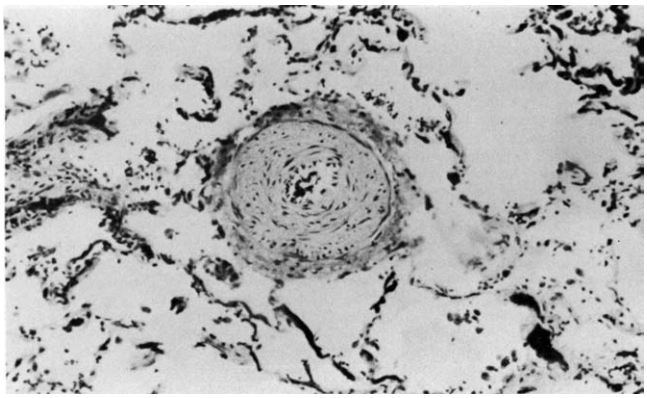


FIGURE 2 Small pulmonary artery in an 18-year-old woman with MCTD showing intimal proliferation and smooth muscle hypertrophy without accompanying inflammation or pulmonary fibrosis, associated with severe pulmonary hypertension. From Alpert *et al.* [145], with permission of the American Heart Association.

bodies may be a marker for increased risk of this complication in MCTD [29].

Heart

Pericarditis, usually responsive to corticosteroid therapy, has been reported to occur in 10–43% of patients with MCTD and may be more frequent in children than in adults [16, 24, 26, 44, 70–72]. Myocarditis has been recognized with increasing frequency and may be due to inflammatory cell infiltrates and/or proliferative vasculopathy of the epicardial and intramural coronary arteries [41, 44, 70–73]. Mitral valve prolapse was detected in 32% of patients with MCTD, 36% of patients with SLE, and 32% of patients with SSc compared with 10% in normal controls in an echocardiographic study [74]. Electrocardiographic abnormalities in MCTD have included conduction disturbances, arrhythmias, and chamber enlargement/hypertrophy [70].

Kidneys

Renal disease occurs in approximately 25% of MCTD patients and may be more frequent in children [24, 27, 28, 29, 39, 75–77]. Often it is asymptomatic. The most prevalent lesion is membranous glomerulonephritis, which can result in the nephrotic syndrome. Of has been reported that renal abnormalities in MCTD respond to treatment with corticosteroids [28, 29]. Focal glomerulonephritis can also occur in MCTD, whereas diffuse proliferative glomerulonephritis is rare [75]. The presence of antibodies against the Sm-D polypeptide and the HLA-DR2 phenotype is, however, a marker of severity for renal disease in MCTD [77, 78]. Proliferative vascular lesions similar to those occurring in the lungs and other tissues may be found in the kidneys. These proliferative vascular lesions have been associated with renovascular hypertension.

Nervous System

About 10% of patients with MCTD have had neurological involvement [21, 24, 27–30, 79], although there was a higher frequency in two reports [27, 79]. The most frequent neurological problem is trigeminal neuropathy [21, 80–82]. Vascular headaches are also relatively common [83]. Aseptic meningitis, which can occur in MCTD [80] must be distinguished from bacterial meningitis, which can occur unexpectedly in patients who appear otherwise healthy and are not on immunosuppressive medications [84]. Organic mental syndromes, encephalopathy, and seizures are less frequent than in SLE [16, 24, 71, 79]. Other neurologic manifestations of MCTD have included peripheral neuropathies, cauda equina syndrome, transverse myelitis, retinal vasculitis, and cerebral hemorrhage or infarction [16, 24, 71–79, 85–88].

Hematologic Findings

About one-third of patients with MCTD have anemia and leukopenia [16, 21, 28, 29]. The anemia is usually consistent with the anemia of chronic inflammation; although the Coombs' test may be positive, a frank hemolytic anemia is rare [16, 89]. Severe thrombocytopenia appears to be more common in children that in adults with MCTD [16, 21, 24, 90]. In one report of 14 children with MCTD, 6 had severe thrombocytopenia with 2 requiring splenectomy, and 1 died of an intracranial hemorrhage [24]. Red cell aplasia and thrombotic thrombocytopenia are rarely seen in MCTD [89, 90–92].

Miscellaneous Features

Sjögren's syndrome may occur in conjunction with MCTD [93, 94]. It has been reported that secondary Sjögren's syndrome occurred in 42% of a well-characterized population of 55 MCTD patients [93]. In contrast to primary Sjögren's syndrome, even massively enlarged lymph nodes in MCTD when biopsied have revealed only lymphoid hyperplasia and not lymphoma [16]. Fever and lymphadenopathy occur in about one-third of MCTD patients [16, 21, 25]. Persistent hoarseness and Hashimoto's thyroiditis are occasionally observed in MCTD. There is limited information on pregnancy in MCTD; one study showed a high risk of fetal loss and disease exacerbation similar to SLE, whereas two studies showed only a slight risk of fetal loss or maternal disease flares [30, 95, 96].

Laboratory Features

Polyclonal hypergammaglobulinemia is present in the majority of patients with MCTD [16, 21, 28, 29]. Rheumatoid agglutinins, often at very high levels, are detected in over 50% of MCTD patients. False-positive Venereal Disease Research Laboratory (VDRL) tests occur in about 10% of patients, with anticardiolipin antibodies also detected in some of these patients [38, 69]. In a comparative study in the author's laboratory, 41% of 59 SLE patients and 15% of 48 MCTD patients had antiphospholipid (anticardiolipin) antibodies; there was an association with 26 clotting events or other features of the antiphospholipid syndrome in the SLE patients, but these clinical complications were not noted in the MCTD patients [69]. Antiphospholipid antibodies may, however, be associated with pulmonary hypertension [29]. In a long-term outcome study, when pulmonary hypertension was present in 38% of the population studied, anticardiolipin antibodies did appear to be linked to pulmonary hypertension [29]. Antilymphocyte antibodies are noted much less frequently in MCTD than in SLE [97]. Circulating immune complexes have been noted in active MCTD [98], but their levels are generally lower and their RES clearance is reported to be less prolonged than in active SLE [36]. Serum complement levels are usually normal in MCTD.

Patients with MCTD characteristically have a high titer (often >1:1,000,000) of antibody to the RNase-sensitive (RNP) component of ENA, and anti-RNP antibodies are detected by Ouchterlony double immunodiffusion or ELISA [16, 21, 28, 29]. Antibodies to single-stranded DNA (ssDNA) can be detected [17], but in contrast to SLE high antidouble-stranded DNA (dsDNA) antibody titers and anti-Sm antibodies are unusual and transient in MCTD [28, 29]. Patients with anti-Sm antibodies by Ouchterlony immunodiffusion or reactivity with the Sm-D polypeptide by immunoblotting, however, are at substantial risk of nephritis and other SLE spectrum clinical manifestations of disease [77, 78]. Rarely, patients who appear clinically to have MCTD have been seen who will initially not have detectable RNP antibodies and then will become positive, typically following treatment with corticosteroids [16, 21, 26, 99]. This serologic pattern of high titers of anti-RNP antibodies usually persists for years during both active and inactive disease, but ultimately antibody levels may fall or become undetectable during sustained clinical remission [28, 29, 100].

Although immunologically distinct, a close physical association of RNP and Sm antigens was shown in early biochemical studies by Tamako *et al*. [101]. Lerner and Steitz [102] and Lerner *et al*. [103] subsequently further elucidated the nature of the small nuclear ribonucleoproteins (snRNPs) reacting with RNP and Sm antibodies. Their studies showed that anti-RNP antibodies immunoprecipitate uridine-rich U1 snRNA–protein complexes whereas anti-Sm antibodies immunoprecipitate snRNA–protein complexes containing U1, U2, and U4–U6 snRNAs [104]. Immunoblotting studies by Pettersson *et al*. [100] revealed that anti-U1 RNP antibodies react with snRNP polypeptides with molecular masses 70 kDa (sometimes designated 70 K and previously referred to as 68 K), 33 kDa (A), and 22 kDa (C), whereas anti-Sm antibodies react predominantly with 28- to 29 kDa (B/B′, now B1/B2) and 16-kDa (D, now D1) polypeptides [100] (Table 5). Subsequently, Takeda *et al*. [78] biochemically purified these snRNPs and developed a very sensitive ELISA permitting quantitative assays for autoantibodies to the individual snRNPs (Fig. 3). More recent studies have revealed that there are at least three Sm-B (B1, B2, and B3) and three distinct Sm-D (Sm-D1, D2, and D3) polypeptides in addition to the newly described Sm-E, F, and G snRNP polypeptides [105–107].

TABLE 5 Relationship of Antibodies to ENA, U1 RNP, Sm, and snRNPs

RNase-sensitive ENA	RNase-resistant ENA
U1 RNP	Sm
70 kDa, A, and C snRNPs	B/B′ and D snRNPs

Longitudinal studies have shown persistence high levels of anti-U1 RNP and anti-U1-70-kDa antibodies for many years in MCTD and their subsequent decline and disappearance in patients during prolonged remission [28, 29, 78, 100] (Fig. 4).

In 1988, Deutscher and Keene [108] reported the identification and characterization of antibodies in the serum of a patient with an SLE overlap syndrome that recognized the naked U1 RNA itself rather than the snRNP polypeptides. A sequence-specific conformational epitope on U1 RNA is recognized by a unique autoantibody. The author's laboratory recently analyzed serum snRNP autoantibody patterns, immunogenetic specificities, and clinical features of patients with autoantibodies to U1 RNP who were positive or negative for anti-U1 RNA antibodies [109]. The very high frequency and high level of anti-U1-70kDa antibodies, HLA-DR4, or HLA-DR2, and clinical features of Raynaud's phenomenon, swollen hands, swelling of three or more joints, and myositis were all significantly more predominant among the anti-U1 RNA-positive

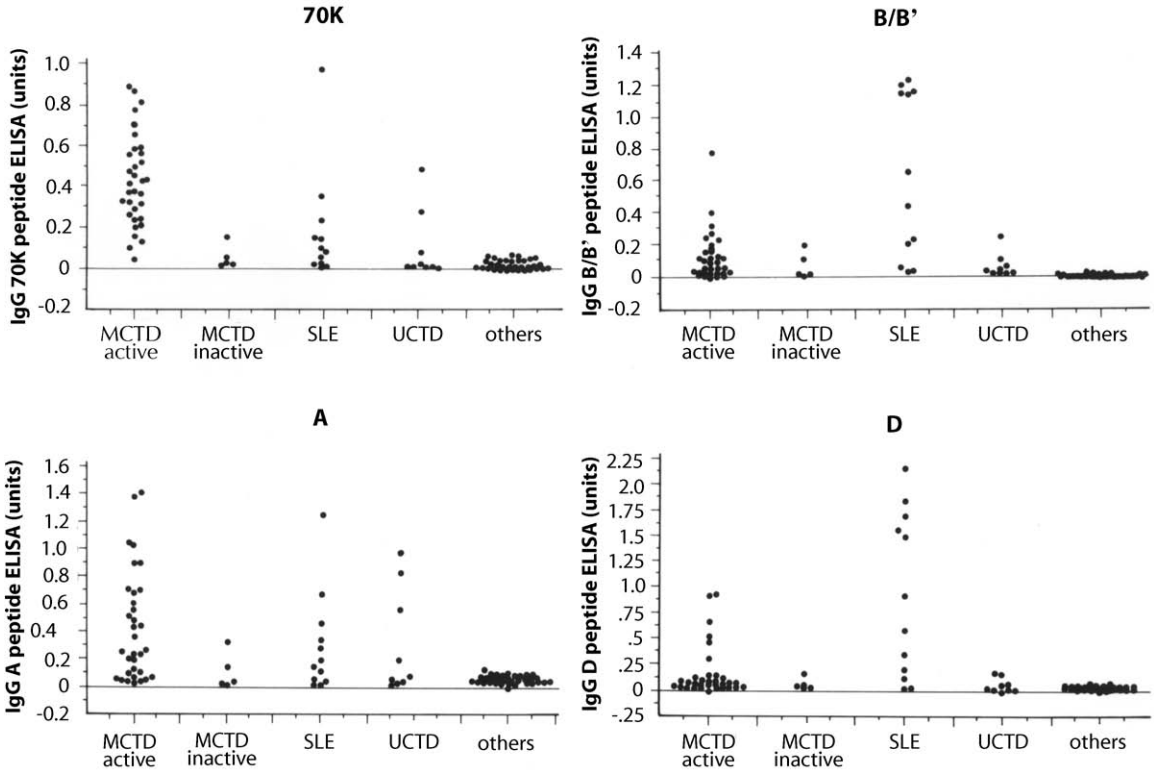


FIGURE 3 Diagnosis in patients whose sera were tested for immunoglobulin G (IgG) reactivity with individual U1 snRNP polypeptides by ELISA. Note that sera of patients with active MCTD showed higher anti-70k (70 kDa) reactivity in the ELISA than sera of patients with inactive MCTD, active SLE, and UCTD. Anti-B/B′ and anti-D reactivity was higher in sera of patients with active SLE than in sera of patients with active MCTD, inactive MCTD, and UCTD. SLE, active SLE, others, 14 patients negative for anti-RNP antibodies but positive for autoantibodies to other cellular components and 20 normal controls. From Takeda *et al*. [78], with permission.

group. In studies from The Netherlands, it was found that increases in anti-U1 RNA antibody levels correlated closely with disease flares, although we have been unable to definitively confirm these results in our patients [29, 110]. Thus, the presence of anti-U1 RNA antibodies may be a specific marker for MCTD and may reflect disease severity and/or activity. Autoantibodies to heterogeneous nuclear RNA, which is associated with nuclear matrix and antibodies to nucleosomes, have also been detected in MCTD [111–113]. These are detected in 20–40% of patients with MCTD, and epitope mapping has shown that there is preferential targeting in MCTD against select parts of the RNA-binding domains.

Histopathology and Immunopathology

The first comprehensive histopathologic investigation of MCTD included observations from three autopsies and five renal biopsies in a study group of 15 children [44]. Although earlier reports had mentioned vascular changes, this report was the first to emphasize the occurrence of widespread proliferative vascular lesions in MCTD. The young age of these patients, short duration of disease, and lack of corticosteroid therapy underscored the likelihood that these vascular changes reflected the natural course of the CTD and were not due to other possible causes that might have complicated the interpretation in an older population. From these autopsies, 31 of 58 organs (53%) had intimal proliferative changes and 9 organs (16%) had medial hypertrophy in blood vessel walls. These obliterative vascular changes were noted in large vessels (e.g., aorta, renal and coronary arteries) and within small arterioles. Particularly striking was the frequency and severity of this vasculopathy in the kidney, lungs, myocardium, intestinal tract, aorta, and coronary vessels, often in the absence of overt clinical manifestations.

Similar obliterative, proliferative vascular lesions have now been described in adults with MCTD, and it has been noted that vascular compromise may lead to organ failure and hemorrhage [28, 29, 41, 42, 64]. In four

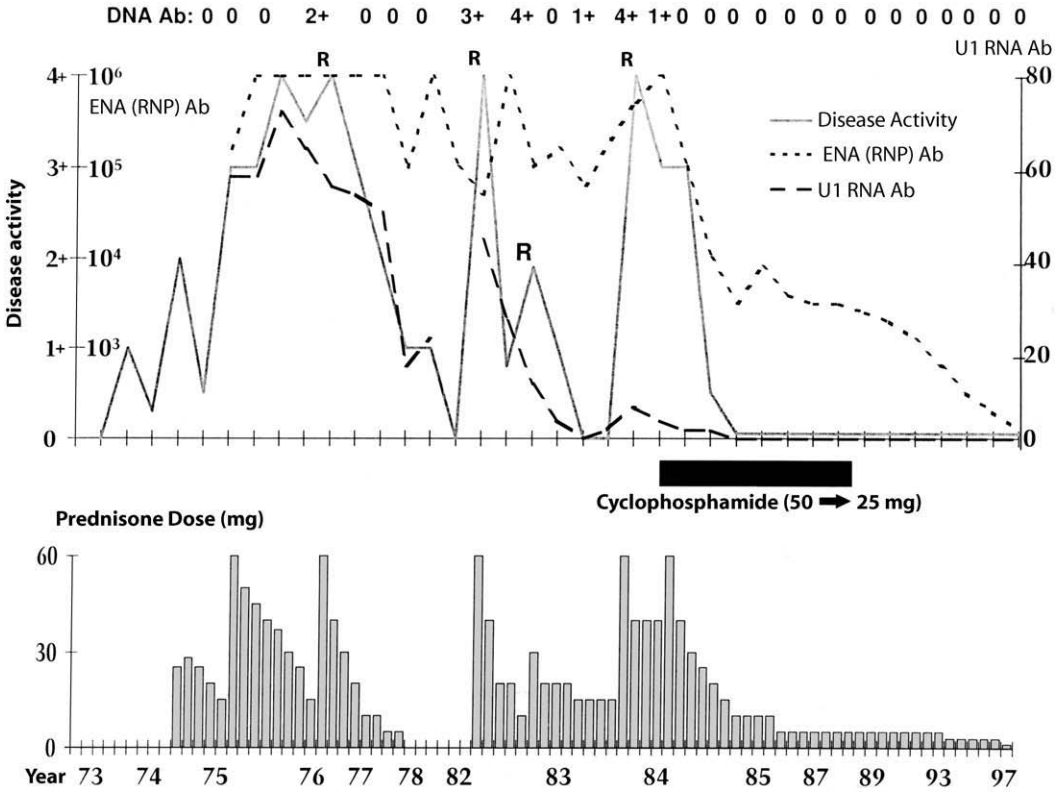


FIGURE 4 Correlation of autoantibodies to native DNA by *Crithidia luciliae*, RNase-sensitive ENA (U1 RNP) by passive hemagglutination, and U1 RNA by radioactive immunoprecipitation with disease activity in a woman with MCTD who was 16 years old in 1973. Note that DNA antibodies were transiently present on four occasions in association with active renal disease (R). Antibodies to U1 RNP remained positive at a very high level over 10 years during periods of both active and inactive disease. The MCTD went into a sustained remission following the addition of cyclophosphamide to the prednisone therapy, and anti-U1 RNP levels finally began to decline in 1985. In contrast to the prolonged high levels of anti-U1 RNP, levels of anti-U1 RNA increased and decreased in close correlation with disease activity and have remained undetectable during her sustained remission.

adult patients with MCTD who had died at the University of Missouri, autopsies revealed this same obliterative vasculopathy with intimal proliferation and medial hypertrophy of arteries in the lungs, kidney, heart, adrenals, and spleen. These proliferative vascular lesions so prominent in MCTD are in distinct contrast to the inflammatory infiltration of blood vessels typically seen in the vasculitis of SLE, RA, and polyarteritis [41].

Characteristic findings in the lungs in MCTD are intimal thickening and medial muscular hypertrophy of the pulmonary arteries and arterioles with little or no interstitial fibrosis (see Fig. 2) [28, 29]. This is in contrast to SSc in which the most characteristic histologic finding is diffuse interstitial fibrosis [43]. Ultrastructural analysis of pulmonary arterial lesions in at least one study in MCTD has shown an abundance of intimal ground substance, increased fibroblast production of collagen, a disordered array of collagen fibers, perforated basal lamina, and hypertrophy of muscle fibers in the media [28, 29]. When analyzed biochemically, these lung specimens showed a selective increase of type III collagen synthesis, resulting in an abnormal type I–type III ratio [28]. This is in contrast to the lungs in SSc where the type I–type III collagen ratio is the same as in normal lungs [114]. Thus, pulmonary hypertension in MCTD is related to proliferative vasculopathy with narrowing of the vessel lumens without significant interstitial fibrosis [41]. Plexiform vascular lesions have also been described in association with pulmonary hypertension in MCTD [41]. Less frequently at autopsy a mixed picture with marked vascular changes and an interstitial fibrosing pneumonitis has been noted [41].

Vascular abnormalities also occur in both limited and diffuse SSc [43]. Obliterative vasculopathy in both MCTD and SSc has also been shown angiographically

in radial, ulnar, and digital arteries [115]. The absence of fibrinoid change, predilection for large vessels, and minimal fibrosis in MCTD seem to distinguish it from SSc [28, 44]. Although medial hypertrophy is the characteristic vascular change in MCTD, there is often a decreased thickness with areas of focal medial atrophy in limited SSc [43]. Replacement of the inner and outer muscle layers of the esophagus, pylorus, and colon with a hyaline material in MCTD also appears to be distinct from the muscle atrophy and fibrosis noted in SSc [44].

The most frequent renal glomerular lesions in MCTD are membranous and mesangioproliferative glomerulonephritis [29, 116, 117], whereas diffuse proliferative glomerulonephritis is seen less frequently than in SLE. Intramembranous, mesangial, and subepithelial electron-dense deposits have been seen on electron microscopy [39, 118, 119]. When diffuse proliferative glomerular changes are mainly inflammatory with minimal chronic changes (crescents, hyaline thrombi, tubular atrophy, and fibrosis), even this more severe renal disease usually responds to corticosteroid therapy in MCTD. Proliferative vascular changes are also seen frequently in the arcuate and interlobular arteries of the kidney as well as in other organs [28] (Figs. 5 and 6).

In a study of skeletal muscle biopsies from 13 patients with MCTD [57], 8 showed a lymphocytic infiltrate either perivascularly or within the perimysium, endomysium, or vessel walls (vasculitis), often in association with focal fiber necrosis. Perifascicular atrophy was seen in 3 cases, and 5 of 11 biopsies that had histochemical determinations showed type I fiber predominance. These findings are similar to the histopathologic changes seen in polymyositis and in the myopathy associated in SLE. Of particular note, however, direct immunofluorescence performed in 10 cases revealed selective deposition of IgG in 7 cases—within normal-appearing vessels, within normal-appearing muscle fibers, around or on the sarcoplasmic membrane, or within the perimysial connective tissue—suggesting that immune mechanisms may be responsible for the myopathy that is so prominent in MCTD. A more recent study of various forms of myositis noted that infiltrating lymphocytes were confined to the endomysium in polymyositis and had a perimysial distribution in MCTD [47].

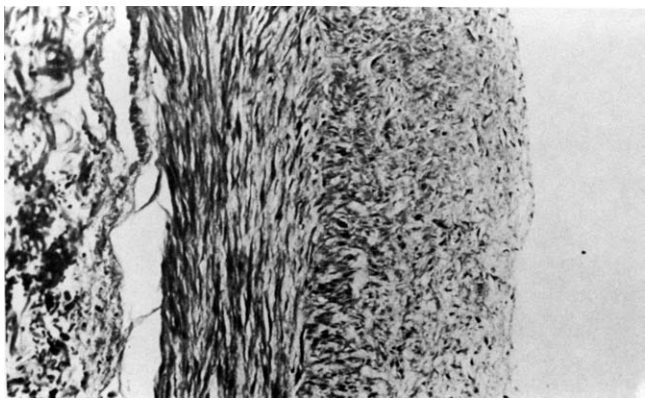


FIGURE 5 Cross section of an intramural coronary artery in an 18-year-old woman with MCTD showing marked intimal hyperplasia and smooth muscle hypertrophy. Cellular elements in the intima are surrounded by a loose fibrillar connective tissue matrix. Acellular elements in the media (presumably mucopolysaccharides) are also increased quantitatively. From Alpert *et al*. [145], with permission of the American Heart Association.

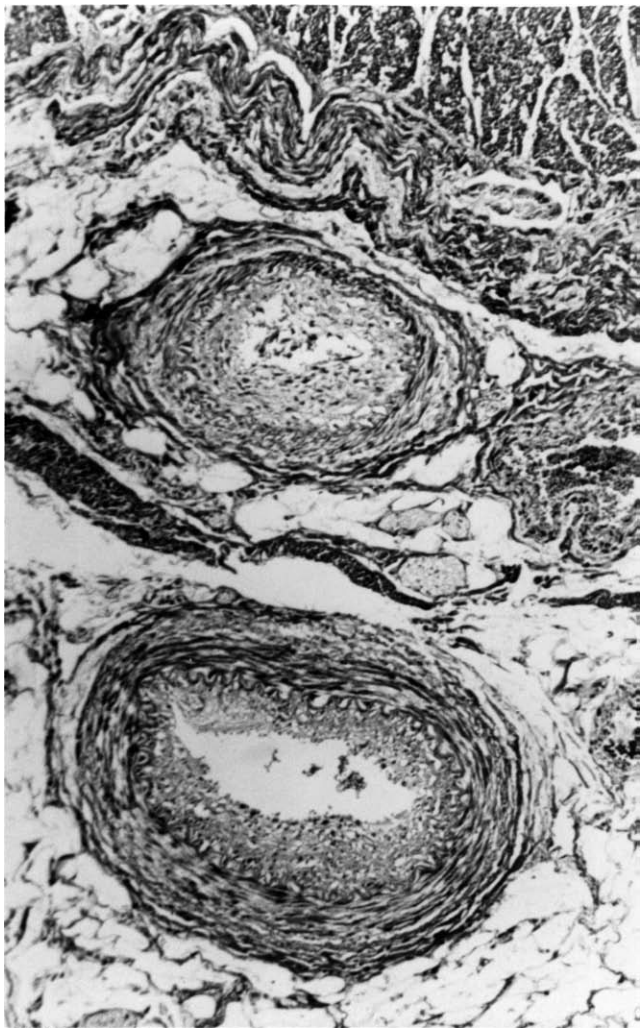


FIGURE 6 Two medium-sized arteries from the ilium of a 15-year-old female with MCTD. These arteries demonstrate intimal proliferation, medial hypertrophy, and luminal narrowing that are characteristic of MCTD. From Sharp and Singsen [119a], with permission.

Immunogenetics

Population immunogenetic studies from Germany, England, Finland, the United States, Japan, and Mexico have shown association between HLA-DR4 and the presence of anti-RNP antibodies or MCTD per se (for review, see Hoffman and Sharp [120] and Sharp and Hoffman [121]). Studies from Japan have also reported association of HLA-DR2 with MCTD [120, 121]. It has been demonstrated that the presence of anti-U1-70kDa autoantibodies is independently associated with select genotypes of HLA-DR4 and HLA-DR2 [11]. Furthermore, a comprehensive analysis of HLA-DR, HLA-DQ, and HLA-DP genes among these anti-U1-70kDa autoantibody-positive CTD patients has been performed [10, 11, 120–122]. It was found that a shared cluster of amino acids, of the sequence FDYFYAQ at positions 26, 28, 30–32, 70, and 73 of the HLA-DRB1, was increased in frequency among patients (Fig. 7). It was concluded that this shared cluster of amino acids, or so-called "shared epitope," might be important in regulating the immune response against the U1-70-kDa autoantigen.

Other genes of the immune system have also been examined in anti-RNP-positive CTD. Polymorphism of genes encoding the heavy and light chains of Ig can be detected by assaying for the distribution of Km and Gm allotypes, respectively, among patients versus healthy controls. Genth *et al*. [9] found that among German patients, the Gm 1,3;5,21 was increased among the anti-RNP-antibody-positive group. Black *et al*. [8] reported the Gm 1,3;5,21 allotype increased among anti-RNP positive patients from the United Kingdom. In a study among anti-U1-70kDa-positive CTD patients from the United States, no Gm or Km allotype associations were found [123].

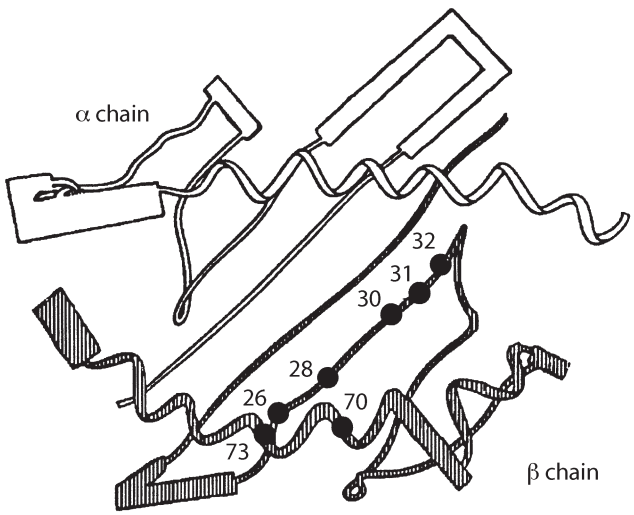


FIGURE 7 Shared epitope of HLA in MCTD. Model of HLA-DR as deduced from X-ray crystallography. Amino acids in positions 26, 28, 30–32, 70, and 73 are shared by HLA-DR4- and HLA-DR2-positive MCTD patients. Amino acid residues in these positions, FDYFYQA (Phe, Asp, Tyr, Phe, Tyr, Gln, Ala), form the shared epitope identified to be common among MCTD patients [11]. From Brown *et al*. [122a], with permission.

Controversy of MCTD as a Distinct Entity

The concept of MCTD has evolved since the original description in 1972 [16]. MCTD is now recognized to occur throughout the United States and the world, with large series of patients described from the United States, Japan, Mexico, Sweden, and France (reviewed in Sharp and Hoffman [121]). Furthermore, controversy over whether MCTD should be considered as a distinct entity has been the subject of several reviews [124–128]. Three major classification schemes have been proposed for MCTD (see Table 4). In recent years, it has been appreciated that there are substantial numbers of patients who manifest Raynaud's phenomenon, arthralgias-arthritis, diffusely swollen hands, and esophageal hypomotility, with high titers of antibodies against the U1-70-kDa polypeptide of RNP. These patients also have distinctive immunogenetics, with population studies demonstrating that they have an increased presence of select genotypes of HLA-DR4 and/or HLA-DR2. It now appears that it is this constellation of clinical manifestations, associated with both anti-U1-70-kDa and anti-U1 RNA autoantibodies and select genotypes of HLA, that most typifies MCTD [120, 121].

Patients with MCTD may also meet classification criteria for other rheumatic diseases, including SLE, RA, polymyositis, or SSc. This appears to be the basis for much of the confusion and controversy surrounding MCTD [121–128]. Thus, based only on clinical manifestations of disease, these patients may be included among patients classified as SLE, RA, or SSc by some authors. Other authors have recognized that a distinct group of patients with features of MCTD exists but have chosen to call them UCTD [129]. Until the etiology of MCTD, SLE, and other CTDs is known, any classification scheme may be flawed and is subject to challenge. We believe, however, that the synthesis of current knowledge on the distinctive features of autoantibody specificity and immunogenetics does support the concept of MCTD as presented here [120, 122, 124–128].

Model for Pathophysiology

A central paradigm of modern immunology is that antigen is processed into small peptide fragments and

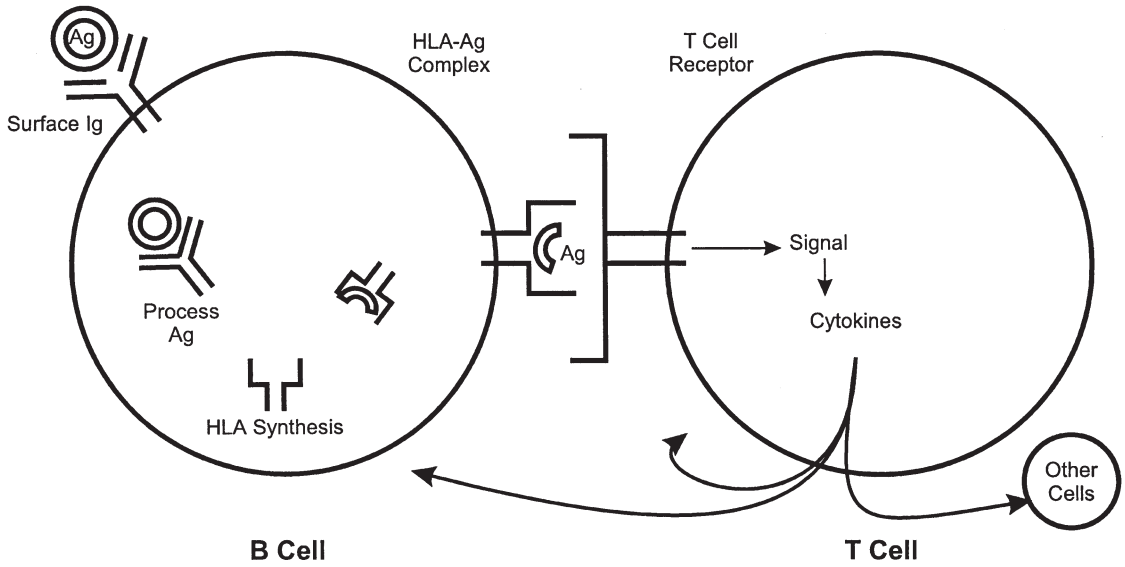


FIGURE 8 Role of T and B cells in MCTD. A central paradigm of modern immunology is that the antigen is processed into small peptide fragments and presented to T cells, bound noncovalently in the antigen-binding cleft of HLA molecules, on the surface of the antigen-presenting cell. This concept is illustrated where surface immunoglobulin (Ig) on B cells binds autoantigens. The antigen is then processed by proteolytic cleavage into small peptide fragments that bind to HLA molecules synthesized recently in the cell. This Ag–HLA complex is presented to autoreactive T cells. T cells that possess the appropriate T-cell receptor are stimulated, which in turn produce and release cytokines. These cytokines can act in both an autocrine and a paracrine manner to expand the autoreactive T cell, as well as to promote proliferation and differentiation of the autoantigen-presenting B cells. B and T cells act in concert to promote autoantibody formation and to promote affinity maturation of autoantibodies of the IgG isotype.

presented to T cells, noncovalently bound in the antigen-binding cleft of HLA molecules, on the surface of the antigen-presenting cell [130]. This is illustrated in Fig. 8. In select instances that may be very germane to autoimmunity, B cells can serve as the antigen-presenting cell. Hypothetically, surface Ig on B cells could be highly efficient in binding autoantigens released from cells undergoing cell death by necrosis or apoptosis. Such free nuclear antigens could then be processed and presented to autoreactive T cells, which in turn produce and release cytokines. The cytokines expand the autoreactive T cell. These cytokines also serve to promote proliferation and differentiation of the autoantigen-presenting B cells, which are in close physical proximity to the responding T cells. In this model, both B and T cells act in concert to promote autoantibody formation and to promote affinity maturation of autoantibodies of the IgG isotype.

T Cells in MCTD

One of the hallmarks of MCTD is the presence of high titers of anti-RNP antibodies of the IgG isotype present in patients' sera. In some cases, these anti-RNP antibodies have been demonstrated to account for one-third of the total serum Ig [131]. The presence of high levels of autoantibodies of the IgG isotype is indirect evidence that T cells are providing help to autoantibody-producing B cells *in vivo*. Furthermore, the association of select HLA alleles with autoantibody production implies a direct role for T cells in autoantibody production.

One hypothesis, for the preferential association of certain HLA-DR4 and HLA-DR2 alleles with the production of anti-RNP and anti-U170-kDa autoantibodies, is that this is an autoantigen-driven immune response. In such a model, the autoantigen (e.g., the U1-70-kDa polypeptide of the RNP complex) binds preferentially to select HLA-DR4 and/or HLA-DR2 alleles and this Ag–HLA complex then stimulates autoreactive T cells to expand and produce cytokines (see Fig. 8). To test this hypothesis, peripheral blood mononuclear cells have been examined for the presence of T cells that react against the U1-70-kDa antigen [132–139]. Fenning *et al.* [134], Hoffman *et al.* [135], and Wolff-Vorbeck *et al.* [136] have reported that human T-cell clones reactive against the U1-70-kDa antigen can be cloned from

peripheral blood mononuclear cells. Holyst *et al.* [137] extended this work and demonstrated that CTD patients have clonable circulating U1-70-kDa-reactive T cells that parallel the specificity of antibodies found in their sera. Furthermore, they have demonstrated that U1-70-kDa-reactive T cells exhibited a T helper cell phenotype and produced cytokines that are important in B-cell help and differentiation. Talken *et al.* [138] examined the T-cell receptor from U1-70-kDa-reactive human T-cell clones and reported that there is nonrandom T-cell receptor usage among T cells reactive against the U1-70-kDa autoantigen, consistent with the selection of T-cell receptors directed against a limited number of antigen epitopes [138, 139]. Taken together, these results suggest that genetic or nongenetic factors determine which T cells are expanded when stimulated with the U1-70-kDa autoantigen. Interestingly, these findings raise the possibility that targeted therapy could be directed effectively against select T cells that are important in the anti-U1-70-kDa response. Such an approach could potentially be effective in suppressing the disease, while leaving T cells with other receptor specificities available to respond to viral and bacterial pathogens.

Distinctive Clinical, Immunologic, and Genetic Features

There are distinctive clinical, immunologic, histopathologic, and genetic features of MCTD that can be identified. These are summarized in Table 6. Clinical features that predominate in MCTD are the constellation of arthritis-arthralgia, swollen hands, sclerodactyly, Raynaud's phenomenon, and abnormal esophageal motility. The development of pulmonary hypertension occurs in a minority of patients, but is often a devastating clinical event. It has also been appreciated that pulmonary disease may frequently be present. Pulmonary hypertension and heart failure are the most frequent disease-associated cause of death in MCTD. Immunologically, B-cell responses are characterized by the presence of high titers of autoantibodies directed against RNP and are particularly distinguished by the presence of high titers of autoantibodies against the U1-70-kDa polypeptide and U1 RNA of the snRNP complex. The presence of antibodies against U1-70-kDa and U1 RNA are useful clinical diagnostic markers in MCTD. Histopathology in MCTD shows that salivary glands, muscle, and other tissues may be massively infiltrated with lymphocytes and plasma cells. There also may be massive lymphoproliferation in MCTD. Histopathology (discussed previously) is characterized by proliferative vascular lesions, including intimal thickening and medial hyperplasia, that may resemble vascular lesions in SSc; however, in the lungs in MCTD there is vasculopathy with virtual absence of interstitial pulmonary fibrosis and selective increase of type III collagen in contrast to SSc [28]. Distinctive genetic (discussed previously) and additional immunologic and histopathologic features are shown in Table 6.

TABLE 6 Distinctive Clinical, Immunologic, and Genetic Features of MCTD

Clinical	Constellation of arthralgia/arthritis, swollen hands, Raynaud's phenomenon, abnormal esophageal motility, sclerodactyly, myositis, pulmonary hypertension
B-cell responses	High-titer U1 RNP antibodies and particularly anti-U1-70-kDa and anti-U1 RNA autoantibodies, typically in the absence of other autoantibodies
Genetic	Association with HLA-DR4 and shared epitope on HLA-DRB1 found on haplotypes bearing HLA-DR4 and HLA-DR2
T-cell responses	snRNP U1-70-kDa and other snRNP-reactive T cells found in the peripheral blood of patients
Immune complexes	Lower levels of immune complexes found in the circulation than in SLE, more rapid RES clearance of immune complexes than from the circulation in SLE
Histopathology	Proliferative vasculopathy in the virtual absence of fibrosis and widespread lymphocytic and plasmacytic infiltrates

Model of Disease: A Synthesis

A working model of disease susceptibility and pathogenesis of MCTD is shown in Fig. 9. It is unclear whether an exogenous agent(s) plays a role in triggering MCTD. Although speculative, it is plausible that an environmental factor(s) may play a role in triggering the disease. It has been proven that genetic factors do contribute to disease [121]. The emerging model, based on work in both human disease and murine models of autoimmunity, would suggest that major histocompatibility complex genes are a major disease-susceptibility factor for disease and that other genes also contribute to disease susceptibility [140–142]. Developing disease might, for example, involve interaction of multiple unlinked susceptibility and resistance genes. Stochastic or random events in immune development, such as those that affect the T-cell repertoire, may also play an important role in whether an individual is prone to development of the disease. The final effector pathway of disease appears to require the interaction of both autoreactive B and T cells. Other immune effector cells

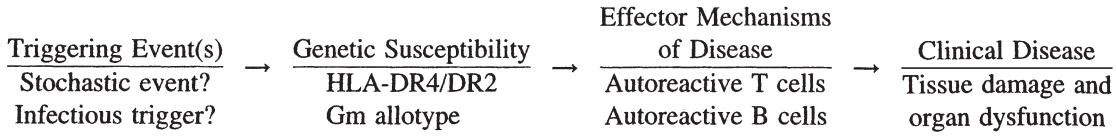


FIGURE 9 A hypothetical model of disease in MCTD. An exogenous agent(s) may play a role in initiating MCTD. An environmental factor(s) may play a role in triggering the disease. It has been proven that genetic factors do contribute to disease. HLA genes and immunoglobulin (Ig) genes have been demonstrated to be disease susceptibility factors, and other yet unidentified genes may also contribute to disease susceptibility. Developing disease might, for example, involve the interaction of multiple unlinked susceptibility and resistance genes. Stochastic or random events in immune development may influence the development of disease. The final effector pathway of disease appears to require the interaction of both autoreactive B and T cells. Other immune effector cells may also then be recruited in the final pathologic response.

TABLE 7 Treatment of MCTD[a]

Type of disease activity	Recommendations
Mild UCTD (Raynaud's phenomenon, arthralgias, myalgias, fatigue, mild synovitis)	NSAIDs, antimalarials, low-dose corticosteroids, avoid cold exposure, calcium channel blockers as tolerated if Raynaud's phenomenon is severe
Disease activity limited to erosive RA-like joint involvement	NSAIDs, antimalarials; low-dose corticosteroids or methotrexate if more severe
Mild to moderate SLE-like involvement (rash, serositis, anemia, leukopenia, fever, lymphadenopathy)	Avoid sun exposure, topical corticosteroids, antimalarials, low to intermediate doses of corticosteroids
SLE-like involvement of major organ systems (pericarditis, myocarditis, thrombocytopenia, glomerulonephritis, aseptic meningitis[b]), myositis, progressive involvement of lug, dysfunction of upper esophageal sphincter with dysphagia and/or aspiration pneumonia	Moderate to high doses of corticosteroids depending on severity, and cyclophosphamide or other cytotoxic drug if steroid dependent
Esophageal reflux without upper sphincter involvement	Raise head of bed, avoid caffeine, discontinue smoking, H2 blockers, H^+ proton pump blockers, metoclopramide, corticosteroids may be used if severe dysphagia related to lower esophageal involvement is not responsive to these conservative measures
Asymptomatic pulmonary hypertension	Right heart catheterization and monitoring pressures in response to therapeutic agents may be a guide to therapy, calcium channel blockers, angiotensin-converting enzyme inhibitors, vasodilators, and possibly cycledophosphamide
Symptomatic pulmonary hypertension	Calcium channel blockers, angiotensin-converting enzyme inhibitors, vasodilators, corticosteroids, cyclophosphamide, or other cytotoxic drugs

[a] NSAIDs, nonsteroidal anti-inflammatory drugs; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; UCTD, undifferentiated connective tissue disease.

[b] Ibuprofen and Sulindac have been associated with a hypersensitivity aseptic meningitis in MCTD so NSAIDs should be discontinued.

may also then be recruited in the final pathologic response. Some aspects of disease may be mediated solely by antibody, others may be mediated primarily by autoreactive T cell, and others by both B and T cells.

Treatment and Prognosis

Since controlled clinical therapeutic trials have not been performed, the treatment of MCTD is based on information derived from clinical experience. The original report of MCTD suggested that the disease frequently appeared to be responsive to treatment with corticosteroids and/or cyclophosphamide [16]. Subsequently, recognition of severe complications such as pulmonary disease, pulmonary hypertension, and proliferative vascular lesions has led to the appreciation that the outcome is not always favorable [28, 29].

Guidelines for treatment of specific clinical problems in MCTD are presented in Table 7 [128, 143]. A mild UCTD syndrome or arthritis presenting without major organ system involvement can often be treated conservatively with nonsteroidal anti-inflammatory drugs or antimalarials without resorting to corticosteroids; however, if the synovitis is more refractory or bone erosions occur, low doses of corticosteroids and/or methotrexate may be required. Antimalarials and/or

low to intermediate doses of corticosteroids will usually control mild to moderate SLE-like features. Higher doses of corticosteroids (e.g., prednisone 1 mg/kg) may be required for the treatment of more serious systemic involvement (e.g., myositis, myocarditis, pericarditis, glomerulonephritis, or thrombocytopenia).

It has been shown that high titers of anti-U1 RNP with anti-U1-70-kDa antibodies are associated with a high risk of esophageal dysfunction, myositis, pulmonary disease, and pulmonary hypertension, which can be detected by functional tests and biopsies early in the evolution of MCTD when patients are still asymptomatic [28, 29, 58]. Thus, detection of this serologic pattern might guide the clinician to evaluate even asymptomatic patients with studies of esophageal motility, muscle enzymes, EMG, muscle biopsy, pulmonary function (including DLCO), and chest X-ray; depending on the results of these studies, an echocardiogram and possibly a right heart catheterization might be performed.

If abnormalities are detected in these organ systems, early therapeutic intervention may be indicated and may control the disease with more selective or shorter courses of drug therapy than would be required if the disease were more advanced or due to a different CTD. Thus, early pulmonary DLCO abnormalities in MCTD may respond to corticosteroids alone or in combination with cyclophosphamide [28, 29, 58] in contrast to SSc. Early esophageal dysfunction may respond to proton pump blockers. Early treatment of myositis in MCTD may require lower doses of corticosteroids for a shorter duration than in the case of polymyositis [47]. Early pulmonary hypertension in a mildly to moderately symptomatic phase in MCTD may be treated successfully with nifedipine, captopril, or hydralazine in addition to immunosuppressive drugs (the author's experience and uncontrolled trials in the literature appear to favor the use of cyclophosphamide) [28, 29, 143, 144] and potentially avoid more severe disease. When pulmonary hypertension is more advanced and very severe, corticosteroids, cytotoxic drugs, calcium channel blockers, and angiotensin-converting enzyme (ACE) inhibitors have been employed as therapeutic agents, but the responses have not always been favorable [28, 29, 67, 68, 145–148].

The initial report of MCTD suggested a favorable prognosis [16]. Subsequent reports described serious clinical problems due to proliferative vascular lesions, renal disease, cardiac disease, and thrombocytopenia in childhood MCTD. Causes of death were meningococcal or pneumococcal infections in three patients and cerebral hemorrhage associated with severe thrombocytopenia in one patient. A follow-up study of the initially reported MCTD patients related that 8 of the 25 patients had died, although 6 of the 8 deaths were not directly related to their rheumatic disease [37]. Inflammatory tissue manifestations in these patients appeared to have responded to corticosteroid therapy while scleroderma-spectrum features had persisted.

In a initial prospective longitudinal study, almost all of the 34 patients had significant major organ system involvement and were treated with moderate to high doses of corticosteroids and/or cyclophosphamide [28]. Two-thirds responded favorably to treatment, whereas one-third with very severe disease were less responsive to corticosteroids, and four patients died. As in other MCTD reports, inflammatory changes such as rash, pleuritis, pericarditis, and myositis responded to corticosteroids, whereas pulmonary and scleroderma-like features were less likely to respond. A good long-term prognosis for some patients with MCTD is demonstrated by the 38% of these patients studied whose disease became inactive, including 10 in a sustained remission (9 off all therapy) for 7–20 years (mean 16 years).

Subsequent longitudinal studies in MCTD patients in prolonged remission, following intensive corticosteroid and cyclophosphamide therapy, have revealed the eventual disappearance of serum anti-U1 RNP autoantibodies, including the anti-U1-70-kDa snRNP reactivity that had usually persisted for years [29]. Thus, the basic autoimmune process appears to have been altered in these patients. The combined mortality rate from seven long-term studies of MCTD including almost 300 patients was 14%, with a mean duration of disease of over 10 years [27–30, 37, 77, 149]. Of these deaths, 10% were directly related to the disease. Disease-related deaths were associated most frequently with proliferative vascular lesions, pulmonary hypertension, cor pulmonale, pericardial tamponade, and sepsis, with intravascular coagulation, perforated bowel, and renal failure noted less frequently. The prognosis in MCTD appears similar to that of SLE but better than that of SSc.

Relationship of MCTD to SLE

The ultimate conclusion on the relationship of MCTD to SLE awaits the determination of the etiology of these entities. Comparison of the clinical, serologic, and immunogenetic features of MCTD and SLE reveals a number of important differences in addition to several obvious similarities. The contrasting features of MCTD and SLE are summarized in Table 8. Esophageal hypomotility, sclerodactyly, and pulmonary hypertension are common and clinically distinctive features of MCTD, whereas renal disease and serious central nervous system disease are distinctive features of SLE. Serologic

TABLE 8 Relationship of Clinical, Serologic, and Immunogenetic Features of MCTD to SLE

Clinical, serologic, or immunogenetic features of disease	Prevalence in disease[a]	
	MCTD	SLE
Raynaud's phenomenon	++++	++
Esophageal hypomotility	++++	–
Swollen hands	++++	+
Sclerodactyly	++++	–
Myositis	+++	+
Pulmonary hypertension	+++	–
Rheumatoid-like joint changes	++	–
Dermatomyositis-like skin abnormalities	++	–
Renal disease	+	++
Central nervous system disease	–	++
Anti-U1-70-kDa antibodies	++++	–
Anti-RNP antibodies	++++	+
Anti-U1 RNA antibodies	+++	–
Anti-Sm antibodies	–	++
Anti-D polypeptide antibodies	–	++
Anti-B polypeptide antibodies	++	++
Anti-dsDNA antibodies	–	+++
Depressed serum C3 or C4 levels	+	+++
HLA-DR4	++++	–
HLA-DR2	+++	+++
HLA-DR3	–	++++

[a] Ranked on a scale of 4 + (most patients) to – (negative or rarely found).

features unique to MCTD include anti-U1 RNA and anti-U1-70-kDa autoantibodies; anti-Sm and anti-dsDNA antibodies are unique to SLE. When comparing immunogenetic markers, patients with both MCTD and SLE may possess HLA-DR2. The presence of the HLA-DR4 phenotype and the shared epitope on select HLA-DR4 and/or HLA-DR2 genotypes are distinctive immunogenetic features of MCTD [10, 11]. Finally, the HLA-DR3 phenotype is common in SLE and almost always absent in MCTD [10, 120, 121].

SJÖGREN'S SYNDROME

As with MCTD there is no uniformly accepted classification scheme for Sjögren's syndrome. Proposed classification systems for Sjögren's syndrome are as shown in Table 9. Many clinical features of Sjögren's syndrome overlap with those of SLE and other rheumatic diseases. Sjögren's syndrome may coexist in patients who also can be classified as SLE [150]. The incidence of Sjögren's syndrome in SLE has been reported to range from 6 to 90% [150, 151]. It has been reported that 42% of MCTD patients have sicca symptoms compatible with secondary Sjögren's syndrome [93]. Patients with Sjögren's syndrome and a second CTD are said to have "secondary Sjögren's syndrome," whereas those without another identifiable disease are said to have "primary Sjögren's syndrome" [152]. Sjögren's syndrome has been reported to coexist in patients with a number of other diseases, such as SSc, polymyositis, polyarteritis, and primary biliary cirrhosis [152]. Studies on Sjögren's syndrome have reported that it may occur in patients with multiple sclerosis-like demyelinating disease [153, 154], hepatitis C [155–157], human T-cell leukemia virus (HTLV)-I infection, and fibromyalgia [158]. The etiological relationship between Sjögren's syndrome and these entities is controversial, however [159, 160]. The current view favors the concept that hepatitis C and HTLV-I virus can be associated with secondary Sjögren's syndrome. Furthermore, MS-like syndromes and fibromyalgia have been described among patients with SLE [161–163].

Clinical Features

The hallmarks of Sjögren's syndrome are signs and symptoms resulting from a distinctive form of ocular inflammation (keratoconjunctivitis sicca) and salivary gland destruction. Inflammation and destruction of exocrine glands in Sjögren's syndrome result in the sicca complex of ocular dryness (xero-ophthalmia) and oral dryness (xerostomia). Sicca symptoms can also be the result of anticholinergic side effects of many medications, impaired neural innervation of glands, or a defective response to stimuli. In SLE it has been reported that autonomic neuropathy is frequent and may also underlay sicca symptoms in some patients [164]. These observations further complicate the relationship between SLE and Sjögren's syndrome. The term Sjögren's syndrome is reserved by most authors [165] to describe the syndrome caused by the inflammatory destruction of salivary and lacrimal glands.

The ocular symptoms of Sjögren's syndrome that are reported frequently by patients are protean in nature [166, 167]. Common ocular symptoms that have been reported include foreign-body sensation, burning, inability to tear, tiring, soreness, pain, redness, itching, photosensitivity, film over the eyes, change in vision, excess secretions, and difficulty moving the lids [161–168]. Objective ocular findings of keratoconjunctivitis sicca include a positive Schirmer's I test of ≤8 mm of wetting of Schirmer paper in 5 min, abnormal rose

TABLE 9 Proposed Classification Criteria of Sjögren's Syndrome

San Diego criteria[a]

I. Primary Sjögren's syndrome
   A. Symptoms and objective signs of ocular dryness
      1. Schirmer's test less than 8 mm wetting per 5 min
      2. Positive rose bengal or staining of cornea or conjunctiva to demonstrate keratoconjunctivitis sicca
   B. Symptoms and objective signs of dry mouth
      1. Decreased parotid flow rate using Lashley cups or other methods
      2. Abnormal biopsy of minor salivary gland (focus score of ≥2 based on average of four evaluable lobules)
   C. Evidence of a systemic autoimmune disorder
      1. Elevated rheumatoid factor ≥1:320
      2. Elevated ANA ≥1:32
      3. Presence of Anti-SSA (Ro) or anti-SSB (La) antibodies

II. Secondary Sjögren's syndrome
   Characteristic signs and symptoms of Sjögren's syndrome (described earlier) plus clinical features sufficient to allow a diagnosis of RA, SLE, polymyositis, scleroderma, or biliary cirrhosis

III. Exclusions
   Sarcoidosis, preexistent lymphoma, acquired immunodeficiency disease, and other known causes of keratitis sicca or salivary gland enlargement

European Epidemiologic Center criteria

I. Primary Sjögren's syndrome (if at least four items present)
   A. Ocular symptoms (at least one present)
      1. Daily, persistent, troublesome dry eyes for more than 3 months
      2. Recurrent sensation of sand or gravel in the eyes
      3. Use of a tear substitute more than three times a day
   B. Oral symptoms (at least one present)
      1. Daily feeling of dry mouth for at least 3 months
      2. Recurrent feeling of swollen salivary glands as an adult
      3. Drinking liquids to aid in washing down dry foods
   C. Objective evidence of dry eyes (at least one present)
      1. Schirmer I test
      2. Rose bengal
      3. Lacrimal gland biopsy with focus score ≥1
   D. Objective evidence of salivary gland involvement (at least one present)
      1. Salivary gland scintigraphy
      2. Parotid sialography
      3. Unstimulated whole sialometry (≤1.5 ml per 15 min)
   E. Laboratory abnormality (at least one present)
      1. Anti-SSA/Ro or anti-SSB/La antibody
      2. ANA
      3. IgM rheumatoid factor (anti-IgG Fc)

[a] Definite Sjögren's syndrome requires objective evidence of dryness of eyes and/or mouth and a systemic autoimmune process including a characteristic minor salivary gland biopsy (criteria IA, IB, and IC). Probable Sjögren's syndrome does not require a minor salivary gland biopsy but can be diagnosed with demonstration of decreased salivary function (criteria IA, Ib-1, and IC).

bengal or fluorescein staining of the interpalpebral conjunctiva of the eye, and dullness of the conjunctiva and/or dullness of the cornea. Oral symptoms that have been reported commonly by patients include oral dryness, difficulty with mastication or swallowing dry foods such as crackers, oral soreness, dental symptoms, and increased fluid intake, oral fissures, or ulcers of the mouth and lips [167, 169]. Signs of oral dryness may include geographic tongue, lack of salivary pooling under the tongue, poor dentition, angular cheilitis, and oral candidiasis. Otorhinolaryngeal symptoms reported in Sjögren's syndrome include nasal dryness and crusting, epistaxis, decreased smell and taste, persistent hoarseness, chronic cough, crusting serous otitis, and hearing loss due to eustatian tube occlusion. Cutaneous manifestations of Sjögren's syndrome include dry skin, vulvo-vaginal dryness, nonthrombocytopenic purpura, Raynaud's phenomenon, and cutaneous vasculitis. Fatigue is a prominent and often problematic feature of Sjögren's syndrome [170, 171]. Patients may experience arthralgias and myalgias; arthritis and inflammatory myopathy are uncommon except in secondary Sjögren's syndrome associated with RA or polymyositis. Interstitial nephritis and renal tubular defects are frequent in Sjögren's syndrome, although proliferative glomerulonephritis is rare, being seen essentially only in

Sjögren's syndrome with cryoglobulinemia [172]. A range of neurologic manifestations have been reported in patients with Sjögren's syndrome. These include mononeuritis multiplex, symmetric polyneuropathy, trigeminal neuropathy, cerebral vasculitis, progressive myelopathy, transverse myelitis, and myasthenia gravis [173]. A putatively distinctive syndrome of sicca complex presenting as peripheral neuropathy has been reported [174]. Additional uncommon extraglandular manifestations of Sjögren's syndrome that have been described include Hashimoto's thyroiditis, mixed cryoglobulinemia, atrophic gastritis, biliary cirrhosis, sclerosing cholangitis, and lymphocytic infiltration of the lungs. Many of the clinical manifestations described here for Sjögren's syndrome also occur in SLE.

Primary and secondary Sjögren's syndromes have both been associated with an increased risk for developing cancer, especially lymphomas [175]. The risk of developing non-Hodgkin's lymphoma in Sjögren's syndrome is increased substantially over the risk for both the general population and patients with other CTDs [175]. Angioblastic lymphadenopathy, pseudolymphoma to lymphoma in Sjögren's syndrome can be problematic, and a number of publications have addressed this issue [176–182].

Laboratory Features

Hypergammaglobulinemia, a positive FANA antibody test, the presence of rheumatoid factor, often at very high levels, and an elevated erythrocyte sedimentation rate are common in Sjögren's syndrome. Anemia, leukopenia, and eosinophilia occur in approximately one-third of patients. Antibodies to the Ro/SSA antigen, composed of snRNP with molecular masses of 52 and 60 kDa, are found in over three-fourths of patients with primary Sjögren's syndrome [14, 183]. Antibodies to the La/SSB antigen, a 48-kDa phosphoprotein found in association with RNA polymerase III transcripts, are present in over half of patients with primary Sjögren's syndrome [184, 185]. Patients with anti-La/SSB antibodies have been reported to be more likely to have clinical features of Sjögren's syndrome than those with anti-Ro/SSA [183, 184, 186]. The anti-Ro/SSA and anti-La/SSB B-cell responses appear linked, with patients who have anti-La/SSB antibodies present in their sera almost always having detectable anti-Ro/SSA antibodies as well.

Histopathology

The characteristic histopathologic findings in Sjögren's syndrome are focal to diffuse lymphocytic infiltrates with a concomitant destruction of lacrimal and salivary glands. A biopsy of labial minor salivary glands, such as those in labial or conjunctival tissue, has been proposed to be useful in the diagnosis Sjögren's syndrome [187]. Criteria have been published to assist in evaluating and classifying biopsy specimens [188].

Immunogenetics

Susceptibility to Sjögren's syndrome has not yet been mapped to a single allele or locus across all ethnic groups. In Caucasians, alleles associated with Sjögren's syndrome include HLA-B8, DR2, DR3, DRw52, DQw1, DQw2, and the extended haplotype HLA-B8, DR3, DRw52, DQw2 [15]. The presence of high titers of anti-Ro/SSA autoantibody is associated with HLA-DQw1/DQw2 heterozygosity. Molecular genetic studies have mapped disease susceptibility among some ethnic groups to glutamine in position 34 of the HLA-DQ α chain and leucine in position 26 of the HLA-DQ β chain [15, 189]. Furthermore, polymorphisms in the T-cell receptor β chain are independently associated with increased levels of Ro/SSA autoantibodies, and it has been reported that T cells infiltrating the salivary gland in Sjögren's syndrome have similar T-cell receptors (so-called common T-cell clonotypes) [189, 190, 191]. The topics of pathogenesis and treatment in Sjögren's syndrome have been reviewed [192].

Relationship of Sjögren's Syndrome to Lupus

Patients with SLE can have secondary Sjögren's syndrome [193]. In some clinical series, this has been reported to be as high as 90% of SLE patients [12, 194]. Furthermore, many of the clinical features of SLE and Sjögren's syndrome do overlap and therefore classification of the primary disease in such patients can be problematic. In addition, there are overlaps of both autoantibodies and immunogenetics in the two diseases. For example, HLA-DR2- and HLA-DR3-bearing haplotypes are both associated with susceptibility to SLE and Sjögren's syndrome [195], and antibodies to SSA/Ro are associated with the subacute cutaneous lupus subset of SLE, C-2 deficiency-associated lupus syndrome, neonatal lupus syndrome, and Sjögren's syndrome. Provost *et al*. [196] have identified what they believed to be a new subset of patients with late-onset disease who have overlapping features of SLE–Sjögren's syndrome and the presence of anti-SSA/Ro antibodies.

OTHER OVERLAP SYNDROMES

The literature is replete with case descriptions and small clinical series of patients said to have overlapping

features of various CTD. Classification of such patients is subject to the problems already discussed. There are, however, several of these overlap syndromes that deserve specific comment.

Patients with PM-Scl/PM-1 autoantibodies have been identified as having a polymyositis-scleroderma overlap syndrome. Raynaud's phenomenon, muscle weakness, pulmonary disease, tendon involvement, and increased risk of severe renal disease have been reported to characterize this syndrome [48, 196]. Widespread sclerodermatous features are uncommon [48]. The presence of this autoantibody is associated with HLA-DR3 [197].

Patients described with autoantibodies reactive against the Ku antigen were found in the original study to have a polymyositis-scleroderma overlap syndrome [198, 199]. Subsequently, it has been found that anti-Ku antibodies can also be detected in the sera of patients with SLE, MCTD, Sjögren's syndrome, SSc, and polymyositis. The prevalence of anti-Ku reactivity is, however, low in these other groups of patients. The prevalence of the antibodies in clinical studies appears related to the method used for detection [198, 199]. The presence of anti-Ku autoantibodies is associated with HLA-DQw1 [198, 199].

A distinctive group of patients has been recognized by the presence of autoantibodies against various transfer RNA (tRNA) synthetases [200, 201]. The first of these antibodies described was anti-Jo-1, which is directed against histidyl-tRNA synthetases. These anti-aminoacyl tRNA synthetase antibodies are associated with inflammatory myositis, but have overlapping features with other CTDs in the majority of patients. Clinically, patients may have features of MCTD, Sjögren's syndrome, or SLE. Clinical manifestations that may occur in addition to features of muscle inflammation include Raynaud's phenomenon, arthritis, arthralgia, pulmonary fibrosis, dermatomyositis rash, sicca, telangiectasia, and dysphagia. Patients with anti-Jo-1 antibodies have been reported to have an erosive arthritis that may mimic RA and frequently have secondary Sjögren's syndrome. Immunogenetics have shown association of anti-Jo-1 with HLA-DR3 [199].

The presence of anti-RNA polymerase II antibodies, in the absence of scleroderma-specific antibodies to RNA polymerase I and III, has been associated with an SLE-overlap syndrome [202]. These anti-RNA polymerase antibodies are frequently accompanied by the presence of other autoantibodies, including anti-RNP, anti-Ku, and antitopoisomerase I antibodies [203].

Patients initially classified as UCTD may ultimately evolve into diseases that can be classified as SLE or another currently recognized disease category. One large multicenter study is examining the progression of early UCTD. Interim analyses of this patient cohort have been published [204]. It was found that patients with well-established CTD, such as SLE, RA, SSc, or polymyositis-dermatomyositis, at the time of entry into the study had more stable diagnoses than those with early UCTD. They reported that 90–100% of the patients with established diagnoses retained the same diagnosis over 1–5 years, whereas of those with UCTD, only 64% retained the same diagnosis at 1 year and only 47% by 5 years [205].

Acknowledgment

Dr. Hoffman's work was supported by the Medical Research Service, Department of Veterans Affairs, and NIH Awards AR43308 and AR48055.

References

1. Klemperer, P., Pollack, A. D., and Baehr, G. (1987). Diffuse collagen disease: Acute disseminated lupus erythematosus and diffuse scleroderma. *JAMA* **119**, 331.
2. Alarcón-Segovia, D. A., and Villareal, M. (1987). Classification and diagnostic criteria for mixed connective tissue disease. pp. 33–40. *In* "Mixed Connective Tissue Disease and Anti-nuclear Antibodies" (R. Kasukawa and G. C. Sharp, eds.), pp. 33–40. Elsevier Science, Amsterdam.
3. Kasukawa, R., Tojo, T., Miyawaki, S., *et al.* (1987). Preliminary diagnostic criteria for classification of mixed connective tissue disease. *In* "Mixed Connective Tissue Disease and Anti-nuclear Antibodies" (R. Kasukawa and G. C. Sharp, eds.), pp. 41–47. Elsevier Science, Amsterdam.
4. Sharp, G. C. (1987). Diagnostic criteria for classification of MCTD. *In* "Mixed Connective Tissue Disease and Anti-nuclear Antibodies" (R. Kasukawa and G. C. Sharp, eds.), pp. 23–32. Elsevier Science, Amsterdam.
5. Manthorpe, R., Oxholm, P., Prause, J. U., and Schiödt M. (1986). The Copenhagen criteria for Sjögren's syndrome. *Scand. J. Rheumatol.* **61**(Suppl.), 19.
6. Vitali, C., Bombardieri, S., Moutsopoulos, H. M., *et al.* (1993). Preliminary criteria for the classification of Sjögren's syndrome: Results of a prospective concerted action supported by the European Community. *Arthritis Rheum.* **36**, 340.
7. Vitali, C., Moutsopoulos, H. M., and Bombardieri, S. (1994). The European Community Study Group on diagnostic criteria for Sjögren's syndrome: Sensitivity and specificity of tests for ocular and oral involvement in Sjögren's syndrome. *Ann. Rheum. Dis.* **53**, 637.
8. Black, C. M., Maddison, P. J., Welsh, K. I., *et al.* (1998). HLA and immunoglobulin allotypes in mixed connective tissue disease. *Arthritis Rheum.* **31**, 131.
9. Genth, E., Zarhowski, H., Mierau, R., Wohltmann, D., and Hartl, P. W. (1987). HLA-DR4 and Gm(1,3;5,21) are associated with U1-nRNP antibody positive connective tissue. *Ann. Rheum. Dis.* **46**, 189.

10. Hoffman, R. W., Rettenmaier, L. J., Takeda, Y., *et al.* (1990). Human autoantibodies against the 70 kD polypeptide of (U1) small nuclear RNP are associated with HLA-DR4 among connective tissue disease patients. *Arthritis Rheum*. **33**, 666.
11. Kaneoka, H., Hsu, K.-C., Takeda, Y., Sharp, G. C., and Hoffman, R. W. (1992). Molecular genetic analysis of HLA-DR and HLA-DQ genes among anti-U1-70 kD autoantibody positive connective tissue disease patients. *Arthritis Rheum*. **35**, 83.
12. Arnett, F. C., and Reveille, J. D. (1992). Genetics of systemic lupus erythematosus. *Rheum. Dis. Clin. North Am*. **18**, 865.
13. Harley, J. B., Reichlin, M., Arnett, F. C., *et al.* (1986). Gene interaction at HLA-DQ enhances autoantibody production in primary Sjögren's syndrome. *Science* **232**, 1145.
14. Harley, J. B., Sestak, A. L., Willis, L. G., *et al.* (1989). A model for disease heterogeneity in systemic lupus erythematosus: Relationships between histocompatibility antigens, autoantibodies, and lymphopenia or renal disease. *Arthritis Rheum*. **32**, 826.
15. Reveille, J. D., MacLeod, M. J., Whittington, K., and Arnett, F. C. (1991). Specific amino acids residues in the second hypervariable region of HLA-DQA1 and DQB1 genes promote the Ro (SS-A)/La (SS-B) autoantibody response. *J. Immunol*. **146**, 3871.
16. Sharp, G. C., Irvin, W. S., Gould, R. G., Holman, H. R., and Tan, E. M. (1972). Mixed connective tissue disease: An apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med*. **52**, 148.
17. Sharp, G. C., Irvin, W. S., LaRoque, R. L., *et al.* (1971). Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. *J. Clin. Invest*. **50**, 350.
18. Northway, J. S., and Tan, E. M. (1972). Differentiations of antinuclear antibodies giving speckled staining patterns in immunofluorescence. *Clin. Immunol. Immunopathol*. **1**, 140.
19. Parker, M. D. (1973). Ribonucleoprotein antibodies: Frequencies and clinical significance in systemic lupus erythematosus, scleroderma and mixed connective tissue disease. *J. Lab. Clin. Med*. **82**, 769.
20. Reichlin, M., and Mattioli, M. (1972). Correlation of a precipitin reaction to an RNA protein antigen and low prevalence of nephritis in patients with systemic lupus erythematosus. *N. Engl. J. Med*. **286**, 908.
21. Sharp, G. C., Irvin, W. S., May, C. M., *et al.* (1974). Association of antibodies to ribonucleoprotein and Sm antigens with mixed connective tissue disease, systemic lupus erythematosus and other rheumatic diseases. *N. Engl. J. Med*. **295**, 1149.
22. Bridges, A. J., Anderson, J. D., McKay, J., *et al.* (1993). Antinuclear antibody testing in a referral laboratory. *Lab. Med*. **24**, 345.
23. Notman, D. D., Kurata, N., and Tan, E. M. (1975). Profiles of antinuclear antibodies in systemic rheumatic diseases. *Ann. Intern. Med*. **83**, 464.
24. Singsen, B. H., Bernstein, B. H., Kornreich, H. K., King, K. K., and Hanson, V. (1977). Mixed connective tissue disease in childhood: A clinical and serologic survey. *J. Pediatr*. **90**, 893.
25. Gilliam, J. N., and Prystowsky, S. D. (1977). Mixed connective tissue disease syndrome: The cutaneous manifestations of patients with epidermal nuclear staining and high titer antibody to Rnase sensitive extractable nuclear antigen (ENA). *Arch. Derm*. **113**, 583.
26. Grant, K. D., Adams, L. E., and Hess, E. V. (1981). Mixed connective tissue disease: A subset with sequential clinical and laboratory features. *J. Rheumatol*. **8**, 587.
27. Lundberg, I., and Hedfors, E. (1991). Clinical course of patients with anti-RNP antibodies: A prospective study of 32 patients. *J. Rheumatol*. **18**, 1511.
28. Sullivan, W. D., Hurst, D. J., Harmon, C. E., *et al.* (1984). A prospective evaluation emphasizing pulmonary involvement in patients with mixed connective tissue disease. *Medicine* **63**, 92.
29. Burdt, M., Hoffman, R. W., Deutscher, S. L., Wang, G. S., Johnson, J. C., and Sharp, G. C. (1999). Long-term outcome in mixed connective tissue disease: Longitudal clinical and serologic findings. *Arthritis Rheum*. **42**, 899.
30. Bennett, R. M., and O'Connell, D. J. (1980). Mixed connective tissue disease: A clinicopathologic study of 20 cases. *Semin. Arthritis Rheum*. **10**, 25.
31. Nakae, K., Furusawa, F., Kasukawa, R., *et al.* (1987). A nationwide epidemiological survey on diffuse collage diseases: Estimation of prevalence rate in Japan. *In* "Mixed Connective Tissue Disease and Anti-nuclear Antibodies" (R. Kasukawa and G. C. Sharp, eds.), pp. 9–13. Elsevier Science, Amsterdam.
32. Horn, J. R., Kapur, J. J., and Walker, S. E. (1978). Mixed connective tissue disease in siblings. *Arthritis Rheum*. **21**, 709.
33. Alarcón-Segovia, D., and Cardiel, M. H. (1989). Comparison between 3 diagnostic criteria for mixed connective tissue disease: Study of 593 patients. *J. Rheumatol*. **16**, 328.
34. Doria, A., Ghirardello, A., deZambias, P., Ruffatti, A., and Gambari, P. F. (1992). Japanese diagnostic criteria for mixed connective tissue disease in caucasian patients. *J. Rheumatol*. **19**, 259.
35. Porter, J. F., Kingsland, L. C., III, Lindberg, D. A. B., *et al.* (1988). The AI/Rheum knowledge-based computer consultant system in rheumatology: Performance in the diagnosis of 59 connective tissue disease patients from Japan. *Arthritis Rheum*. **31**, 219.
36. Frank, M. M., Lawley, T. J., Hamberger, M. I., and Brown, E. J. (1983). Immunoglobulin G: Fc receptor-mediated clearance in autoimmune disease. *Ann. Intern. Med*. **98**, 206.
37. Nimelstein, S. H., Brody, S., McShane, D., and Holman, H. R. (1980). Mixed connective tissue disease: A subsequent evaluation of the original 25 patients. *Medicine* **59**, 239.
38. Hainaut, P., Lavenne, E., Magy, J. M., and Lebacq, E. G. (1986). Circulating lupus type anticoagulant and pul-

monary hypertension associated with mixed connective tissue disease. *Clin. Rheum.* **5**, 96.
39. Kitridou, R. C., Akmal, M., Turkel, S. B., *et al.* (1986). Renal involvement in mixed connective tissue disease: A longitudinal clinicopathologic study. *Semin. Arthritis Rheum.* **16**, 135.
40. Manthorpe, R., Elling, H., van der Meulen, J. T., and Sorensen, S. F. (1980). Two fatal cases of mixed connective tissue disease: Description of case histories terminating as progressive systemic sclerosis. *Scand. J. Rheumatol.* **9**, 7.
41. Hosoda, Y. (1987). Review on pathology of mixed connective tissue disease. *In* "Mixed Connective Tissue Disease and Anti-nuclear Antibodies" (R. Kasukawa and G. C. Sharp, eds.), pp. 281–290. Elsevier Science, Amsterdam.
42. Jones, M. B., Osterholm, R. K., Wilson, R. B., *et al.* (1978). Fatal pulmonary hypertension and resolving immune-complex glomerulonephritis in mixed connective tissue disease: A case report and review of the literature. *Am. J. Med.* **65**, 855.
43. Salerni, R., Rodnan, G. P., Leon, D. F., and Shave, J. A. (1977). Pulmonary hypertension in the crest syndrome variant of progressive systemic sclerosis (scleroderma). *Ann. Intern. Med.* **86**, 394.
44. Singsen, B. H., Swanson, V. L., Bernstein, B. H., *et al.* (1980). A histologic evaluation of mixed connective tissue disease in childhood. *Am. J. Med.* **68**, 710.
45. Asherson, R. A., Higenbottam, T. W., Dinh Xuan, A. T., Khamashta, M. A., and Hughes, G. R. V. (1990). Pulmonary hypertension in a lupus clinic: Experience with twenty-four patients. *J. Rheumatol.* **17**, 1292.
46. Greidinger, E. L., Hoffman, R. W., Casciola-Rosen, L., and Rosen, A. (2000). Autoantibody recognition of distinctly modified forms of the U1-70 kDa antigen are associated with different clinical disease manifestations. *Arthritis Rheum.* **43**, 881.
47. Lundberg, I., Nennesmo, I., and Hedfors, E. (1992). A clinical, serological, and histopathological study of myositis patients with and without anti-RNP antibodies. *Semin. Arthritis Rheum.* **2**, 27.
48. Treadwell, E. L., Alspaugh, M. A., Wolfe, J. F., and Sharp, G. C. (1984). Clinical relevance of PM-1 antibody and physiochemical characterization of PM-1 antigen. *J. Rheumatol.* **11**, 658.
49. Wasicek, C. A., Reichlin, M., Montes, M., and Raghu, G. (1984). Polymyositis and interstitial lung disease in the patient with the anti-Jo1 prototype. *Am. J. Med.* **76**, 538.
50. Rasmussen, E. K., Ullman, S., Hoier-Madsen, M., Sorensen, S. F., and Halberg, P. (1987). Clinical implications of ribonucleoprotein antibody. *Arch. Derm.* **123**, 601.
51. Bennett, R. M., and O'Connell, D. J. (1978). The arthritis of mixed connective tissue disease. *Ann. Rheum. Dis.* **37**, 397.
52. Martinez-Cordero, E., and Lopez-Zepeda, J. (1990). Resorptive arthropathy and rib erosions in mixed connective tissue disease. *J. Rheumatol.* **17**, 719.
53. O'Connell, D. J., and Bennett, R. M. (1977). Mixed connective tissue disease: Clinical and radiological aspects of 20 cases. *Br. J. Radiol.* **50**, 620.
54. Piirainen, H. I. (1990). Patients with arthritis and anti-U1-RNP antibodies: A 10-year follow-up. *Br. J. Rheum.* **29**, 345.
55. Sargent, E. N., Turner, A. F., and Jacobson, G. (1969). Superior marginal rib defects: An etiologic classification. *Am. J. Roentgenol.* **106**, 491.
56. Lewis, R. A., Adams, J. P., Gerber, N. L., Decker, J. L., and Parsons, D. B. (1978). The hand in mixed connective tissue disease. *J. Hand. Surg.* **3**, 217.
57. Oxenhandler, R., Hart, M., Corman, L., Sharp, G., and Adelstein, E. (1977). Pathology of skeletal muscle in mixed connective tissue disease. *Arthritis Rheum.* **20**, 985.
58. Marshall, J. B., Kretschmar, J. M., Gerhardt, D. C., *et al.* (1990). Gastrointestinal manifestations of mixed connective tissue disease. *Gastroenterology* **98**, 1232.
59. Gutierrez, F., Valenzuela, J. E., Ehresmann, G. R., Quismorio, F. P., and Kitridou, R. C. (1982). Esophageal dysfunction in patients with mixed connective tissue disease and systemic lupus erythematosus. *Digest. Dis. Sci.* **27**, 592.
60. Cosnes, J., Levy, R. A., and Darnis, F. (1980). Budd-Chiari syndrome in a patient with mixed connective tissue disease. *Dig. Dis. Sci.* **25**, 467.
61. Lynn, J. T., Gossen, G., Miller, A., and Russell, I. J. (1984). Pneumatosis intestinalis in mixed connective tissue disease: Two case reports and literature review. *Arthritis Rheum.* **27**, 1186.
62. Norman, D. A., and Fleischmann, R. M. (1978). Gastrointestinal systemic sclerosis in serologic mixed connective tissue disease. *Arthritis Rheum.* **21**, 811.
63. Rosenberg, A. M., Petty, R. E., Cumming, G. R., and Koehler, B. E. (1979). Pulmonary hypertension in a child with mixed connective tissue disease. *J. Rheumatol.* **6**, 700.
64. Wiener-Kronish, J. P., Solinger, A. M., Warnock, M. L., *et al.* (1981). Severe pulmonary involvement in mixed connective tissue disease. *Am. Rev. Respir. Dis.* **124**, 499.
65. Udaya, B. S., and Prakash, M. D. (1985). Intrathoracic manifestations in mixed connective tissue disease. *Mayo Clin. Proc.* **60**, 813.
66. Germain, M. J., and Davidman, M. (1984). Pulmonary hemorrhage and acute renal failure in a patient with mixed connective tissue disease. *Am. J. Kidney Dis.* **3**, 420.
67. Martens, J., and Demedts, M. (1992). Diaphragm dysfunction in mixed connective tissue disease. *Scand. J. Rheumatol.* **11**, 165.
68. Sanchez-Guerrero, J., Cesarman, G., and Alarcón-Segovia, D. (1989). Massive pulmonary hemorrhage in mixed connective tissue disease. *J. Rheumatol.* **16**, 1132.
69. Komatireddy, G. R., Wang, G. S., Sharp G. C., and Hoffman, R. W. (1997). Antiphospholipid antibodies among anti-U1-70 kDa antibody positive patients with mixed connective tissue disease. *J. Rheumatol.* **24**, 319.

70. Alpert, M. A., Goldberg, S., Singsen, B. H., *et al.* (1983). Cardiovascular manifestations of mixed connective tissue disease in adults. *Circulation* **68**, 1182.
71. Cryer, P. F., and Kissane, J. M. (1978). Clinicopathologic Conference: Mixed connective tissue disease. *Am. J. Med*. **65**, 833.
72. Oetgen, W. J., Mutter, M. L., Davia, J. E., and Lawless, O. J. (1983). Cardiac abnormalities in mixed connective tissue disease. *Chest* **2**, 185.
73. Lash, A. D., Wittman, A. L., and Quismorio, F. P., Jr. (1986). Myocarditis in mixed connective tissue disease: Clinical and pathologic study of three cases and review of the literature. *Semin. Arthritis Rheum*. **15**, 288.
74. Comens, S. M., Alpert, M. D., Sharp, G. C., *et al.* (1989). Frequency of mitral valve prolapse in systemic lupus erythematosus, progressive systemic sclerosis and mixed connective tissue disease. *Am. J. Cardiol*. **63**, 369.
75. Hoffman, R. W. (2001). Mixed connective tissue disease. *In* "Textbook of Nephrology" (S. G. Massry and R. J. Glassock, eds.), pp. 787–790. Lippincot, Williams and Wilkins, PA.
76. Bresnihan, B., Bunn, C., Snaith, M. L., and Hughes, G. R. V. (1977). Antiribonucleoprotein antibodies in connective tissue diseases: Estimation by counter immunoelectrophoresis. *Br. Med. J*. **1**, 610.
77. Hoffman, R. W., Cassidy, J. T., Takeda, Y., Smith-Jones, E. I., Wang, G. S., and Sharp, G. C. (1993). U1-70 kD autoantibody-positive connective tissue disease in children: A longitudinal clinical and serologic analysis. *Arthritis Rheum*. **36**, 1599.
78. Takeda, Y., Wang, G. S., Wang, R. J., *et al.* (1989). Enzyme-linked immunosorbent assay using isolated (U) small nuclear ribonucleoprotein polypeptides as antigens to investigate the clinical significance of autoantibodies to these polypeptides. *Clin. Immunol. Immunopathol*. **50**, 213.
79. Bennett, R. M., Bong, D. M., and Spargol, B. H. (1978). Neuropsychiatric problems in mixed connective tissue disease. *Am. J. Med*. **65**, 955.
80. Searles, R. P., Mladinich, E. K., and Messner, R. P. (1978). Isolated trigeminal sensory neuropathy: Early manifestation of mixed connective tissue disease. *Neurology* **28**, 1286.
81. Varga, E., Field, E. A., and Tyldesley, W. R. (1978). Orofacial manifestations of mixed connective tissue disease. *Neurology* **28**, 1286.
82. Vincent, R. M., and Van Housen, R. N. (1980). Trigeminal sensory neuropathy and bilateral carpal tunnel syndrome: The initial manifestation of mixed connective tissue disease. *J. Neurol. Neurosurg. Psych*. **43**, 458.
83. Bronshvas, M. M., Prystowsky, S. D., and Traviesa, D. C. (1978). Vascular headaches in mixed connective tissue disease. *Headache* **18**, 154.
84. Hyslop, D. L., Singsen, B. H., and Sharp, G. C. (1980). Leukocyte function, infection and mortality in mixed connective tissue disease. *J. Neurol. Neurosurg. Psych*. **43**, 458.
85. Kappes, J., and Bennett, R. M. (1982). Cauda equina syndrome in a patient with high titer anti-RNP antibodies. *Arthritis Rheum*. **25**, 349.
86. Kraus, A., Cervantes, G., Barojas, E., and Alarcón-Segovia, D. (1985). Retinal vasculitis in mixed connective tissue disease. *J. Rheumatol*. **12**, 1122.
87. Martyn, J. B., Wong, M. J., and Huang, S. H. (1988). Pulmonary and neuromuscular complications of mixed connective tissue disease: A report and review of the literature. *J. Rheumatol*. **15**, 703.
88. Weiss, T. D., Nelson, J. S., Woolsey, R. M., Zuckner, J., and Baldassare, A. R. (1978). Transverse myelitis in mixed connective tissue disease. *Arthritis Rheum*. **21**, 982.
89. Segond, P., Yeni, P., Jacquot, J. M., and Massias, P. (1978). Severe autoimmune anemia and thrombopenia in mixed connective tissue disease. *Arthritis Rheum*. **21**, 995.
90. ter Borg, E. J., Houtman, P. M., Kallenberg, C. G. M., van Leeuwen, M. A., and van Ryswyk, M. H. (1988). Thrombocytopenia and hemolytic anemia in a patient with mixed connective tissue disease due to thrombotic thrombocytopenic purpura. *J. Rheumatol*. **15**, 1174.
91. de Rooij, D. J. R. A. M., vand de Putte, L. B. A., and van Beusekom, H. J. (1982). Severe thromboc. ytopenia in mixed connective tissue disease. *Scand. J. Rheumatol*. **11**, 184.
92. Julkunen, H., Jantti, J., and Pettersson, T. (1989). Pure red cell aplasia in mixed connective tissue disease. *J. Rheumatol*. **16**, 1385.
93. Setty, Y. N., Pittman, C. B., Mahale, A. S., Greidinger, E. L., and Hoffman, R. W. (2003). Sicca symptoms and anti-SSA/Ro antibodies are common in mixed connective tissue disease. *J. Rheumatol*.
94. Fraga, A., Gudino, J., Ramos-Niembro, F., and Alarcón-Segovia, D. (1978). Mixed connective tissue disease in childhood: Relationship with Sjögren's syndrome. *Am. J. Dis. Child*. **132**, 263.
95. Kaufman, R. L., and Kitridou, R. C. (1982). Pregnancy in Mixed connective tissue disease comparison with systemic lupus erythematosus. *J. Rheumatol*. **9**, 549.
96. Lundberg, I., and Hedfors, E. (1991). Pregnancy outcome in patients with high titer anti-RNP antibodies: A retrospective study of 40 pregnancies. *J. Rheumatol*. **18**, 359.
97. Diaz-Jouanen, E., Llorente, L., Ramos-Niembro, F., and Alarcón-Segovia, D. (1977). Cold-reactive lymphocytotoxic antibodies in mixed connective tissue disease. *J. Rheumatol*. **4**, 4.
98. Halla, J. T., Schronhenloher, R. E., Hardin, J. G., and Volanakis, J. E. (1978). Circulating immune complexes in mixed connective tissue disease (MCTD). *Arthritis Rheum*. **21**, 562.
99. Alarcón-Segovia, D. (1979). Mixed connective tissue disease: Appearance of antibodies to ribonucleoprotein following corticosteroid treatment. *J. Rheumatol*. **6**, 694.
100. Pettersson, I., Wang, G., Smith, E. I., *et al.* (1986). The use of immunoblotting and immuoprecipitation of (U) small nuclear ribonucleoproteins in the analysis of sera of patients with mixed connective tissue disease and sys-

temic lupus erythematosus: A cross-sectional, longitudinal study. *Arthritis Rheum*. **29**, 986.
101. Takano, M., Golden, S. S., Sharp, G. C., and Agris, P. F. (1981). Molecular relationships between two nuclear antigens and their biochemical characterization. *Biochemistry* **21**, 5929.
102. Lerner, M. R., and Steitz, J. A. (1979). Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA* **76**, 5495.
103. Lerner, M. R., Boyle, J. A., Hardin, J. A., and Steitz, J. A. (1981). Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science* **221**, 400.
104. Pettersson, I., hinterberger, M., Mimori, T., Gottlieb, E., and Steitz, J. A. (1984). The structure of mammalian small nuclear ribonucleoproteins: Identification of multiple protein and anti-Sm autoantibodies. *J. Biol. Chem*. **259**, 5907.
105. Hoch, S. O. (1994). The Sm antigens. *In* "Manual of Biological Markers of Disease" (W. J. van Venrooij and R. N. Maini, eds.), pp. B24/1–29. Kluwer Academic, Dordrecht, The Netherlands.
106. Zieve, G. W., and Sauterer, R. A. (1990). Cell biology of the snRNP particles. *Crit. Rev. Biochem. Mol. Biol*. **25**, 1–46.
107. Holyst, M.-M., and Hoffman, R. W. (1998). U-small nuclear ribonucleoprotein (RNP)-reactive autoantibodies: Diagnostic testing and clinical interpretation. *Clin. Immunol. Newsletter* **18**, 53–57.
108. Deutscher, S. L., and Keene, J. D. (1988). A sequence-specific conformational epitope on U1 RNA is recognized by a unique autoantibody. *Proc. Natl. Acad. Sci. USA* **85**, 3299.
109. Hoffman, R. W., Sharp, G. C., and Deutscher, S. L. (1995). Analysis of anti-U1-RNA antibodies in connective tissue disease patients: Association with HLA and clinical manifestations of disease. *Arthritis Rheum*. **38**, 1837.
110. Hoet, R. M., Koornneef, I., de Rooij, D. J., van de Putte, L. B., and van Venrooij, W. J. (1992). Changes in anti-U1 RNA antibody levels correlate with disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum*. **35**, 1202.
111. Fritzler, M. J., Ali, R., and Tan, E. M. (1984). Antibodies from patients with mixed connective tissue disease react with heterogeneous nuclear ribonucleoprotein or ribonucleic acid (hnRNP/RNA) of the nuclear matrix. *J. Immunol*. **132**, 1216.
112. Salden, M. H. L., Van Eekelen, C. A. G., Habets, W. J. A., *et al.* (1982). Antinuclear matrix antibodies in mixed connective tissue disease. *Eur. J. Immunol*. **12**, 783.
113. Skriner, K., Sommergruber, W. H., Tremmel, V., Fischer, I., Barta, A., Smolen, J. S., and Steiner, G. (1997). Anti-A2/RA33 autoantibodies are directed to the RNA binding region of the A2 protein of the heterogeneous nuclear ribonucleoprotein complex: Differential epitope recognition in rheumatoid arthritis, systemic lupus erythematosus, and mixed connective tissue disease. *J. Clin. Invest*. **100**, 127.
114. Seyer, J. M., Kang, A. H., and Rodnan, G. (1981). Investigation of type I and type III collagens of the lung in progressive systemic sclerosis. *Arthritis Rheum*. **24**, 625.
115. Peller, J. S., Gabor, G. T., Porter, J. M., and Bennett, R. M. (1985). Angiographic findings in mixed connective tissue disease. *Arthritis Rheum*. **23**, 768.
116. Bennett, R. M., and Spargo, B. H. (1977). Immune complex nephropathy in mixed connective tissue disease. *Am. J. Med*. **63**, 534.
117. Cohen, I. M., Swerdlin, I. H. R., Steinberg, S. M., and Stone, R. A. (1980). Mesangial proliferative glomerulonephritis in mixed connective tissue disease (MCTD). *Clin. Nephrol*. **13**, 93.
118. Kobayashi, S., Nagase, M., Kimura, M., *et al.* (1985). Renal involvement in mixed connective tissue disease. *Am. J. Nephrol*. **5**, 282.
119. Sawai, T., Hara, S., Motomo, N., and Kyrogoku, M. (1987). Histopathological studies on the kidney, skin, and synovial tissue of MCTD. *In* "Mixed Connective Tissue Disease and Anti-nuclear Antibodies" (R. Kasukawa and G. C. Sharp, eds.), pp. 291–296. Elsevier Science, Amsterdam.
119a. Sharp, G. C., and Sinapen, B. H. (1985). Mixed connection tissue disease, *In* "the Autoimmune Diseases" (N. Roseard, I. Mackay, eds.), p. 105. Academic Press, Orlando, FL.
120. Hoffman, R. W., and Sharp, G. C. (1995). Is anti-U1-RNP autoantibody positive connective tissue disease genetically distinct? *J. Rhematol*. **22**, 586.
121. Sharp, G. C., and Hoffman, R. W. (1995). Mixed connective tissue disease. *In* "Connective Tissue Disease" (J. F. Belch and R. B. Zurier, eds.), pp. 151–178. Chapman and Hall, New York.
122. Hsu, K. C., Hill, D., and Hoffman, R. W. (1992). HLA-DPB1*0401 is associated with the presence of autoantibodies reactive with the U1-70kD polypeptide antigen of U1-small nuclear ribonucleoprotein among connective tissue disease patients. *Tissue Antigens* **39**, 272.
122a. Brown, J. H., Jardetzlu, T., Saper, M. A., *et al*. (1988). A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature* **332**, 845.
123. Hoffman, R. W., Sharp, G. C., Irvin, W. S., *et al.* (1991). Association of immunoglobulin Km and Gm allotypes with specific antinuclear antibodies and disease susceptibility among connective tissue disease patients. *Arthritis Rheum*. **34**, 453.
124. Smolen, J. S., and Steiner, G. (1998). Mixed connective tissue disease: To be or not to be? *Arthritis Rheum*. **41**, 768.
125. Farhey, Y., and Hess, E. V. (1997). Mixed connective tissue disease. *Arthritis Care Res*. **10**, 333.
126. Sharp, G. C., and Hoffman, R. W. (1999). Clinical, immunologic, and immunogenetic evidence that mixed connective tissue disease is a distinct entity: Comment on the article by Smolen and Steiner. *Arthritis Rheum*. **42**, 190.
127. Hoffman, R. W., and Greidinger, E. L. (2000). Mixed connective tissue disease. *Curr. Opin. Rheumatol*. **12**, 386.

128. Hoffman, R. W., and Greidinger, E. L. (2001). Mixed connective tissue disease. *In* "Modern Therapeutics in Rheumatic Diseases" (G. C. Tsokos, L. W. Moreland, G. M. Kamen, J. P. Pelletier, J. Martel-Pelletier, and S. Gay, eds.), pp. 347–357. Humana Press, New Jersey.
129. Gendi, N. S. T., Welsh, K. I., van Venrooij, W., *et al.* (1995). HLA type as a predictor of mixed connective tissue disease differentiation: Ten-year clinical and immunogenetic followup of 46 patients. *Arthritis Rheum*. **38**, 259.
130. Unanue, E. R. (1993). Macrophages, antigen-presenting cells and the phenomenon of antigen handling and presentation. *In* "Fundamental Immunology" (W. E. Paul, ed.), 3rd Ed., pp. 111–144. Raven Press, New York.
131. Maddison, P. J., and Reichlin, M. (1977). Quantitation of precipitating antibodies to certain soluble nuclear antigens in SLE: Their contibution to hypergammaglobulinemia. *Arthritis Rheum*. **20**, 819.
132. Hoffman, R. W. (2001). T cells in the pathogenesis of systemic lupus erythematosus. *Front. Biosci*. **6D**, 1369.
133. O'Brien, R. M., Cram, D. S., Coppel, R. L., and Harrison, L. C. (1990). T-cell epitopes on the 70-kDa protein of the (U1)RNP complex in autoimmune rheumatologic disorders. *J. Autoimm*. **3**, 858.
134. Fenning, S., Wolff-Vorbeck, G., Hackl, W., *et al.* (1995). T cell lines recognizing the 70kD protein of U1 small nuclear ribonucleoprotein (U1snRNP). *Clin. Exp. Immunol*. **101**, 408.
135. Hoffman, R. W., Takeda, Y., Sharp, G. C., *et al.* (1993). Human T cell clones reactive against U-small nuclear ribonucleoprotein autoantigens from connective tissue disease patients and healthy individuals. *J. Immunol*. **151**, 6460.
136. Wolff-Vorbeck, G., Hackl, W., Fenning, S., *et al.* (1994). Characterization of an HLA-DR4-restricted T cell clone recognizing a protein moiety of small nuclear ribonucleoproteins (UsnRNP). *Clin. Exp. Immunol*. **95**, 378.
137. Holyst, M.-M., Hill, D. L., Hoch, S. O., and Hoffman, R. W. (1997). Analysis of human T cell and B cell responses against U small nuclear ribonucleoprotein 70-kd, B, and D polypeptides among patients with systemic lupus erythematosus and mixed connective tissue disease. *Arthritis Rheum*. **40**, 1493.
138. Talken, B. L., Holyst, M.-M., Hill, D. L., and Hoffman, R. W. (1999). Non-random T cell receptor usage among U-small nuclear ribonucleoprotein specific human T cell clones from connective tissue disease patients. *Arthritis Rheum*. **42**, 703.
139. Talken, B. L., Bailey, C. W., Reardon, S. L., Caldwell, C. W., and Hoffman, R. W. (2001). Structural analysis of T cell receptor (TCR) alpha and beta chains from human T cell clones specific for small nuclear ribonucleoprotein polypeptides Sm-D, Sm-B, and U1-70kD: TCR complementarity determining region 3 usage appears highly conserved. *Scand. J. Immunol*. **54**, 204.
140. Morel, L., Mohan, D., Yu, Y., *et al.* (1997). Functional dissection of systemic lupus erythematosus using congenic mouse strains. *J. Immunol*. **158**, 6019.
141. Harley, J. B., Moser, K. L., Gaffney, P. M., and Behrens, T. W. (1998). The genetics of human systemic lupus erythematosus. *Curr. Opin. Immunol*. **10**, 690.
142. Wakeland, E. K., Liu, K., Graham, R. R., and Behrens, T. W. (2001). Delineating the genetic basis of systemic lupus erythematosus. *Immunity* **15**, 397.
143. Hoffman, R. W., and Greidinger, E. L. (2001). Mixed connective tissue disease. *Medicine*. **2**.
144. Takada, K., Illei, G. G., and Boumpas, D. T. (2001). Cyclophosphamide for the treatment of systemic lupus erythematosus. *Lupus* **10**, 154.
145. Alpert, M. A., Pressly, T. A., Mukerji, V., *et al.* (1991). Acute and long-term effects of nifedipine on pulmonary and systemic hemodynamics in patients with pulmonary hypertension associated with diffuse systemic sclerosis, the CREST syndrome and mixed connective tissue disease. *Am. J. Cardiol*. **68**, 1987.
146. Alpert, M. A., Pressly, T. A., Mukerji, V., Lambert, C. R., and Mukerji, B. (1992). Short- and long-term hemodynamic effects of captopril in patients with pulmonary hypertension and selected connective tissue disease. *Chest*. **102**, 1407.
147. Dahl, M., Chalmers, A., Wade, J., Calverley, D., and Munst, B. (1992). Ten year survival of a patient with advanced pulmonary hypertension and mixed connective tissue disease treated with immunosuppressive therapy. *J. Rheumatol*. **19**, 1807.
148. Friedman, D. M., Mitnick, H. J., and Danilowicz, D. (1992). Recovery from pulmonary hypertension in an adolescent with mixed connective tissue disease. *Ann. Rheum. Dis*. **51**, 1001.
149. Cassidy, J. T., Wortmann, D. W., Nelson, A. M., *et al.* (1992). Clinical outcome of children with mixed connective tissue disease (MCTD). *J. Rheumatol*. **19**(Suppl. 33), 121.
150. Heaten, J. M. (1959). Sjögren's syndrome and systemic lupus erythematosus. *Br. Med. J*. **1**, 466.
151. Steinberg, A. D., and Talal, N. (1971). The coexistence of Sjögren's syndrome and systemic lupus erythematosus. *Ann. Intern. Med*. **74**, 55.
152. Strand, V., and Talal, N. (1980). Advances in the diagnosis and concept of Sjögren's syndrome autoimmune exocrinopathy. *Bull. Rheum. Dis*. **30**, 1046.
153. Alexander, E. L., Malinow, K., Lejewski, J. E., *et al.* (1986). Primary Sjögren's syndrome with central nervous system disease mimicking multiple sclerosis. *Ann. Intern. Med*. **104**, 323.
154. Alexander, E. L. (1993). MS and Sjögren's syndrome. *Neurology* **43**, 455.
155. Mariette, X., Zerbib, M., Jaccard, A., *et al.* (1993). Hepatitis C virus and Sjögren's syndrome. *Arthritis Rheum*. **36**, 280.
156. Ramos-Casals, M., Garcia-Carrasco, M., Cervera, R., Rosas, J., Trejo, O., de la Red, G., Sanchez-Tapias, J. M., Font, J., and Ingelmo, M. (2001). Hepatitis C virus infection mimicking primary Sjögren's syndrome: A clinical and immunologic description of 35 cases. *Medicine* **80**, 1.
157. Wattiaus, M. J. (1997). Gougerot Sjogrens syndrome and hepatitis C virus: What relationship? *Presse Med*. **26**, 652.

158. Bonafede, R. P., Downey, D. C., and Bennett, R. M. (1995). An association of fibromyalgia with primary Sjögren's syndrome: A prospective study of 72 patients. *J. Rheumatol*. **22**, 133.
159. Simmons, O., Brien, E., Chen, S., *et al.* (1995). One hundred anti-Ro (SS-A) antibody positive patients: a 10-year follow-up. *Medicine* **74**, 109.
160. Vitali, C., Sciuto, M., Neri, R., *et al.* (1992). Anti-hepatitis C virus antibodies in primary Sjögren's syndrome: False positive results are related to hyper gamma globulinemia. *Clin. Exp. Rheum*. **10**, 103.
161. Moriwaka, F., Tashiro, K., Fukawawa, T., *et al.* (1990). A case of systemic lupus erythematosus: Its clinical and MRI resemblance to multiple sclerosis. *Jpn. J. Psychiat. Neurol*. **44**, 601.
162. Hammoudeh, M., and Khan, M. A. (1982). Clinical variant of systemic lupus erythematosus resembling multiple sclerosis. *J. Rheumatol*. **9**, 336.
163. Middleton, G. D., McFarlin, J. E., and Lipsky, P. E. (1994). The prevalence and clinical impact of fibromyalgia in systemic lupus erythematosus. *Arthritis Rheum*. **37**, 1181.
164. Liote, F., and Osterland, C. (1994). Autonomic neuropathy in SLE: Cardiovascular autonomic functional assessment. *Ann. Rheum. Dis*. **53**, 671.
165. Fox, R. (1994). Classification criteria for Sjögren's syndrome. *Rheum. Dis. Clin. North. Am*. **20**, 391.
166. Kincaid, M. C. (1987). The eye in Sjögren's syndrome. *In* "Sjögren's Syndrome" (N. Talal, H. M. Moutsopoulos, and S. S. Kassan, eds.), pp. 25–33. Springer-Verlag, New York.
167. Whaley, K., Williamson, J., Chisholm, D. M., *et al.* (1973). Sjögren's syndrome. I. Sicca components. *Quart. J. Med*. **42**, 279.
168. Block, K. J., Buchanan, W. W., Wohl, M. R., and Bunim, J. J. (1965). Sjögren's syndrome: A clinical, pathologic and serologic study of sixty-two cases. *Medicine* **44**, 187.
169. Daniels, T. E. (1987). Oral manifestations of Sjögren's syndrome. *In* "Sjögren's Syndrome" (N. Talal, H. M. Moutsopoulos, and S. S. Kassan, eds.), pp. 15–24. Springer-Verlag, New York.
170. Calabrese, L. H., Davis, M. E., and Wilke, W. S. (1994). Chronic fatigue syndrome and a disorder resembling Sjögren's syndrome: Preliminary report. *Clin. Infect. Dis*. **18**(Suppl.), S28.
171. Gudbjorsson, B., Broman, J. E., Hetta, J., and Hallgren, R. (1993). Sleep disturbances in patients with primary Sjögren's syndrome. *Br. J. Rheumatol*. **32**, 1072.
172. Kassan, S. S., and Talal, N. (1987). Renal disease with Sjögren's syndrome. *In* "Sjögren's Syndrome" (N. Talal, H. M. Moutsopoulos, and S. S. Kassan, eds.), pp. 96–101. Springer-Verlag, New York.
173. Alexander, E. L. (1987). Neuromuscular complications of primary Sjögren's syndrome. *In* "Sjögren's Syndrome (N. Talal, H. M. Moutsopoulos, and S. S. Kassan, eds.), Springer-Verlag, New York.
174. Grant, I. A., Hunder, G. C., Homburger, H. A., and Dyck, P. J. (1997). Peripheral neuropathy associated with sicca complex. *Neurology* **48**, 855.
175. Kassan, S. S., Thomas, T. L., Moutsopoulos, H. M., *et al.* (1978). Increased risk of lymphoma in Sjögren's syndrome. *Ann. Intern. Med*. **89**, 888.
176. DeVita, S., Ferraccioli, G., De Re, V., *et al.* (1994). The polymerase chain reaction detects B cell clonalities in patients with Sjögren's syndrome and suspected malignant lymphoma. *J. Rheumatol*. **21**, 1497.
177. Fishleder, A., Tubbs, R., Hesse, B., and Levin, H. (1987). Immunoglobulin-gene rearrangement in benign lymphoepithelial lesions. *N. Engl. J. Med*. **316**, 1118.
178. Freimark, B., Fantozzi, R., Bone, R., Bordin, G., and Fox, R. (1994). Detection of clonally expanded salivary gland lymphocytes in Sjögren's syndrome. *Arthritis Rheum*. **37**, 1441.
179. Kipps, T. J., Tomhave, E., Chen, P. P., and Fox, R. I. (1989). Molecular characterization of a major autoantibody-associated cross-reactive idiotype in Sjögren's syndrome. *J. Immunol*. **142**, 4261.
180. Pablos, J. L., Carreira, P. E., Morillas, L., *et al.* (1994). Clonally expanded lymphocytes in the minor salivary glands of Sjögren's syndrome patients without lymphoproliferative disease. *Arthritis Rheum*. **37**, 1441.
181. Pisa, E. K., Pisa, P., Kang, H. I., and Fox, R. I. (1991). High frequency of t(14;18) translocation in salivary gland lymphomas from Sjögren's syndrome patients. *J. Exp. Med*. **174**, 1245.
182. Speight, P. M., Jordan, R., Colloby, P., Nandha, H., and Pringle, J. H. (1994). Early detection of lymphomas in Sjögren's syndrome by in situ hybridization for kappa and lambda light chain mRNA in labial salivary glands. *Eur. J. Cancer* **4**, 244.
183. Ben-Chetrit, E., Fox, R. I., and Tan, E. M. (1990). Dissociation of immune responses to the SS-A (Ro) 52kd and 60kd polypeptides in systemic lupus erythematosus and Sjögren's syndrome. *Arthritis Rheum*. **33**, 349.
184. Chan, E. K., Francoeur, A. M., and Tan, E. M. (1986). Epitopes, structural domains and asymmetry of amino acids residues in SS-B/La nuclear protein. *J. Immunol*. **136**, 3744.
185. Chambers, J. C., and Keene, J. D. (1985). Isolation and analysis of cDNA clones expressing human lupus La antigen. *Proc. Natl. Acad. Sci. USA* **82**, 2115.
186. Rader, M. D., Codding, C., and Reichlin, M. (1989). Differences in the fine specificity of anti-Ro (SS-A) in relation to the presence of other precipitating autoantibodies. *Arthritis Rheum*. **32**, 1563.
187. Daniels, T. E. (1984). Labial salivary gland biopsy in Sjögren's syndrome: Assessment as a diagnostic criteria in 362 suspected cases. *Arthritis Rheum*. **27**, 147.
188. Daniels, T. E., and Whitcher, J. P. (1994). Association of patterns of labial salivary gland inflammation with keratoconjunctivitis sicca: Analysis of 618 patients with suspected Sjögren's syndrome. *Arthritis Rheum*. **37**, 869.
189. Scofield, R. H., Frank, M. B., Neas, B. R., *et al.* (1994). Cooperative association of T cell beta receptor and HLA-DQ alleles in the production of anti-Ro in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **72**, 335.

190. Frank, M. B., McArthur, R., Harley, J. B., and Fujisaku, A. (1990). Anti-Ro autoantibodies are associated with T cell receptor beta genes in systemic lupus erythematosus patients. *J. Clin. Invest*. **85**, 33.
191. Sasaki, M., Nakamura, S., Ohyama, Y., Shinohara, M., Ezaki, I., Hara, H., Kadena, T., Kishihara, K., Yamamoto, K., Nomoto, K., and Shirasuna K. (2000). Accumulation of common T cell clonotypes in the salivary glands of patients with human T lymphotropic virus type I-associated and idiopathic Sjögren's syndrome. *J. Immunol*. **164**, 2823.
192. Hoffman, R. W., and Greidinger, E. L. (2001). Sjögren's syndrome. *In* "Modern Therapeutics in Rheumatic Diseases" (G. C. Tsokos, L. W. Moreland, G. M. Kamen, J. P. Pelletier, J. Martel-Pelletier, and S. Gay, eds.), pp. 337–345. Humana Press, New Jersey.
193. Steinberg, A. D., and Talal, N. (1971). The coexistence of Sjögren's syndrome and systemic lupus erythematosus. *Ann. Intern. Med*. **74**, 55.
194. Grennan, D. M., Ferguson, M., Ghobarey, A. E., *et al.* (1977). Sjögren's syndrome in SLE. An examination of the clinical signficance of Sjögren's syndrome by comparison of its frequency in typical and atypical forms of SLE, overlap syndrome and scleroderma. *N. Zeal. Med. J*. **86**, 376.
195. Reveille, J. D., and Arnett, F. C. (1992). The immunogenetics of Sjögren's syndrome. *Rheum. Dis. Clin. North Am*. **18**, 539.
196. Provost, T. T., Watson, R., and Simmons-O'Brien, E. (1997). Anti-Ro(SS-A) antibody positive Sjögren's/lupus erythematosus overlap syndrome. *Lupus* **6**, 105.
197. Reimber, G., Steen, V. D., Penning, C. A., Medsger, T. A., Jr., and Tan, E. M. (1988). Correlates between autoantibodies to nucleolar antigens and clinical features in patients with systemic sclerosis (scleroderma). *Arthritis Rheum*. **31**, 525.
198. Genth, E., Miera, R., Genetzky, P., *et al.* (1990). Immunogenetic associations of scleroderma-related antinuclear antibodies. *Arthritis Rheum*. **33**, 657.
199. Mimori, T., Hardin, J. A., and Steitz, J. A. (1986). Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders. *J. Biol. Chem*. **261**, 2274.
200. Yaneva, M., and Arnett, F. C. (1989). Antibodies against Ku protein in sera from patients with autoimmune disease. *Clin. Exp. Immunol*. **76**, 366.
201. Marguerie, C., Bunn, C. C., Beyon, H. L., *et al.* (1990). Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. *Quart. J. Med*. **77**, 1019.
202. Arnett, F. C., Hirsch, T. J., Bias, W. B., Nishikai, M., and Reichlin, M. (1981). The Jo-1 antibody system in myositis: Relationships to clinical features and HLA. *J. Rheumatol*. **8**, 925.
203. Satoh, M., Ajmani, A. K., Ogasawara, T., *et al.* (1994). Autoantibodies to RNA polymerase II are common in systemic lupus erythematosus and overlap syndrome. *J. Clin. Invest*. **94**, 1981.
204. Alarcon, G. S., Williams, G. V., Singer, J. Z., *et al.* (1991). Early undifferentiated connective tissue disease. I. Early clinical manifestations in a large cohort of patients with undifferentiated connective tissue disease compared with cohorts of patients with well-established connective tissue disease. *J. Rheumatol*. **18**, 1332.
205. Alarcon, G. S., Wilkens, R. F., Ward, J. R., *et al.* (1996). Early undifferentiated connective tissue disease. IV. Musculoskeletal manifestations in a large cohort of patients with undifferentiated connective tissue diseases: Follow-up analyses in patients with unexplained polyarthritis and patients with rheumatoid arthritis at baseline. *Arthritis Rheum*. **39**, 403.

25

SYSTEMIC LUPUS ERYTHEMATOSUS AND FIBROMYALGIA

John B. Winfield

ABSTRACT

The fibromyalgia syndrome with its associated chronic pain and multiple painful tender points occurs in more than 25% of patients with systemic lupus erythematosus (SLE). Variables that contribute to pain sensitivity and other symptomatology in fibromyalgia include female gender, poor sleep, deconditioning, a variety of psychological elements, negative beliefs and attributions, and loss of control. Adverse childhood experiences, especially sexual abuse, contribute to vulnerability to chronic, unrelieved stress and psychological distress as adults. Psychological stress in turn leads to dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) axis, the autonomic nervous system, and central nociceptive processing. Proinflammatory cytokines are important mediators, both centrally and peripherally, of fatigue, poor sleep, musculoskeletal pain, and "sickness" in fibromyalgia and SLE. Although the psychological stress and distress that accompanies comorbid fibromyalgia in SLE negatively influences functional impairment and quality of life, and contributes importantly to fatigue and cognitive impairment, the available evidence does not support a role in flares of SLE disease activity or in major organ system injury over time. Optimum clinical assessment and therapy of SLE requires recognition of the contribution of fibromyalgia to overall morbidity in SLE. © 2003, Elsevier Science (USA).

INTRODUCTION

Pain, fatigue, sleep, and quality of life in SLE are strongly influenced by comorbid fibromyalgia and related chronic pain and fatigue syndromes. This chapter reviews the clinical classification of fibromyalgia, its high prevalence in people with SLE, evidence concerning the pathophysiology of pain and fatigue, and the extremely important contribution of psychological stress and distress to overall morbidity in this disorder. While organ system damage in SLE appears to be a consequence of strictly biological disease variables, the possibility that psychological distress contributes to the genesis of autoimmunity and to SLE flares remains an open question.

CLINICAL CLASSIFICATION OF FIBROMYALGIA

Fibromyalgia is a syndrome of chronic widespread pain, fatigue, poor sleep, and multiple somatic complaints. A generalized decrease in the pain perception threshold and in the threshold for pain tolerance is present, which reflect altered central nociceptive processing (see [1] for review). The 1990 American College of Rheumatology (ACR) classification criteria for fibromyalgia specify (1) presence of widespread pain for

>3 months and (2) pain, not just tenderness, that can be elicited by manual pressure of ~4 kg/cm^2 at 11 or more defined tender points [2]. Fibromyalgia is not a discrete entity, however. Because of the strong correlation of the number of painful tender points with psychological distress in fibromyalgia [3], in SLE [4] and other rheumatic disease, and in the general population [5, 6], fibromyalgia as classified by the ACR criteria is the tip of the iceberg. Thus, "fibromyalgia" represents the extreme end of a *continuum* of psychological distress and pain in the population. Clinicians experienced in this area do not slavishly apply the ACR criteria for diagnosis in practice. Most people with fibromyalgia will meet classification criteria for chronic fatigue syndrome and multiple other regional pain syndromes. At our current level of understanding, fibromyalgia is best classified as one of a series of largely overlapping "symptom-based conditions" [7], "functional somatic syndromes" [8], or "unexplained clinical conditions" [8a] that can be grouped together with mood and anxiety disorders.

PREVALENCE OF FIBROMYALGIA IN THE GENERAL POPULATION AND IN SYSTEMIC LUPUS ERYTHEMATOSUS

Chronic pain and fatigue are extremely prevalent in the general population, especially among women, and constitute a huge societal burden: regional pain, 20%; widespread pain, 11%; fibromyalgia by ACR criteria, 3–5% in females, 0.5–1.6% in males; chronic fatigue, ~20% [9–11]. In the United States, fibromyalgia as classified by the ACR criteria appears to be especially common in SLE: prevalence of fibromyalgia in clinic SLE populations is consistently ~20–25% [4, 12–14]. In other countries, for example, India, Spain, and the United Kingdom, fibromyalgia by ACR criteria occurs in 8–10% of SLE patients [15–17]. These may all be underestimates. Thus, in a study of coping responses in 173 patients with SLE in the Hopkins Lupus Cohort, 17% had >11 painful tender points (met ACR criteria) and 45% had 1–10 painful tender points. The latter shared psychological characteristics of the SLE/fibromyalgia patients, but not of the SLE patients without tender points [4]. These findings are similar to an earlier study in which 22% of SLE patients met ACR criteria and another 23% had features of fibromyalgia ("probable fibromyalgia") [12]. Why fibromyalgia should be so common in SLE is less clear. Ascertainment bias undoubtedly contributes in this regard, as does the unrelieved stress and distress associated with a chronic illness like SLE. Additional research in the field of psychoneuroimmunology should provide answers to whether SLE predisposes to fibromyalgia and precisely how and to what extent psychological distress contributes to altered immune system function and autoimmunity (see [18–21] for recent reviews).

PATHOPHYSIOLOGY OF PAIN AND FATIGUE IN FIBROMYALGIA

The hallmarks of fibromyalgia are widespread chronic pain and reduction of pain thresholds. The latter renders peripheral stimuli painful that ordinarily are not painful, such as light touch (*allodynia*). The International Association for Study of Pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"[22]. The neurophysiologist views pain as a complex sensation–perception interaction involving simultaneous parallel processing of nociceptive input from the spinal cord that activates a central network encompassing the pain experience in multiple regions of the brain. These regions include sensory-discriminative elements of nociception, afferent input from somatic reflexes, and major contributions from pathways and regions of the brain concerned with emotional/motivational and cognitive aspects of pain, which determine the subjective intensity of pain. The hypothalamic–pituitary–adrenocortical axis and the sympathetic nervous system (principal effectors of the stress response) are activated, as well. Normally adaptive, the stress response may become maladaptive in chronic pain and fatigue syndromes, such as fibromyalgia.

Emotions, cognitive elements (what does the pain mean?), and other psychological factors (coping, self-efficacy for control of pain) represent the psychological correlates of biological systems attempting to restore homeostasis in response to the pain experience. Negative emotions (depression and anxiety), negative beliefs and attributions, and loss of control or unpredictability in one's environment all can function as stressors with actions in these systems. Important biological elements here include cytokines (IL-1 (interleukin-1), IL-6, TNF-α (tumor necrosis factor-α), others), the HPA axis, and the autonomic nervous system which, together with the immune system and the endocrine system, functions as a complex bidirectionally communicating network. Melzak has developed a *neuromatrix* model [23] to explain how the brain functions as a neural network to integrate endocrine, immune, autonomic, afferent, inflammatory, social and behavioral, and pain

inhibitory inputs that influence pain perception and pain behaviors.

In the aggregate, the above psychological elements constitute *psychological stress*, which can downregulate or dysregulate immune responses and contribute to the development of actual flulike "sickness." This sickness is characterized by synthesis of acute phase reactants by the liver and behavioral changes, including increased pain sensitivity, decreased activity, decreased social interaction, reduced cognitive function and vigilance, depressed mood, decreased sexual behavior, and subtle endocrinologic and immunologic correlates. Conversely, peripheral immune stimulation, either by infection or inflammation associated with active SLE, communicates with the brain using the same network to produce a sickness response [24, 25].

BIOPSYCHOSOCIAL MODEL OF CHRONIC PAIN AND FATIGUE

A useful approach to understanding fibromyalgia is based on Engel's biopsychosocial model of chronic illness: health status and outcomes in chronic illness are influenced by the interaction of biological, psychological, and sociological factors [26]. Important biological variables are gender, sleep, physical condition, stress/neuroendocrine and autonomic dysregulation, and central sensitization to pain. Fibromyalgia, like SLE occurs predominately in females, and female gender is associated with increased pain sensitivity generally (see [27] for review). In clinical and experimental studies comparing males and females, females have more pain at all sites and more use of analgesic medications, are more sensitive to pain stimuli, and have central pain modulatory systems that are influenced by phasic alterations in reproductive hormone levels. Of interest with respect to the latter gender characteristic is a recent observation that serum IL-6 levels are significantly increased during the luteal phase of the menstrual cycle [28]. Such observations are of obvious relevance to the oft-noted changes in well-being in healthy women and in disease activity in women with chronic conditions such as SLE and fibromyalgia during this phase of the menstrual cycle. Aversive stimuli and stressful tasks are more likely to evoke sympathetic nervous system and HPA axis responses and greater psychological responses in females than in males. Although data are conflicting [29], it has been observed that woman report essentially all symptoms, including unexplained (somatoform) symptoms, at least 50% more than men [30]. This gender effect, although correlated with anxiety and depressive disorders, remains significant after controlling for psychiatric morbidity.

Poor sleep is almost universal in fibromyalgia and is directly associated with perception of pain during sleep [31, 32]. While not the proximate cause of fibromyalgia, abnormal sleep affects both limbs of the stress response system and contributes to negative mood and cognitive difficulties [33, 34]. As already discussed, a growing body of evidence suggests that in fibromyalgia and related disorders, psychological stress initiates or perpetuates functional alterations of the stress response system, with multiple adverse neuroendocrine, autonomic nervous system, and immunologic effects [18–21, 35–38]. Therefore, it is plausible that neuroendocrine and autonomic dysregulation (sympathetic hyperactivity) contribute to diverse clinical manifestations in fibromyalgia and SLE, including psychological elements, for example, anxiety and depression. As discussed in detail later, however, current knowledge does not permit firm conclusions regarding the impact of psychological variables on more strictly biological aspects of clinical disease expression in SLE.

Cognitive-behavioral variables operant in the chronic pain and fatigue of fibromyalgia include pain beliefs and attributions, hypervigilance (expectancy), active and passive coping strategies, perceived self-efficacy for pain control, mood, depression and anxiety, personality traits and disorders, and pain behaviors. The repertoire of cognitive-behavioral variables in an adult have antecedents in earlier life that determine vulnerability to stress and influence the cognitive, affective, and behavioral aspects of the pain experience. These include poor health in parents, parental pain history, poor family environment, and childhood abuse, particularly sexual abuse [39, 40] (see [41] for review).

Environmental and sociocultural variables are the multiple experiences and forces in the environment and in life and culture that influence the course of chronic pain and fatigue. These include the psychosocial experiences during childhood discussed previously, spousal and family support, work environment/job satisfaction, ethnological factors, focus on definable causes, media hype, and primary and secondary gain. Influences here can be positive and helpful, for example, job satisfaction, or negative, for example, diagnostic "waffling" and inappropriate diagnostic testing. Environmental and cultural variables clearly impel the high prevalence of chronic pain disorders in Western societies.

PSYCHOLOGICAL STRESS IN FIBROMYALGIA

Persons with fibromyalgia often carry a huge psychological burden of stress and distress, and certain data

suggest that psychological distress can precede pain, although this remains an incompletely resolved question. Depression, anxiety, and psychiatric disorders are extremely common. Fibromyalgia patients in tertiary care centers have very high rates of lifetime (81%) and current (48%) psychiatric disorders, and elevations on self-report measures of depression, anxiety, hypochondriasis, and neuroticism [42]. Scores on the Rand 36-item Health Survey (SF-36), a self-report questionnaire measuring functional impairment and well-being, usually are in the severely impaired range in all areas (physical functioning, pain, role limitations, emotional well-being, social functioning, energy/fatigue, general health perception). Depression, maladaptive coping, low self-efficacy, and anxiety contribute to a vicious cycle of negative beliefs, distress, pain and fatigue, somatization, and functional impairment.

DOES PSYCHOLOGICAL STRESS CAUSE OR EXACERBATE SYSTEMIC LUPUS ERYTHEMATOSUS?

The idea that particularly significant psychological stressors, such as death or divorce, might be related causally to onset of SLE or flares in SLE disease activity was raised almost 50 years ago [43]. As reviewed by Herrmann and colleagues [44], stress seems to be one of the factors that can provoke juvenile chronic arthritis, and prospective data support the concept that minor daily stress can aggravate symptomatology and joint inflammation during the course of rheumatoid arthritis. From limited early data, these authors conclude, "[psychological] stress seems to act as an exacerbating factor in SLE, precipitating flares." More extensive information that became available during the past decade argues against this proposition, however, at least with respect to our current methods for assessing SLE disease activity.

In most studies comparing subjects with SLE and fibromyalgia "vs" subjects with SLE and no associated fibromyalgia, no differences between the groups with respect to clinical and laboratory measures of SLE disease activity are observed [12–14, 16]. This is especially apparent in studies using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and/or British Isles Lupus Activity Group (BILAG), which rely on more objective measures of actual inflammation [14, 16]. In a seminal study by Middleton *et al*. [12], Systemic Lupus Activity Measure (SLAM) scores were higher in the SLE/fibromyalgia group, but differences disappeared when the SLAM scores were recalculated after elimination of subjective symptoms that could be attributed to fibromyalgia, such as fatigue. Data regarding levels of anti-double-stranded (ds) DNA in SLE subjects with and without associated fibromyalgia are inconsistent [12, 45]. Associated fibromyalgia appears not to contribute to elevation of the SLICC/ACR damage index [14] or specific organ system involvement, but does make it more difficult for physicians to rate SLE disease activity [13]. Comorbid fibromyalgia in SLE is highly correlated with all domains of the SF-36 indicating worse quality of life (QOL) [14]. Fatigue, which may be the most disabling physical symptom in SLE, is associated with depression and abnormal SF-36 scores, but not with abnormal laboratory values and objective clinical measures of SLE disease activity or damage [14, 46, 47]. Finally, Akkasilpa *et al*. have observed a strong association in SLE of tender points with uncertainty and lack of "hardiness" [4]. In this study, patients with SLE were examined for tender points and assessed by the Health-Related Hardiness Scale (HRHS) and the Mishel Uncertainty in Illness Scale (MUIS). Thirty-eight percent of the subjects had no tender points, 45% had 1–10 tender points, and 17% had > or = 11 TP (tender points), thus meeting one of the ACR criteria for classification as fibromyalgia. Scores on HRHS and MUIS reflecting less hardiness and greater uncertainty correlated highly with increasing numbers of tender points, a measure of psychological distress [3].

The experience of psychosocial stress and attendant psychological distress appear to be more related to *patient perception of illness* in SLE, rather than to the expression of clinically measurable SLE disease activity [46, 48–51]. Adams *et al*. [50] observed that major life events, daily stressors, depression, anxiety, and anger were associated with severity of self-reported symptomatology, such as joint pain and abdominal distress. This was especially true in a subset of subjects, termed stress responders. In a very recent, prospective study, Ward and colleagues [51] directly approached the question of whether changes in depressive symptoms or anxiety led to changes in SLE disease activity. Outcome measures were the SLAM, the SLEDAI, and the European Consensus Lupus Activity Measure (ECLAM). Although as previously noted in an earlier study of the association of comorbid fibromyalgia with SLE disease activity [12], changes in depression and anxiety were positively correlated with SLAM scores, but not when SLAM scores were modified to exclude ratings of depression and fatigue. There was no association with SLEDAI or ECLAM scores or with physician global assessment of disease activity. These investigators found no evidence that psychological distress leads to increased SLE disease activity and concluded that "depression and anxiety scores parallel changes in *patients' assessments* of the activity of their SLE."

From the above, one should not conclude that fatigue and pain sensitivity in SLE or fibromyalgia have no biological basis. A large body of neuroscience research is beginning to clarify the bases for altered central nociceptive processing in chronic pain disorders. Altered cytokine profiles have been identified in fibromyalgia and chronic fatigue syndrome [52–54]. Fatigue certainly can be a symptom associated with systemic inflammation in SLE in the absence of fibromyalgia [16], although it continues to be difficult to precisely define its biological bases [55]. Certain data suggest that prolactin [56] and proinflammatory cytokines, such as TNF-α and IL-6 [52], provide a link between immune stimulation in the periphery, that is, *intrinsic* to SLE, and central nervous system (CNS)-mediated sleep, somnolence, and fatigue. The key concept with respect to symptomatology in SLE, particularly for those persons with SLE who are overly susceptible to psychological stress, is that input to central mechanisms that underlie fatigue and pain sensitivity can be peripheral inflammation, psychological distress, or both. This is especially true for persons with coexistent fibromyalgia. For example, Bruce *et al*. [47] found fatigue severity to be highly correlated with the tender point count (a "sedimentation rate" for distress irrespective of a diagnosis of fibromyalgia [3] in consecutive patients attending an academic Lupus Clinic ($r = 0.46$, $p < 0.001$).

STRESS AND IMMUNE RESPONSES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Only limited data are available concerning the effects of psychological stress on aspects of the immune response in SLE. Attenuated mobilization of leukocytes after acute (10 min of unpleasant environmental noise) or longer-term (administration of a two-h long battery of neuropsychological tests) psychological stress in SLE relative to healthy subjects or non-SLE subjects receiving corticosteroids has been observed [57]; the catecholamine response was normal in this study. Pawlak and colleagues [58] reported that public speaking, an acute psychological stressor, induces similar psychological, cardiovascular, and neuroendocrine responses in patients and controls, but SLE patients exhibit less pronounced increases in natural killer (NK) cell numbers and NK activity than do control subjects, and failure to express increased numbers of β(2)-adrenoreceptors on peripheral blood mononuclear cells. This group also found a stress-induced increase in IL-4-producing cells in SLE patients only, whereas IFN γ^+ cell numbers increased due to stress in SLE subjects and control groups [59]. Consistent with the idea that there is altered sympathetic regulation in SLE, is evidence that baseline and post-hCRH testing in moderately active SLE shows sympathetic hyperactivity as assessed by blood pressure and heart rate measurements [60]. Such data obviously raise the question of whether changes in cytokine patterns might be responsible for stress-induced exacerbation of disease activity in SLE.

In a prospective study of the effect of psychosocial stress carried out over nine time points at 6-week intervals, [48], a clear relationship with laboratory and clinical indices of disease activity in SLE was not evident. The static design of this study has been criticized by Schubert *et al*. [61] as unsuitable for revealing true effects because of the enormous individual variation in the psychosocial, laboratory, and clinical variables and the long intervals between assessments. In an attempt to clarify this issue, this group investigated the dynamic interactions among psychosocial, emotional, and physiological variables in single subject with SLE on a *daily* basis for 63 days according to a time-series analytic approach. Although SLE disease activity remained quiescent throughout this period, an association of psychosocial stressors and urine neopterin levels was observed. A similar study using "integrative single case studies" as a new biopsychosocial approach to the psychoneuroimmunology of SLE found interesting associations of daily psychosocial stressors and associated emotions with urine neopterin and changes in urine cortisol [62]. Urine neopterin appears to be a marker of SLE disease activity [63], raising the possibility, at least, of clinically significant effects of psychological stress. Of interest in this regard is a recent report that urine neopterin is increased in major depression, but not in fibromyalgia without major depression [64].

FIBROMYALGIA AND ITS IMPACT ON HEALTH OUTCOMES AND QUALITY OF LIFE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Overall health outcome and QOL in SLE is strongly influenced by interactions of individual psychosocial variables (socioeconomic status, education, insurance, social network), stressors of daily life and stress inherent in living with a chronic illness like SLE, and societal factors. While there is considerable variation in patient vulnerability in this regard, low self-efficacy for disease management [65], daily stress (hassles) and level of social support [66, 67], and style of coping [68] have been identified as important variables associated with disease activity, physical function, and mental health. It is less clear that such factors contribute to organ system damage over time, however. For example, in a random

sample of SLE patients at five academic medical centers [69], there was evidence of organ system damage measured by the SCLICC/ACR damage index within a mean of 4 years after disease onset. Older age at diagnosis, greater disease activity at diagnosis, and longer disease duration correlated with greater damage in different organ systems, but race and socioeconomic status did not. These findings are consistent with a dominant effect of more strictly clinical variables, rather than social and behavioral variables, on organ system damage in SLE, as discussed previously.

Comorbid fibromyalgia can amplify the negative influence of social and behavioral variables on QOL in SLE. When fibromyalgia is present, patients with SLE have increased pain, fatigue, and insomnia, more impairment in functional status, decreased sense of well-being, poorer perception of health, and increased rates of unemployment, divorce, and welfare/disability payments [12, 14]. Because psychological distress and pain sensitivity exist as a continuum in the population, many persons with SLE, that is, "stress responders" [50] who do not meet the ACR classification criteria as fibromyalgia (some tender points present, but fewer than 11) are subject to the adverse effects of stress on QOL. Stress here includes major stress (life events), minor stress (daily hassles), less than optimum self-efficacy and self-esteem, and maladaptive coping skills. Taking this approach, Da Costa and colleagues [70] studied the association of stress with changes in functional impairment, as assessed by the Stanford Health Assessment Questionnaire (HAQ). More negative life events and depressed mood were correlated with more abnormal HAQ scores. Changes in the HAQ scores were not related to SLE disease duration, activity, or organ system damage. It follows that reduction of stress, enhancement of social support, preservation of the patient's social roles, and improving coping skills and self-efficacy should all be part of the comprehensive management of SLE [16, 66–68, 71]. Similarly, potentially modifiable sociodemographic variables, such as low education, Medicaid or no health insurance, low income, and having a physically demanding job, predict which ~40% of people with SLE who quit work within a few years after diagnosis [72].

Adaptation of self-report questionnaires as a routine element in outpatient clinical care is an efficient and, in the author's experience, exceedingly effective method for assessing QOL and functional impairment and the social and behavioral variables that influence QOL and functional impairment. In addition, longitudinal monitoring of health status and response to therapy is possible. An example of such an instrument is the Multidimensional Health Assessment Questionnaire (MDHAQ) [73] originally developed by Pincus and Callahan. The MDHAQ takes only a few minutes to complete while patients wait to be seen by the physician, and screens for difficulties with activities of daily living, for psychological distress with Likert scales for sleep, stress, anxiety, and depression, and for somatization via a checklist of current symptoms. Ten-centimeter visual analog scales for pain, fatigue, and global self-assessment of overall health provide additional useful information. If the physician reviews the findings from this questionnaire in the examining room with the patient, patient acceptance of this instrument is universally excellent. Patient/physician communication is enhanced by assuring that the physician is aware of what in the patient's life and illness is most distressing to them. The author combines the MDHAQ with pressure algometry (dolorimetry) at four fibromyalgia tender points in the routine assessment of all patients at each clinic visit, an approach that is highly sensitive for detection of comorbid fibromyalgia in SLE and other rheumatologic disorders.

RELEVANCE OF STRESS, DISTRESS, AND FIBROMYALGIA TO NEUROPSYCHIATRIC MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Cognitive impairment is one of the most common and distressing neuropsychiatric problems in both fibromyalgia and SLE. Using the ACR case definitions for neuropsychiatric syndromes in SLE in a prospective Texas cohort study of 128 subjects, 79% exhibited cognitive impairment by standardized neuropsychological testing and in approximately one-third, impairment was rated as moderate or severe [74]. Anxiety and mood disorders also were prevalent: anxiety disorder, 24%; major depressive-like episode, 28%; mood disorder, 23%. In fibromyalgia, there is a general consensus that cognitive dysfunction is related to psychological stress and distress, rather than any irreversible brain injury. In SLE, a variety of pathogenetic variables have been proposed: psychosocial variables, psychiatric disorders independent of SLE [75], intrathecal cytokines [76], extra-CNS immune activation-derived cytokines acting on the brain [77], and autoantibody or immune complex-mediated brain injury [78, 79]. The exact contributions of these putative variables remain to be clarified. Cognitive impairment in SLE is particularly difficult to assess and study, and most studies have not clearly defined the possible contribution of comorbid fibromyalgia and its associated "fibrofog." Hay *et al.* [75] observed a point prevalence of ~20–25% for both psychiatric disorder and cognitive impairment in a prospec-

tive cohort of 49 patients with SLE at two time points. Only 1 of 9 patients with cognitive impairment at first interview was still impaired at second interview, suggesting absence of any irreversible or subclinical brain injury. Rather, change in cognitive function mirrored change in psychiatric status.

Certain data suggest that the high prevalence of cognitive impairment in SLE is independently associated with depression, but not with other clinical variables [80, 81]. Others have not observed a strong association of cognitive impairment with depression [82]. While cognitive impairment, along with anxiety and depression may reflect the psychological impact (stress and distress) of active SLE, with improvement during periods of remission [83], most investigators have found cognitive difficulties to be unassociated with clinically active SLE [82, 84]. The role of proinflammatory cytokines, which have been associated with cognitive impairment and pain sensitivity in both SLE and fibromyalgia [77] and intrathecal anti-phospholipid antibodies are currently under active investigation. The author's bias is that much of the cognitive and emotional difficulties in SLE derive from the same biopsychosocial variables operant in fibromyalgia. In other words, cognitive impairment in SLE reflects fibrofog.

CONCLUSIONS

Fibromyalgia and related chronic pain and fatigue disorders are exceedingly common in SLE. The biopsychosocial variables that underlie chronic pain and fatigue are becoming increasingly well understood, both with respect to their relative contributions and in terms of psychoneuroimmunological mechanisms. The psychological stress and distress that accompanies comorbid fibromyalgia in SLE strongly influences quality of life, functional impairment, rates of unemployment, divorce, and welfare/disability payments. Current evidence does not support the idea that fibromyalgia contributes measurably to clinical and laboratory measures of disease activity in this disorder. Similarly, organ system damage over time appears to be primarily a consequence of more strictly biological disease variables. Fatigue, mood and anxiety disorders, and cognitive impairment are manifestations of comorbid fibromyalgia, not SLE. Optimum clinical assessment and therapy of SLE requires recognition of the contribution of fibromyalgia to overall morbidity in SLE.

References

1. Winfield, J. B. (1999). Pain in fibromyalgia. *Rheum. Dis. Clin. North Am.* **25**, 55–79.
2. Wolfe, F., Smythe, H. A., Yunus, M. B., Bennett, R. M., Bombardier, C., Goldenberg, D. L., Tugwell, P., Campbell, S. M., Abeles, M., and Clark, P. (1990). The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum.* **33**, 160–172.
3. Wolfe, F. (1997). The relation between tender points and fibromyalgia symptom variables: Evidence that fibromyalgia is not a discrete disorder in the clinic. *Ann. Rheum. Dis.* **56**, 268–271.
4. Akkasilpa, S., Minor, M., Goldman, D., Magder, L. S., and Petri, M. (2000). Association of coping responses with fibromyalgia tender points in patients with systemic lupus erythematosus. *J. Rheumatol.* **27**, 671–674.
5. Croft, P., Schollum, J., and Silman, A. (1994). Population study of tender point counts and pain as evidence of fibromyalgia. *Br. Med. J.* **309**, 696–699.
6. Croft, P., Burt, J., Schollum, J., Thomas, E., Macfarlane, G., and Silman, A. (1996). More pain, more tender points: Is fibromyalgia just one end of a continuous spectrum? *Ann. Rheum. Dis.* **55**, 482–485.
7. Hyams, K. C. (1998). Developing case definitions for symptom-based conditions: The problem of specificity. *Epidemiol. Rev.* **20**, 148–156.
8. Barsky, A. J., and Borus, J. F. (1999). Functional somatic syndromes. *Ann. Intern. Med.* **130**, 910–921.

8a. Aaron, L. A., and Buchwald, D. (2001). A review of evidence for overlap among unexplained clinical conditions. *Ann. Intern. Med.* **134**, 868–881.

9. Wolfe, F., Ross, K., Anderson, J., Russell, I. J., and Hebert, L. (1995). The prevalence and characteristics of fibromyalgia in the general population. *Arthritis Rheum.* **38**, 19–28.
10. Buchwald, D., Umali, P., Umali, J., Kith, P., Pearlman, T., and Komaroff, A. L. (1995). Chronic fatigue and the chronic fatigue syndrome: Prevalence in a Pacific Northwest health care system. *Ann. Intern. Med.* **123**, 81–88.
11. White, K. P., Speechley, M., Harth, M., and Ostbye, T. (1999). The London Fibromyalgia Epidemiology Study: The prevalence of fibromyalgia syndrome in London, Ontario. *J. Rheumatol.* **26**, 1570–1576.
12. Middleton, G. D., McFarlin, J. E., and Lipsky, P. E. (1994). The prevalence and clinical impact of fibromyalgia in systemic lupus erythematosus. *Arthritis Rheum.* **37**, 1181–1188.
13. Morand, E. F., Miller, M. H., Whittingham, S., and Littlejohn, G. O. (1994). Fibromyalgia syndrome and disease activity in systemic lupus erythematosus. *Lupus* **3**, 187–191.
14. Gladman, D. D., Urowitz, M. B., Gough, J., and MacKinnon, A. (1997). Fibromyalgia is a major contributor to quality of life in lupus. *J. Rheumatol.* **24**, 2145–2148.
15. Handa, R., Aggarwal, P., Wali, J. P., Wig, N., and Dwivedi, S. N. (1998). Fibromyalgia in Indian patients with SLE. *Lupus* **7**, 475–478.
16. Taylor, J., Skan, J., Erb, N., Carruthers, D., Bowman, S., Gordon, C., and Isenberg, D. (2000). Lupus patients with

fatigue—is there a link with fibromyalgia syndrome? *Rheumatology* (*Oxford*) **39**, 620–623.
17. Lopez-Osa, A., Jimenez-Alonso, J., Garcia-Sanchez, A., Sanchez-Tapia, C., Perez, M., Peralta, M. I., Gutierrez-Cabello, F., and Morente, G. (1999). Fibromyalgia in Spanish patients with systemic lupus erythematosus. *Lupus* **8**, 332–333.
18. Chikanza, I. C., and Grossman, A. B. (2000). Reciprocal interactions between the neuroendocrine and immune systems during inflammation. *Rheum. Dis. Clin. North Am.* **26**, 693–711.
19. Yang, E. V., and Glaser, R. (2002). Stress-induced immunomodulation and the implications for health. *Int. Immunopharmacol.* **2**, 315–324.
20. Elenkov, I. J., Chrousos, G. P., and Wilder, R. L. (2000). Neuroendocrine regulation of IL-12 and TNF-alpha/IL-10 balance. Clinical implications. *Ann. N. Y. Acad. Sci.* **917**, 94–105.
21. Elenkov, I. J., Wilder, R. L., Chrousos, G. P., and Vizi, E. S. (2000). The sympathetic nerve—an integrative interface between two supersystems: The brain and the immune system. *Pharmacol. Rev.* **52**, 595–638.
22. Merskey, H. (1986). Classification of chronic pain: Description of chronic pain syndromes and definition of pain terms. *Pain* **3**, S1–S226.
23. Melzack, R. (1999). From the gate to the neuromatrix. *Pain* **6**, S121–S126.
24. Maier, S. F., and Watkins, L. R. (1998). Cytokines for psychologists: Implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol. Rev.* **105**, 83–107.
25. Watkins, L. R., and Maier, S. F. (1999). Implications of immune-to-brain communication for sickness and pain. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7710–7713.
26. Engel, G. L. (1977). The need for a new medical model: A challenge for biomedicine. *Science* **196**, 129–136.
27. Fillingim, R. B., and Maixner, W. (1995). Gender differences in the responses to noxious stimuli. *Pain Forum* **4**, 209–221.
28. Konecna, L., Yan, M. S., Miller, L. E., Scholmerich, J., Falk, W., and Straub, R. H. (2000). Modulation of IL-6 production during the menstrual cycle in vivo and *in vitro*. *Brain Behav. Immun.* **14**, 49–61.
29. Piccinelli, M., and Simon, G. (1997). Gender and cross-cultural differences in somatic symptoms associated with emotional distress. An international study in primary care. *Psychol. Med.* **27**, 433–444.
30. Kroenke, K., and Spitzer, R. L. (1998). Gender differences in the reporting of physical and somatoform symptoms. *Psychosom. Med.* **60**, 150–155.
31. Affleck, G., Urrows, S., Tennen, H., Higgins, P., and Abeles, M. (1996). Sequential daily relations of sleep, pain intensity, and attention to pain among women with fibromyalgia. *Pain* **68**, 363–368.
32. Agargun, M. Y., Tekeoglu, I., Gunes, A., Adak, B., Kara, H., and Ercan, M. (1999). Sleep quality and pain threshold in patients with fibromyalgia. *Comp. Psychiatry* **40**, 226–228.
33. Drewes, A. (1999). Pain and sleep disturbances with special reference to fibromyalgia and rheumatoid arthritis. *Rheumatology* **38**, 1035–1044.
34. Vgontzas, A. N., Tsigos, C., Bixler, E. O., Stratakis, C. A., Zachman, K., Kales, A., Vela-Bueno, A., and Chrousos, G. P. (1998). Chronic insomnia and activity of the stress system: A preliminary study. *J. Psychosom. Res.* **45**, 21–31.
35. Clauw, D. J., and Chrousos, G. P. (1997). Chronic pain and fatigue: Overlapping clinical and neuroendocrine features and potential pathogenic mechanisms. *Neuroimmunomodulation* **4**, 134–153.
36. Demitrack, M. A., and Crofford, L. J. (1998). Evidence for and pathophysiologic implications of hypothalamic–pituitary–adrenal axis dysregulation in fibromyalgia and chronic fatigue syndrome. *Ann. N.Y. Acad. Sci.* **840**, 684–697.
37. Gold, P. W., and Chrousos, G. P. (1999). The endocrinology of melancholic and atypical depression: Relation to neurocircuitry and somatic consequences. *Proc. Assoc. Am. Physicians* **111**, 22–34.
38. Martinez-Levin, M., and Hermosillo, A. (2000). Autonomic nervous system dysfunction may explain the multisystem features of fibromyalgia. *Semin. Arthritis Rheumat.* **29**, 197–199.
39. McBeth, J., Macfarlane, G. J., Benjamin, S., Morris, S., and Silman, A. J. (1999). The association between tender points, psychological distress, and adverse childhood experiences: A community-based study. *Arthritis Rheum.* **42**, 1397–1404.
40. Fillingim, R. B., Wilkinson, C. S., and Powell, T. (1999). Self-reported abuse history and pain complaints among young adults. *Clin. J. Pain* **15**, 85–91.
41. Winfield, J. B. (2000). Psychological determinants of fibromyalgia and related syndromes. *Curr. Rev. Pain* **4**, 276–286.
42. Epstein, S. A., Kay, G., Clauw, D., Heaton, R., Klein, D., Krupp, L., Kuck, J., Leslie, V., Masur, D., Wagner, M., Waid, R., and Zisook, S. (1999). Psychiatric disorders in patients with fibromyalgia. A multicenter investigation. *Psychosomatics* **40**, 57–63.
43. McClary, A. R., Meyer, E., and Weitzman, E. L. (1955). Observations on the role of the mechanisms of depression in some patients with disseminated lupus erythematosus. *Psyschosom. Med.* **4**, 311–321.
44. Herrmann, M., Scholmerich, J., and Straub, R. H. (2000). Stress and rheumatic diseases. *Rheum. Dis. Clin. North Am.* **26**, 737–763.
45. Yunus, M. B., Hussey, F. X., and Aldag, J. C. (1993). Antinuclear antibodies and connective tissue disease features in fibromyalgia syndrome: A controlled study. *J. Rheumatol.* **20**, 1557–1560.
46. Krupp, L. B., LaRocca, N. G., Muir, J., and Steinberg, A. D. (1990). A study of fatigue in systemic lupus erythematosus. *J. Rheumatol.* **17**, 1450–1452.
47. Bruce, I. N., Mak, V. C., Hallett, D. C., Gladman, D. D., and Urowitz, M. B. (1999). Factors associated with fatigue in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **58**, 379–381.
48. Wekking, E. M., Vingerhoets, A. J., van Dam, A. P., Nossent, J. C., and Swaak, A. J. (1991). Daily stressors and systemic lupus erythematosus: A longitudinal analysis—first findings. *Psychother. Psychosom.* **55**, 108–113.

49. Wekking, E. M. (1993). Psychiatric symptoms in systemic lupus erythematosus: An update. *Psychosom. Med*. **55**, 219–228.
50. Adams, S. G., Jr., Dammers, P. M., Saia, T. L., Brantley, P. J., and Gaydos, G. R. (1994). Stress, depression, and anxiety predict average symptom severity and daily symptom fluctuation in systemic lupus erythematosus. *J. Behav. Med.* **17**, 459–477.
51. Ward, M. M., Marx, A. S., and Barry, N. N. (2002). Psychological distress and changes in the activity of systemic lupus erythematosus. *Rheumatology* (*Oxford*) **41**, 184–188.
52. Mullington, J. M., Hinze-Selch, D., and Pollmacher, T. (2001). Mediators of inflammation and their interaction with sleep: Relevance for chronic fatigue syndrome and related conditions. *Ann. N. Y. Acad. Sci.* **933**, 201–210.
53. Gur, A., Karakoc, M., Nas, K., Remzi, Cevik, Denli, A., and Sarac, J. (2002). Cytokines and depression in cases with fibromyalgia. *J. Rheumatol.* **29**, 358–361.
54. Wallace, D. J., Linker-Israeli, M., Hallegua, D., Silverman, S., Silver, D., and Weisman, M. H. (2001). Cytokines play an aetiopathogenetic role in fibromyalgia: A hypothesis and pilot study. *Rheumatology* (*Oxford*) **40**, 743–749.
55. Omdal, R., Mellgren, S. I., Koldingsnes, W., Jacobsen, E. A., and Husby, G. (2002). Fatigue in patients with systemic lupus erythematosus: Lack of associations to serum cytokines, antiphospholipid antibodies, or other disease characteristics. *J. Rheumatol.* **29**, 482–486.
56. Jara, L. J., Vera-Lastra, O., Miranda, J. M., Alcala, M., and Alvarez-Nemegyei, J. (2001). Prolactin in human systemic lupus erythematosus. *Lupus* **10**, 748–756.
57. Hinrichsen, H., Folsch, U., and Kirch, W. (1992). Modulation of the immune response to stress in patients with systemic lupus erythematosus: Review of recent studies. *Eur. J. Clin. Invest*. **22** (Suppl. 1), 21–25.
58. Pawlak, C. R., Jacobs, R., Mikeska, E., Ochsmann, S., Lombardi, M. S., Kavelaars, A., Heijnen, C. J., Schmidt, R. E., and Schedlowski, M. (1999). Patients with systemic lupus erythematosus differ from healthy controls in their immunological response to acute psychological stress. *Brain Behav. Immun.* **13**, 287–302.
59. Jacobs, R., Pawlak, C. R., Mikeska, E., Meyer-Olson, D., Martin, M., Heijnen, C. J., Schedlowski, M., and Schmidt, R. E. (2001). Systemic lupus erythematosus and rheumatoid arthritis patients differ from healthy controls in their cytokine pattern after stress exposure. *Rheumatology* (*Oxford*) **40**, 868–875.
60. Gluck, T., Oertel, M., Reber, T., Zietz, B., Scholmerich, J., and Straub, R. H. (2000). Altered function of the hypothalamic stress axes in patients with moderately active systemic lupus erythematosus. I. The hypothalamus—autonomic nervous system axis. *J. Rheumatol.* **27**, 903–910.
61. Schubert, C., Lampe, A., Rumpold, G., Fuchs, D., Konig, P., Chamson, E., and Schussler, G. (1999). Daily psychosocial stressors interfere with the dynamics of urine neopterin in a patient with systemic lupus erythematosus: An integrative single-case study. *Psychosom. Med.* **61**, 876–882.
62. Christian, S., Lampe, A., Rumpold, G., Geser, W., Noisternig, B., Chamson, E., Schatz, D., Konig, P., Fuchs, D., and Schussler, G. (2001). The influence of daily psychosocial stressors and associated emotions on the dynamic course of urine cortisol and urine neopterin in systemic lupus erythematosus: Experience taken from two "integrative single-case studies." *Z. Psychosom. Med. Psychother.* **47**, 58–79.
63. Lim, K. L., Muir, K., and Powell, R. J. (1994). Urine neopterin: A new parameter for serial monitoring of disease activity in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **53**, 743–748.
64. Bonaccorso, S., Lin, A. H., Verkerk, R., van Hunsel, F., Libbrecht, I., Scharpe, S., DeClerck, L., Biondi, M., Janca, A., and Maes, M. (1998). Immune markers in fibromyalgia: Comparison with major depressed patients and normal volunteers. *J. Affect. Disord.* **48**, 75–82.
65. Karlson, E. W., Daltroy, L. H., Lew, R. A., Wright, E. A., Partridge, A. J., Fossel, A. H., Roberts, W. N., Stern, S. H., Straaton, K. V., Wacholtz, M. C., Kavanaugh, A. F., Grosflam, J. M., and Liang, M. H. (1997). The relationship of socioeconomic status, race, and modifiable risk factors to outcomes in patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 47–56.
66. Dobkin, P. L., Fortin, P. R., Joseph, L., Esdaile, J. M., Danoff, D. S., and Clarke, A. E. (1998). Psychosocial contributors to mental and physical health in patients with systemic lupus erythematosus. *Arthritis Care Res.* **11**, 23–31.
67. Bae, S. C., Hashimoto, H., Karlson, E. W., Liang, M. H., and Daltroy, L. H. (2001). Variable effects of social support by race, economic status, and disease activity in systemic lupus erythematosus. *J. Rheumatol.* **28**, 1245–1251.
68. Dobkin, P. L., Da Costa, D., Dritsa, M., Fortin, P. R., Senecal, J. L., Goulet, J. R., Choquette, D., Rich, E., Beaulieu, A., Cividino, A., Edworthy, S., Barr, S., Ensworth, S., Esdaile, J. M., Gladman, D., Smith, D., Zummer, M., and Clarke, A. E. (1999). Quality of life in systemic lupus erythematosus patients during more and less active disease states: Differential contributors to mental and physical health. *Arthritis Care Res.* **12**, 401–410.
69. Rivest, C., Lew, R. A., Welsing, P. M., Sangha, O., Wright, E. A., Roberts, W. N., Liang, M. H., and Karlson, E. W. (2000). Association between clinical factors, socioeconomic status, and organ damage in recent onset systemic lupus erythematosus. *J. Rheumatol.* **27**, 680–684.
70. Da Costa, D., Dobkin, P. L., Pinard, L., Fortin, P. R., Danoff, D. S., Esdaile, J. M., and Clarke, A. E. (1999). The role of stress in functional disability among women with systemic lupus erythematosus: A prospective study. *Arthritis Care Res.* **12**, 112–119.
71. Karasz, A., and Ouellette, S. C. (1995). Role strain and psychological well-being in women with systemic lupus erythematosus. *Women Health* **23**, 41–57.
72. Partridge, A. J., Karlson, E. W., Daltroy, L. H., Lew, R. A., Wright, E. A., Fossel, A. H., Straaton, K. V., Stern, S. H., Kavanaugh, A. F., Roberts, W. N., and Liang, M. H. (1997). Risk factors for early work disability in systemic lupus

erythematosus: Results from a multicenter study. *Arthritis Rheum.* **40**, 2199–2206.
73. Pincus, T., Swearingen, C., and Wolfe, F. (1999). Toward a multidimensional Health Assessment Questionnaire (MDHAQ): Assessment of advanced activities of daily living and psychological status in the patient-friendly health assessment questionnaire format. *Arthritis Rheum.* **42**, 2220–2230.
74. Brey, R. L., Holliday, S. L., Saklad, A. R., Navarrete, M. G., Hermosillo-Romo, D., Stallworth, C. L., Valdez, C. R., Escalante, A., del, R., I, Gronseth, G., Rhine, C. B., Padilla, P., and McGlasson, D. (2002). Neuropsychiatric syndromes in lupus: Prevalence using standardized definitions. *Neurology* **58**, 1214–1220.
75. Hay, E. M., Huddy, A., Black, D., Mbaya, P., Tomenson, B., Bernstein, R. M., Lennox Holt, P. J., and Creed, F. (1994). A prospective study of psychiatric disorder and cognitive function in systemic lupus erythematosus. *Ann. Rheum. Dis.* **53**, 298–303.
76. Trysberg, E., Carlsten, H., and Tarkowski, A. (2000). Intrathecal cytokines in systemic lupus erythematosus with central nervous system involvement. *Lupus* **9**, 498–503.
77. Kozora, E., Laudenslager, M., Lemieux, A., and West, S. G. (2001). Inflammatory and hormonal measures predict neuropsychological functioning in systemic lupus erythematosus and rheumatoid arthritis patients. *J. Int. Neuropsychol. Soc.* **7**, 745–754.
78. Wilson, H. A., Winfield, J. B., Lahita, R. G., and Koffler, D. (1979). Association of IgG anti-brain antibodies with central nervous system dysfunction in systemic lupus erythematosus. *Arthritis Rheum.* **22**, 458–462.
79. Brey, R. L., and Escalante, A. (1998). Neurological manifestations of antiphospholipid antibody syndrome. *Lupus* **7** (Suppl 2), S67–S74.
80. Hay, E. M., Black, D., Huddy, A., Creed, F., Tomenson, B., Bernstein, R. M., and Holt, P. J. (1992). Psychiatric disorder and cognitive impairment in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 411–416.
81. Hanly, J. G. (2001). Neuropsychiatric lupus. *Curr. Rheumatol. Rep.* **3**, 205–212.
82. Gladman, D. D., Urowitz, M. B., Slonim, D., Glanz, B., Carlen, P., Noldy, N., Gough, J., Pauzner, R., Heslegrave, R., Darby, P., and MacKinnon, A. (2000). Evaluation of predictive factors for neurocognitive dysfunction in patients with inactive systemic lupus erythematosus. *J. Rheumatol.* **27**, 2367–2371.
83. Segui, J., Ramos-Casals, M., Garcia-Carrasco, M., de Flores, T., Cervera, R., Valdes, M., Font, J., and Ingelmo, M. (2000). Psychiatric and psychosocial disorders in patients with systemic lupus erythematosus: A longitudinal study of active and inactive stages of the disease. *Lupus* **9**, 584–588.
84. Carbotte, R. M., Denburg, S. D., and Denburg, J. A. (1995). Cognitive dysfunction in systemic lupus erythematosus is independent of active disease. *J. Rheumatol.* **22**, 863–867.

26

NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

Rogelio Garcia-Cavazos
Robin Brey

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disorder affecting multiple organ systems, including the central (CNS) and peripheral nervous systems (PNS). CNS involvement in SLE is a complex diagnostic entity due to its multiple clinical presentations. The American College of Rheumatology ACR [1] established case definitions and diagnostic criteria for 19 CNS and PNS syndromes observed in SLE patients, which collectively are referred to as neuropsychiatric systemic lupus erythematosus (NPSLE) syndromes (Table 1).

The new ACR [1] guidelines for NPSLE eliminate the frequently used term lupus cerebritis. Although cutaneous and visceral vasculitis is not unusual in SLE [2], true cerebral vasculitis is rarely found in SLE despite its popular use as a clinical diagnosis [3–5]. Similarly, because both psychiatric and peripheral nervous system disorders may also be present, a term such as CNS lupus is overly specific and thus should also be avoided in discussions about NPSLE.

Prevalence of NPSLE Syndromes Using the American College of Rheumatology Case Definitions

Systemic lupus erythematosus is a disease with a fluctuating course and NPSLE manifestations can occur as a single or multiple events at any time during the course of the disease, even during periods in which no nonnervous system SLE disease activity is detected [6, 7]. Approximately 40% of the NPSLE manifestations develop before the onset of SLE or at the time of diagnosis and 63% within the first year after diagnosis [7].

Estimates of the prevalence of NPSLE have ranged from 14 to over 80% [1, 8–14], and most are based on research conducted before the introduction of the ACR criteria for NPSLE. The goal of the ACR case definitions, criteria, and nosology guidelines was to increase consistency in the classification of patients and standardize clinical descriptions and reporting requirements in NPSLE research [1]. Encouraging results are beginning to emerge from research efforts conducted in different research centers across geographic regions. At least three studies [12–14] have reported prevalence of NPSLE based on the ACR 1999 criteria. The three detected the presence of 14 to 17 of the 19 syndromes described by the ACR [1] and report identical prevalence of cranial neuropathy (1.5%) and chorea (1%). These studies also report very similar prevalence of five other syndromes: cerebrovascular disease (2%), total spectrum of headache (56–61%), total spectrum of mood disturbances (69–74%), psychosis (5%), and total range of cognitive disorders (75–80%).

It should be noted that agreement on the prevalence of cognitive, mood, and psychotic manifestations was high in these studies and assessment was based on standardized examination instruments. This is a significant methodological improvement, as difficulty in defining psychiatric abnormalities and cognitive dysfunction is likely to be a source of discrepant prevalence rates of NPSLE across studies. Continued, extensive use of the

ACR criteria for NPSLE will help clarify the sources of discrepancies and improve reporting practices in NPSLE research.

Pathogenesis of NPSLE Syndromes

The pathogenic etiology of NPSLE is likely to be multifactorial and may involve autoantibody production, microangiopathy, and intrathecal production of proinflammatory cytokines [15]. Histopathologic studies reveal a wide range of brain abnormalities caused by multifocal microinfarcts, cortical atrophy, gross infarcts, hemorrhage, ischemic demyelination, and patchy multiple-sclerosis-like demyelination [16], but these are not diagnostic for NPSLE [6]. Bland microvasculopathy (characterized by vessel tortuosity, cuffing of small vessels, vascular hyalinization, endothelial proliferation, and perivascular gliosis), formerly attributed to deposition of immune complexes but now suspected to arise from activation of complement, appears to be the most common microscopic finding [16, 17]. However, this too is a nonspecific finding as patients without NPSLE also show these changes [15]. A histologically normal brain with no specific pathognomonic brain lesions is also a common finding in NPSLE [16].

Autoantibody production has been implicated in vasculopathic and autoantibody-mediated neuronal injury mechanisms. Anti-ribosomal P antibodies (anti-P) have been linked to diffuse CNS involvement in NPSLE [18, 19]. Anti-phospholipid (aPL) autoantibodies, detected by immunoreactivity to anticardiolipin (aCL) assays and/or their ability to prolong phospholipid-dependent coagulation assays (lupus anticoagulant, LAC), are implicated in the microvascular thrombo/embolitic and endothelial damage found in the brain of NPSLE patients [6].

Vasculopathy in SLE

Neuropathologic studies in SLE patients have frequently found a small vessel vasculopathy consisting of proliferative changes of the intima, vascular hyalinization, and perivascular lymphocytosis. This small vessel vasculopathy has been seen both in SLE patients with only psychiatric symptoms as well as those with focal NS manifestations [15]. Interestingly, single photon emission computed tomography (SPECT) and MR spectroscopy studies suggest that both cerebral atrophy and cognitive decline in SLE patients may be related to chronic cerebral ischemia [20–22]. It is possible that SPECT and MR spectroscopy studies represent a functional image correlate for cerebral vasculopathic change.

Cytokines

Cytokines appear to have regulatory roles in mediating SLE-disease activity and inflammation in target organs [23, 24]. Work has highlighted the importance of SLE monocytes in the generation of potentially pathogenic cytokines, particularly IL-6 and IL-10 [25]. The balance between proinflammatory and anti-inflammatory cytokines and the degree and extent of inflammation appear to profoundly influence SLE-mediated disease manifestations [23].

Several, largely cross-sectional studies in SLE have suggested that IL-6 is a marker of disease activity [26–28]. There are multiple other mechanisms whereby SLE patients might have higher IL-6 levels. The C5b-9 complex stimulates IL-6 [29]. Diabetes, which is increased in SLE patients taking prednisone, is associated with higher IL-6 levels [30, 31]. Homocysteine, which is elevated in 30% of SLE patients, leads to higher IL-6 levels [32]. Over 33% of SLE patients are morbidly obese, another associate of IL-6 levels [33]. Finally, dehydroepiandrosterone (DHEA) is low in SLE; a low level of DHEA could increase IL-6 secretion [34, 35].

Few studies have investigated the role of cytokine abnormalities in NPSLE patients. Cerebrospinal fluid (CSF) IL-1 and IL-6 levels are increased in SLE patients with CNS involvement compared with non-SLE neurological controls [25, 26]. Both serum and CSF IL-6 levels are elevated in NPSLE patients vs non-NPSLE patients or healthy controls [25, 26]. In one study, however, there was no difference in serum IL-6 levels between NPSLE patients and CNS infection control patients [25]. In another study, CSF IL-6 activity paralleled NPSLE disease activity most consistently [27]. In a study of 15 SLE patients without overt CNS disease (vs rheumatoid and healthy controls), regression analysis showed that serum DHEA-s and IL-6 accounted for unique portions of the variance in measures of learning and attention, after controlling for depression and corticosteroid treatment [28].

While many of these studies suggest that cytokine levels in the CSF may reflect SLE-mediated CNS activity, serum levels also appear to be important in patients with NPSLE manifestations. In addition, cytokine stimulation of peripheral nerves and sensory receptors has been shown to lead to large changes in neural activity and physiological and behavioral responses in the CNS. The synthesis and release of IL-1α, IL-6, and tumor necrosis factor-α (TNF-α) play a prominent role in the mediation of these phenomena [36]. These proinflammatory cytokines do not easily cross the blood–brain barrier, and therefore their effects on neural events are

postulated to occur either by entring into the CNS using specific transport systems or in areas where the blood–brain barrier is more permeable (e.g., circumventricular organs) or by binding to other receptors on endothelial cells of brain vasculature leading to endothelial release of other mediators into brain parenchyma. Another reasonable alternative is that cytokines act by activating afferent neurons in a paracrine action at the site where they are released, leading to neural events within the CNS in the location where these peripheral afferent neurons terminate. Experimental evidence supports this hypothesis, particularly in the case of the vagus nerve [36]. In addition, animal models have demonstrated induction of diverse manifestations of "sickness behavior" such as fever, fatigue/malaise, decreased pain thresholds, and hypocampal-dependent memory impairment by these mechanisms [36].

Adhesion Molecules

Other processes leading to immune-mediated brain dysfunction in SLE probably involve abnormal endothelial–white blood cell interactions that allow proteins or cells access to the CNS. The expression of adhesion proteins on endothelial cells appears to be upregulated in SLE, and facilitates lymphocyte entry in CNS disease [37–46]. Shedding of the active form of these molecules occurs, and soluble levels can be measured in both serum and CSF [45, 46].

Studies of circulating soluble adhesion molecules in SLE have yielded contradictory results [47–49]. In addition, there is disagreement as to whether soluble adhesion molecules are an accurate reflection of membrane bound proteins. Soluble serum levels of intercellular adhesion molecule 1 (ICAM-1) increase with systemic disease activity in patients with SLE [50, 51]. Wellicome and colleagues [52] evaluated soluble serum levels of vascular cell adhesion molecule 1 (VCAM-1) in patients with rheumatoid arthritis and SLE and found elevations in both groups as compared to normal controls. Janssen and colleagues [46] studied soluble serum levels of ICAM-1, VCAM-1, and E-selectin in SLE and found that soluble VCAM-1 levels were elevated during active disease, and normalized with remission in SLE. These findings were replicated by Spronk and colleagues [53] who also described an elevation of soluble VCAM-1 levels with disease activity and a fall with clinical remission in SLE, but no difference in ICAM-1 and E-selectin levels between SLE patients and controls at any time point. Machold *et al*. [54] likewise failed to find a difference in mean ICAM-1 levels between SLE patients, rheumatoid arthritis patients, and normal controls; however, within the patient groups, soluble serum ICAM-1 levels correlated with other markers of disease activity, for example, sedimentation rates and clinical findings. Levels of sICAM-1, but not sE-selectin, may decreased after corticosteroid therapy [55], although this is controversial [56, 57]. In one study, only combined elevation of three adhesion molecules (sCD14, sICAM-1, and sE-selectin) correlated with SLE prognosis [58].

Autoantibodies and NPSLE

Autoantibody production has been implicated in vasculopathic and autoantibody-mediated neuronal injury mechanisms. Anti-ribosomal P antibodies (anti-P) have been linked to diffuse CNS involvement in NPSLE [18, 19].

Anti-phospholipid antibodies (aPL) are a family of antibodies directed against plasma proteins bound to negatively- charged phospholipids that lead to hypercoaguability through effects on the protein C/protein S system, platelets, endothelial cells, and complement activation [59, 60]. APLs, defined as the lupus anticoagulant (LA), anticardiolipin (aCL), and anti-β_2 glycoprotein 1 (anti-β2GP1), are strongly associated with localized NPSLE, including TIA (transient ischemic attack), stroke, seizure, and cerebral vein thrombosis [61].

Multiple studies have also shown an association of aPLs with cognitive dysfunction in SLE [62–65]. Denburg *et al*. [63] found, in a cross-sectional study, that LA-positive patients were two to three times more likely than LA-negative patients to be cognitively impaired, primarily on tasks of verbal memory, cognitive flexibility, and psychomotor speed. In a 5-year prospective study, Hanly *et al*. found that patients who had persistent IgG aCL positivity had a reduction in psychomotor speed, and patients who had persistent IgA positivity had a reduction in conceptual reasoning and executive ability [62]. In a study of 45 SLE patients assessed twice, persistently elevated aCL levels were associated with poorer cognitive function, particularly speed of attention and concentration [64]. High titers of CSF IgG aCL have been detected in SLE patients (vs controls) with lupus headache, acute psychosis, cognitive dysfunction, higher cortical dysfunction, and altered consciousness [65]. Lupus anticoagulant, but not IgG aCL, was associated with reduced regional cerebral blood flow in a SPECT study [66]. However, one comparative study of positron emission tomography (PET), HMPAO-SPECT, and magnetic resonance imaging (MRI) scans did not find any correlation with aCL [67]. Anti-phospholipid (aPL) autoantibodies are also implicated in both microvascular thrombo/embolic episodes and endothelial damage.

Non-Immune-Mediated Mechanisms

Neurological complications of SLE also result from abnormalities either not directly related to the primary disease or as an effect of non-nervous system organ involvement. Many of these processes result in neurological dysfunction, including confusion and seizures, that are indistinguishable from primary immune-mediated damage. The major etiologies in this group are infections, toxins (including medications), and metabolic derangements. Although survival in SLE has improved over the years, infection remains a leading cause of morbidity and mortality in the disease [8]. Meningitis from both bacterial pathogens such as *Neisseria* and opportunistic infections including *Candida* and *Cryptococcus* occur [68]. The spectrum of infections includes bacterial, viral, fungal, and parasitic organisms [69]. Septic emboli continue to be a cause for concern in patients with fever. The high frequency of infections is due to diminished immunocompetence resulting from both medication and the underlying disease. The importance of identifying CNS infections is underscored by studies showing that patients on high-dose corticosteroids for NPSLE have a higher mortality rate than those on lower doses [70].

The toxins most often causing neurological abnormalities in SLE are medications. Corticosteroids and antihypertensive medications can produce neurological and psychiatric symptoms [71–73]. Metabolic abnormalities likely to affect the nervous system include uremia, as well as disorders of calcium, sodium, and magnesium. The rapid reversibility of abnormalities on some neurodiagnostic studies may well reflect transient metabolic abnormalities [74, 75].

Murine Neurobehavioral SLE

A number of animal models exist for various clinical and serological manifestations of SLE. Some of these, such as NZB, (NZB × NZW)F1, MRL/lpr, and BXSB mice represent inbred strains that provide an opportunity to study the evolution of spontaneous SLE disease manifestations [76, 77]. Others, such as MRL/++, another inbred stain that develops mild autoimmune disease manifestations late in life, provide an opportunity to examine agents or environmental manipulations that accelerate selected features of disease expression [76]. Disease manifestations can be induced in "permissive" strains, that is, those that do not ordinarily develop autoimmune disease, using a variety of agents, such as immunization with the 16/6 idiotype anti-DNA antibody (reviewed in Ref, 78), or treatment with human anti-cardiolipin antibody (aCL) [78–80]. Finally, advances in transgenic technology have allowed investigators to study highly selected aspects of the immune response either in disease expression or as therapeutic modalities, for example, by manipulating MHC expression [81], or by inserting "autoimmunity" genes into strains lacking an autoimmune background [82, 83].

None of these models provides us with a perfect reflection of human SLE-related nervous system disease. Nonetheless, important information about immunopathological events leading to brain disease can be gained from studying them. Such information is likely to provide clues about human SLE-related brain disease that can be further evaluated in appropriately designed human studies.

Murine Strains with Spontaneous Disease

All of the inbred autoimmune strains develop a variety of clinical and serological features that are similar to disease manifestations seen in patients with SLE, rheumatoid arthritis, or Sjögren's syndrome [76, 77]. Although the time course and particular expression of disease varies from strain to strain, all have been studied most widely as models for autoimmune renal disease associated with high-serum anti-DNA antibody levels and immune complex formation. Only recently have these strains been investigated more intensively for the development of neurological disease.

In 1973, Lampert and Oldstone described immune complexes in the choroid plexus of (NZB × NZW) F1 mice [84]. Ten years later, Alexander and colleagues described neuropathological changes in MRL-lpr and MRL/++ mice [85] and Rudick and Eskin described similar changes in (NZB × NZW) F1 mice [86]. Although brain inflammation in (NZB × NZW) F1 mice increased steadily with age, it was not consistently present until 14 months. In contrast to a prominent choroid plexus, a meningeal inflammatory response was noted in MRL/lpr mice by 6 months, with widespread brain inflammation sparing the choroid plexus in MRL/++ mice by 12 months of age. Although Alexander and colleagues mentioned apparent weakness in some animals [85], neither study systematically evaluated neurobehavioral dysfunction associated with these neuropathological findings.

During this same time period, it became clear that (NZB × NZW)F1, MRL-lpr, and BXSB mice all develop a variety of autoantibodies, including those that are reactive with brain antigens [87–96]. In addition, learning, behavioral, and sensorimotor abnormalities have been noted in all autoimmune strains. These increase with age and are associated with increases in serum levels of brain-reactive and other antibodies [95, 96] and the magnitude of brain inflammation over time [97]. There are a variety of potential mechanisms

whereby brain inflammation could lead to brain dysfunction: (1) by causing changes in MHC class II expression by cerebrovascular endothelial and other primary brain cells, or (2) by producing proinflammatory cytokines (which could also change neuronal function or lead to disruption of the blood–brain barrier). Many of the less subtle features of neurological dysfunction in all of the autoimmune murine strains appear late in the disease course when many other abnormalities that could affect neurological functioning are present, including renal failure, hemolytic anemia, systemic vasculitis, arthritis, and high levels of many autoantibodies. The careful categorization of this late-onset neurological dysfunction has been important [92, 93] because it can be a useful endpoint in the study of treatment effects. However, the utility of late-onset neurological dysfunction as a model in and of itself is somewhat limited inasmuch as it may fail to identify key features of pathogenic importance occurring early during the onset of neurological dysfunction.

Some groups have found that the appearance of detectable autoantibody levels coincides with the appearance of the first signs of sensorimotor dysfunction between 8 and 10 weeks of age in MRL/lpr mice. In order for autoantibodies to be causally associated with neurological dysfunction, they must first gain access through the blood–brain barrier. Vogelweid and colleagues were able to demonstrate a disrupted barrier using a more sensitive immunohistochemical technique in MRL/lpr mice [98]. They found an age-related increase in the frequency of both CNS inflammation, composed predominantly of $CD4^{+}$ cells, and perivascular leakage IgG around brain vessels of MRL/lpr mice. Brey and colleagues were also able to demonstrate both aCL and brain–reactive antibody-producing monocytes from whole-brain homogenates of individual 18-week-old MRL/lpr mice [99]. Taken together, these data suggest that antibodies reactive to important brain antigens could gain access to the CNS, whether or not they are actually pathogenic. The blood–brain barrier appears to be disrupted early on and thus could play a key role in SLE-related neurological dysfunction in the MRL/lpr model, especially if antibodies or other systemic mediators of inflammation (e.g., cytokines) are causally related to disease manifestations. Antibody-producing cells are present in the brains of MRL/lpr mice late in the disease course and could play a role as well.

Several studies [96, 100–103] raise the possibility that in addition to any deleterious effects of autoantibodies, an even earlier immune-mediated event may be the trigger of some behavioral defects in MRL/lpr mice. These investigators observed that at a young age, before CNS inflammation or even serologic autoantibodies are present, MRL/lpr mice exhibit some signs of behavioral dysfunction in comparison to congenic MRL/++ controls. This antedates other lupus-related manifestations and worsens with age. One interpretation of these results is that the onset of autoimmune disease in MRL/lpr mice is associated with alterations in emotional and motivational aspects of behavior [103, 104]. This hypothesis has also been supported by findings in NZB autoimmune mice [105].

Murine Strains with Accelerated or Induced Disease

One limitation to using autoimmune-prone mouse strains for studying nervous system dysfunction is that often there are other systemic disease manifestations that could effect nervous system function, as is the case in human lupus patients. Another approach has been to induce autoimmune disease in permissive animal strains or to accelerate disease in strains that have mild autoimmune disease that develops later in life. Some of these have been associated with nervous system manifestations.

Both systemic and neurological autoimmune disease manifestations can be seen in mouse and rat strains immunized with human aCL, anti-double-stranded (ds)DNA of the 16/6 idiotype, or antiproteinase 3 antibodies (reviewed in Ref, 72). Immunization with any of these in naïve animals leads first to the production of antiautoantibody (anti-idiotypic antibodies) and later to the production of host autoantibodies with pathogenic potential.

Mice with high anti-phospholipid (aPL) antibody levels develop poor grip strength, trouble with coordination on the rotating bar, and hyperactivity in an open field test [106]. Rats with high levels of aPL had defective special learning in the Morris water maze compared to control rats. Vasculopathy of brain microvessels was demonstrated in immunized animals by immunofluorescence and electron microscopy [106]. This suggests that high levels of aPL in these animals lead to neurological dysfunction at least in part by damaging the blood–brain barrier.

Brey and colleagues demonstrated accelerated neurological, but not systemic, disease manifestations in MRL/++ mice immunized with both apolipoprotein H and neuronal surface proteins extracted from a murine neuroblastoma cell line [107, 108]. Immunization with either antigen resulted in neurological dysfunction that began around 24–26 weeks and slowly progressed until sacrifice at 44–48 weeks.

These studies suggest that antibodies directed against several brain antigens can independently lead to the accelerated development of neurological manifesta-

tions in this mouse model. More work is needed to determine the pathogenic mechanism by which these antibodies interfere with brain function.

CLINICAL FEATURES

An important consideration in the diagnostic approach to a patient with possible NPSLE manifestations is whether the particular clinical syndrome is due to SLE-mediated organ dysfunction, a secondary phenomenon related to infection, medication side-effects or metabolic abnormalities (e.g., uremia), or is due to an unrelated condition. For each NPSLE syndrome, the ACR case definitions give clear guidance regarding the differential diagnosis and evaluation that is required to make a diagnosis of a primary NPSLE syndrome. It cannot be stressed enough that infection is a major cause of central nervous system syndromes in hospitalized SLE patients [8]. Appropriately, the exclusion of infection plays a prominent role in the differential diagnosis of central nervous system NPSLE syndromes in the ACR guidelines. A great deal of thought and effort by a large group of experts made these guidelines possible. The appendix with clear algorithms for the diagnostic approach to each of the 19 recognized NPSLE syndromes can be found on the American College of Rheumatology's website at http://www.rheumatology.org/ar/1999/aprilappendix.html [109]. This is a tremendous resource for all clinicians who care for SLE patients when faced with the possibility of evaluating a patient for an NPSLE syndrome. A summary of these can be found in Table 1.

TABLE 1 Neuropsychiatric Syndromes Associated with SLE[a]

NPSLE Associated with central nervous system
Aseptic meningitis
Cerebrovascular disease
Stroke
Transient ischemic attack
Cerebral venous sinus thrombosis
Cognitive disorders
Delirium (acute confusional state)
Dementia
Mild cognitive disorders
Demyelinating syndrome
Headaches
Tension headaches
Migraine Headaches
Movement disorders (chorea)
Psychiatric disorders
Psychosis
Mood disorders
Anxiety disorder
Seizure disorders
Transverse myelopathy
NPSLE associated with peripheral nervous system
Autonomic neuropathy
Myasthenia gravis
Peripheral neuropathy
Sensorineural hearing loss
Sudden onset
progressive
Cranial neuropathy

[a] Modified from Sibbitt *et al.* [6] and ACR ad hoc committee [1].

Diagnosis and Management of Individual Syndromes

Acute Inflammatory Demyelinating Polyradiculoneuropathy (Guillain-Barré Syndrome)

Polyradiculoneuropathy is a rare and potentially severe manifestation of NPSLE [110]. The ACR nomenclature defines this entity as an acute, inflammatory, and demyelinating syndrome of the spinal roots, peripheral, and occasionally cranial nerves. The onset of the disease may precede, coincide, or follow the diagnosis of SLE [110]. The disease may follow a monophasic course, follow a relapsing–remitting pattern, or in CIDP, the chronic form of the disease, a chronic course with episodic flares [111, 112]. The differential diagnosis of polyradiculoneuropathy includes acute spinal cord disease, botulism, poliomyelitis and other neurotropic infections, and acute myasthenia gravis.

The diagnostic criteria include clinical features of progressive polyradiculoneuropathy that is usually symmetric and ascending and predominantly motor, and may be associated with arreflexia. It usually peaks within 21 days of onset, and may have an acute or insidious onset with an acute, subacute, or chronic course [109].

Other investigations advocated are CSF analysis, which typically shows elevated protein without pleocytosis, and nerve conduction studies (NCS). Electrodiagnostic findings supportive of polyradiculoneuropathy are one or more of the following abnormalities in three or more nerves tested: (a) conduction block with decreasing compound muscle action potential with more proximal testing, (b) absence or prolonged F waves, (c) conduction velocity slowing, and (d) prolonged distal latencies. Since abnormalities may be subtle early in the course of the disease, NCS may need to be repeated.

Aseptic Meningitis

This is a syndrome of acute or subacute onset characterized by headache, fever, and meningeal irritation, with CSF findings of pleocytosis and negative cultures. Typical CSF findings are mild pleocytosis (usually in the 200–300 cells/mm^3, may be much higher in the severely ill) with lymphocytic predominance and mildly elevated protein [113, 114].

Aseptic meningitis is one of the most infrequent manifestations of NPSLE, occurring in up to 1% [113, 115, 116]. Its onset is usually early in the course of SLE and may actually precede the diagnosis [117, 118]. Most cases of aseptic meningitis are associated with NSAID intake, and other drugs like trimethoprim/sulfamethoxazole [113] and recurrent episodes temporally related to azathioprine [117] have been reported.

Alternate diagnostic considerations must include infectious processes (bacterial, mycobacterial, viral, fungal, and parasitic), subarachnoid hemorrhage, malignancy (leukemia, lymphoma, or carcinoma), granulomatous disease (sarcoidosis), and medications like NSAIDs, IVIg, and azathioprine. NPSLE-related aseptic meningitis is responsive to steroid therapy [114]. The clinical course is usually benign [117], but chronic cases have been described [118].

Autonomic Disorder

Autonomic dysfunction is characterized by sympathetic and or parasympathetic impairment with sparing of the somatic sensorimotor function [119]. It is a disorder of the autonomic nervous system with orthostatic hypotension, sphincteric erectile/ejaculatory dysfunction, anhidrosis, heat intolerance, and constipation.

The reported prevalence of autonomic disorders varies from no difference vs. controls, to as high as 88% [120], and no association with anti-cardiolipin (aCL) antibodies was found [121]. Although it can occur in isolation, approximately one-half of the cases reported in the literature were associated with other neurological deficits [119]. There are no studies investigating whether autonomic dysfunction carries prognostic significance in SLE as it does in diabetes mellitus [122, 123].

Noninvasive cardiovascular testing is the mode of choice to investigate for the presence of autonomic disorders [120, 124]. The heart rate response to postural changes and breathing evaluates parasympathetic function, whereas blood pressure response to sustained hand grips is a test of sympathetic efferent function [119, 120]. Accordingly, the ACR diagnostic criteria recommend CV testing, in addition to sweat test [109]. The differential diagnoses include Lambert-Eaton-related autonomic dysfunction, medications (TCAs), organophosphate toxicity, Shy-Drager syndrome, and other neuropathic processes (i.e., elderly, diabetics).

Cerebrovascular Disease

Cerebrovascular disease is defined as neurological deficits due to arterial insufficiency or occlusion, venous occlusive disease, or hemorrhage with usually focal deficits that may be multifocal in recurrent disease.

Cerebral ischemic events may occur early in the course of SLE, or may precede the diagnosis. This may provide a diagnostic clue of underlying SLE in young patients with stroke that would be otherwise unsuspected [125]. The frequency of cerebrovascular disease as a whole has been reported as ranging from 5.3 [113] to 19% [115]. Venous thrombosis specifically is a rare complication of SLE, and may be seen as the presenting manifestation of the disease [126].

The association between anti-phospholipid (aPL) antibodies venous and arterial thrombotic events has been described both in primary anti-phospholipid antibody syndrome and secondary to SLE [127]. The pathogenesis of aPL antibodies is not fully understood, but may include endothelial activation and injury and alteration of the levels and or function of coagulation proteins [128]. There is also an association with Libman-Sacks endocarditis [129], which is a known source of embolic phenomena. Furthermore, in patients with a first stroke and aPL antibodies, the risk of recurrence is high and usually occurs within the first year [129]. This is acknowledged by the Ad Hoc Committee as they advocate routine ascertainment of aPL antibody status.

The evaluation should include obtaining information regarding potential precipitants and stroke risk factors (i.e., cocaine or amphetamine use, atrial fibrillation, septal defects, hypertension, diabetes, cigarette smoking, and lipid status). Imaging studies include CNS imaging (MRI or computed tomography (CT) and angiogram if indicated) and cardiovascular evaluation (EKG, carotid Doppler US, and echocardiogram). Exclusion of space-occupying lesion in the brain (infections, tumor), trauma, vascular malformation, and hypoglycemia are recommended [109].

Cranial and Peripheral Neuropathies

A variety of neuropathic processes of the peripheral nervous system have been described. The ACR nomenclature recognizes cranial, mono, and polyneuropathies, as well as plexopathies [109, 130] which may be sensory or sensorimotor [131], and axonal or demyelinating [109, 130, 132]. Peripheral sensorimotor neuropathy (PN) is the most common, and mononeuritis multiplex

[133], cranial neuropathies, and plexopathies [134] are progressively less frequent [135]. The onset of peripheral neuropathies usually follows the diagnosis of SLE, but it may actually precede it [130, 132]. Peripheral neuropathy has been linked to cutaneous vasculitis, is common among NPSLE patients with renal failure, and may be an important prognostic factor for mortality in SLE [10, 136–139].

Distal PN tends to affect the longer nerves first, hence the earlier involvement of the lower extremities [132]. Neurological exam may reveal hypesthesia to pinprick and light touch hypesthesia testing, impaired temperature, and vibratory perception [140]. Clinical and subclinical cranial and peripheral neuropathies are detected in 12–47% of unselected outpatient SLE referrals by electromyography and nerve conduction velocity studies [10, 136–139]. However, clinical correlation with electrophysiologic testing is equivocal [130–132, 141], and may underestimate the true prevalence [132]. Even different modalities of electrophysiologic testing yield diverse findings [131]. The clinical manifestations of PN are not fixed and the course is not of inexorable progression, instead fluctuating over time [141]. Other causes of neuropathy, including vitamin deficiencies, diabetes mellitus, leprosy, etc., should be excluded.

Histopathology data is scarce [132]. Studies of sural nerve biopsies showed predominantly axonal degeneration with loss of myelinated and unmyelinated fibers with occasional perivascular inflammatory infiltrates [132, 133, 142]. Scheinberg describes the occurrence of vasculopathic changes in the vasa nervorum, and demyelination [143]. Immune complex, complement, IgG, and C1q deposition have been seen in the vasa nervorum and basement membrane of Schwann cells [144].

Cranial neuropathies most commonly involve CN II–VII, with ocular manifestations, either as internuclear ophthalmoplegia or isolated II, IV, or VI palsy [145–150], facial palsy [151], and trigeminal neuralgia [152] being the most common. Even though audiovestibular involvement is considered rare [153], subclinical involvement may be much more common by audiometric studies independently of hydroxychoroquine treatment [154]. Other reported cranial neuropathies include X [155] or its branches [156, 157] and XII [158].

Demyelinating Syndrome

A demyelinating syndrome is defined as an acute or relapsing demyelinating encephalomyelitis with evidence of discrete neurological lesions distributed in place and time [109]. The diagnostic criteria require the occurrence of two or more manifestations or recurrence of at least one manifestation on distinctly separate occasions. The manifestations include (a) multiple areas of damage to white matter within the central nervous system, causing one or more limbs to become weak with sensory loss; (b) transverse myelopathy; (c) optic neuropathy; (d) diplopia due to isolated nerve palsies or internuclear ophthalmoplegia; (e) brain stem disease with vertigo, vomiting, ataxia, dysarthria, or dysphagia; and (f) other cranial nerve palsies [109].

Transverse myelitis, optic neuropathy and cranial neuropathies are also listed as separate case definitions. Guidelines dictate that patients meeting criteria for either of these and for demyelinating syndrome should be classified as having both. Evaluation for the presence of entities with known associations with demyelinating syndrome (i.e., structural lesions, familial disorders like ataxia and leukodystrophies, sarcoidosis, Behçet's, and multiple sclerosis (MS)), and the exclusion of infectious (TB, HIV, syphilis, etc.) and nutritional (vitamin B_{12} deficiency) causes are advocated. Studies recommended in the evaluation of all patients with possible demyelinating syndrome include CSF evaluation for cell count, protein, oligoclonal bands, IgG index, culture and cytology, MRI, and if indicated, evoked potentials [109].

Lupoid Sclerosis

The association of SLE and multiple sclerosis has been described [159–162]. It has been denominated lupoid sclerosis to illustrate the clinical overlap that exists between the two. There is still debate on whether this represents coexistence of two separate illnesses [161]. However, as mentioned previously, the Ad Hoc committee recognizes this as a distinct manifestation of NPSLE.

The onset of demyelinating syndrome may precede the diagnosis of SLE [161, 163] making the distinction from multiple sclerosis in some cases difficult [114, 160, 161]. Adding to the confusion are the high prevalence of positive anti-nuclear antibody (ANA) in MS (about 81%, typically of low titer), the frequent finding of oligoclonal bands in about 50% of cases of NPSLE, and MRI findings that may often be indistinguishable [164]. The presence of other systemic features of SLE like arthritis, rash or alopecia, the presence of extractable nuclear antibodies, an elevated erythrocyte sedimentation rate (ESR) or CSF findings of pleocytosis or elevated protein may be valuable diagnostic clues [164].

Anti-phospholipid antibody syndrome (APS) may sometimes mimic demyelinating syndrome, as they can both present in young females with multifocal relapsing symptoms, visual disturbances, and high T2-weighted signal in CNS MRI. Visual disturbances of retrobulbar neuritis may last days to weeks and may be bilateral

whereas amaurosis fugax is brief and unilateral. Disseminated CNS involvement, relapsing–remitting course, white matter or spinal cord lesions, oligoclonal bands in the CSF, and abnormal evoked potentials all favor demyelinating syndrome. A history of recurrent thrombosis or miscarriages and the presence of abnormal coagulation studies are suggestive of APS [163].

Optic Neuritis

Optic neuritis is one of the major causes of blindness in SLE [165]. It has been associated with aPL [166–168] and with transverse myelitis [166, 168–170]. In fact, out of 51 cases of SLE-related optic neuropathy reported in the literature by 1997, 41% had signs of spinal cord disease [171], and Jabs *et al*. found it to be as high as 54% [172]. The coexistence of optic neuropathy and transverse myelopathy in non-SLE patients is denominated Devic's syndrome [171, 173], and it is considered to be a variant of MS.

Clinical presentation of optic neuritis is acute visual loss that may be painful or painless. Physical examination reveals an afferent pupillary defect evidenced by an escape phenomenon on the swinging light test, known as Marcus-Gunn pupil. Fundoscopic exam often is normal, as the involvement is frequently retrobulbar. As gliosis occurs, central disk pallor, particularly in the temporal side, may later become evident [165, 174, 175].

Optic neuropathy may be the presenting manifestation of SLE in some cases [171, 175]. Since it is most commonly associated with MS [170], this may lead to confusion in previously undiagnosed patients. Findings suggestive of NPSLE are bilateral involvement (sometimes not suspected clinically, but unmasked by visual evoked potentials (VEP) or visual field testing) [165], bilateral, dense, central scotomata (up to 70% of cases) [165, 175], and severe, persistent visual defects after the first attack, rare findings in MS [167]. Other diagnostic clues are the absence of oligoclonal bands in the CSF and typical VEP findings of markedly decreased or absent responses with little or no conduction delay [167].

Transverse Myelitis

Transverse myelitis (TM) is a rare but serious complication of NPSLE that may occur at any point of the disease [113, 176–179]. Thoracic involvement is most common, with associated optic neuritis in almost half [179]. Some postulate the cause of the susceptibility of this region is an anatomical disadvantage in its blood supply taxed by the vasculopathic changes often seen in autopsy [176, 179]. The reported association with aPL could also play a role [179–181]. The evolution is rapid and often complete within a few hours [182]. Prognosis has always been viewed as dismal [113]. However, Kovacs *et al*. reviewed the 105 cases reported in the literature and found favorable outcomes in most, pondering whether the introduction of early, more aggressive treatment may be the cause [179].

The diagnosis of TM is made clinically, which may be supported by serological tests, CSF analysis, and imaging studies [183]. Physical exam typically reveals paraparesis or paraplegia and a sensory level. Sphincter involvement, manifested as urinary retention or fecal incontinence invariably occurs [176, 183, 184], and fever is very often present at the onset [176]. Deep tendon reflexes are most commonly absent, but they may be hyperactive [176, 184]. Other presenting symptoms include low back, mid-scapular or abdominal pain, and lower extremity paresthesia [176].

Cerebrospinal fluid abnormalities are common but nonspecific and often mild [184]. They include elevated protein, pleocytosis, and hypoglycorrhachia [176, 182]. The latter has been described as a finding typical of TM, as it does not occur in idiopathic TM [185] and may be a clue in undiagnosed SLE. MRI is abnormal in about 70% of the cases [179], which may show high T2 signal, spinal cord enlargement, and contrast enhancement. These last two findings have been reported to correlate with the clinical course on serial imaging [186]. An additional and very important role of MRI is to help exclude space-occupying lesions that may have the same clinical manifestations [184].

Headache

The inclusion of headache as a manifestation of NPSLE is a matter of controversy. With a few exceptions [187, 188], most authors agree there is a higher prevalence of headache and its subtypes in SLE vs. controls or population estimates [12, 13, 189–194]. The dispute arises from lack of correlation with clinical, neuropsychiatric, and laboratory features of SLE, as well as imaging and neurochemical abnormalities [187–191, 193–196], raising the question of whether headache or its subtypes even represent active NPSLE. Previous associations with aCL and Raynaud's phenomenon that would seem to make sense pathophysiologically in vascular-type headaches have not been found in a number of studies [187–189, 191, 194, 196].

The definition of headache in SLE is also a matter of controversy. The work of Atkinson and Appenzeller in 1975 [190] and Brandt and Lessell in 1978 [195] described a severe, disabling, persistent headache, not responsive to narcotics that followed disease activity and would subside with corticosteroids that was later named "lupus headache" [187, 197]. This term is part of the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), but was deemed too non-specific for

the ACR NPSLE nomenclature. They instead describe migraine with and without aura, tension headache, cluster headache, intracranial hypertension-related, including pseudotumor cerebri, and nonspecific intractable headache, with strict inclusion parameters [109].

The evaluation of headache in SLE is difficult and the vast differential diagnosis includes very serious conditions. These include brain abscess, infectious meningitis, NSAID-induced, cerebral venous thrombosis, hypertension, acute or chronic sinusitis and intracranial hemorrhage, tumor, aseptic meningitis, and metabolic reversible causes like carbon monoxide exposure and caffeine withdrawal [109]. The evaluation consists of a very detailed clinical interview supplemented by additional tests like brain imaging and lumbar puncture as necessary.

Movement Disorders

Chorea is an uncommon clinical manifestation of SLE, characterized by random, irregular and jerky movements that may occur in any part of the body. Two-third of the cases have a single episode and over one-half have bilateral symptoms [198]. Prevalence is about 1–4% of SLE [13, 198, 199] and is the only movement disorder included in the ACR nomenclature. It is the most common movement disorder, but reports of tremor mimicking essential tremor exist [200]. Estrogen-containing oral contraceptives and pregnancy have been known to precipitate attacks [198, 201]. Chorea has been associated with aPL and anti-phospholipid antibody syndrome (APS) [198, 199, 202–205].

The diagnosis is clinical, but supplemented by studies aiding in the differential diagnosis, which includes Wilson's disease, drugs (illicit or prescription), hyperthyroidism, as well a Huntington's and Sydenham's chorea. Since chorea may precede the onset of other SLE manifestations and is clinically indistinguishable from Sydenham's and Huntington's [198, 202, 206, 207], clinical suspicion is of utmost importance. Neuropathologic studies have revealed basal ganglia abnormalities in the minority of patients [206, 208], and imaging studies are nonspecific except in aiding in the differential diagnosis.

Myasthenia Gravis

Myasthenia gravis (MG) is an autoimmune disorder characterized by antibodies against acetylcholine receptors inducing muscle weakness and fatigability commonly affecting bulbar and other voluntary muscles. It is a purely motor disorder and tendon reflexes are spared. Clinical manifestations include ptosis, diplopia, masticatory weakness, dysphagia, and weakness of the extremities and less commonly of the trunk and neck musculature. History in undiagnosed patients may reveal symptoms occurring in the evening, reflecting the accentuation of symptoms with repeat use typical of the disorder.

The association of MG with SLE has been reported in more than 50 cases [209–217]. Muscle weakness in SLE is often a diagnostic problem; given this known association, MG should be included in the differential diagnosis [213, 214]. Myasthenia gravis may either precede or follow the diagnosis of SLE [209]. The development of SLE produces variable impact in the severity of MG and vice versa [209, 212]. Thirty percent of patients with MG have a positive ANA, increasing to 50% in those with thymoma [213]. Interestingly, homology in a sequence segment of U1 RNP and the main immunogenic region of the acetylcholine receptor has been reported [214].

Diagnosis is made by compatible clinical history in association to the presence of anti-acetylcholine receptor antibodies and/or fatigability evidenced by electrophysiological testing (EMG). A trial of edrophonium (Tensilon) or neostigmine should reversibly eliminate the patient's symptoms. The differential diagnosis includes Eaton-Lambert syndrome (reported in a few SLE patients, yet not included in the ACR nomenclature), MS, Guillain-Barré syndrome (GBS), and its Miller-Fisher variant, steroid, congenital, and inflammatory myopathies, medications, metabolic causes, and CVA.

Seizures

Seizures are defined as spells of brief, involuntary, paroxysmal alterations of behavior lasting seconds to several minutes and associated with a transient hypersynchronous discharge of cortical neurons. The behavioral alterations may or may not include convulsions. Recovery after a seizure is typically rapid and complete. It is important to differentiate seizures from spells that may mimic them such as breath-holding spells, migraine, convulsive syncope, narcolepsy with cataplexy, and psychogenic spells [218]. Next it is important to distinguish between isolated seizures and epilepsy, a disorder characterized by recurrent, unprovoked seizures. Provoked seizures are seen in SLE patients with systemic or CNS infection, renal failure, electrolyte imbalances, drug toxicity, or hypertension [219]. Two-thirds to three-fourths of the seizures in SLE patients are generalized tonic/clonic seizures. These tend to occur for the first time during a disease flare and often do not recur until another flare. If residual cerebral injury is sustained during the flare, the patient might develop epilepsy.

Seizures account for 9–58% of NPSLE manifestations reported in clinical series (reviewed in Refs 220–223). Seizures in SLE patient tend to appear early in the disease course [221–223], and may be the presenting feature of SLE [221]. The frequency varies widely, however, depending on the specific SLE population studied. For example, in studies that include an unselected, primarily outpatient population, the frequency of seizures ranges from 9–20% [12–14, 219, 221, 222]. When the study population includes only hospitalized SLE patients, the reported frequency is higher, as is the proportion of patients with conditions that could provoke seizures [8].

The clinical approach to the evaluation of seizures in any SLE patient is to look for those conditions that could provoke seizures listed previously. In a hospitalized patient, this must include a thorough evaluation for infection. A brain MRI scan is done to evaluate for focal cerebral lesions, and an electroencephalogram (EEG) is done to evaluate for epileptiform activity. A lumbar puncture should be performed after brain imaging is obtained in any patient in whom encephalitis or meningitis is suspected, but does not need to be performed routinely.

Cognitive Dysfunction

The etiology of cognitive dysfunction in SLE remains an active research question, but it is clear that it cannot be fully accounted for by past or current corticosteroid treatment, disease duration, disease activity (SLEDAI) or its associated psychological/emotional distress, or sociodemographic factors [7, 13, 224]. The detection of cognitive disorders has been regarded as time-consuming and therefore impractical for routine testing [12, 13], and yet mild to severe cognitive dysfunction remains the most common type of NPSLE manifestation with a prevalence of up to 75% [12–14]. It is most reliably detected and monitored through neuropsychological examination, often revealing a subcortical cognitive syndrome with most prominent compromise in the areas of processing efficiency/speed and attention/concentration, memory function, conceptual reasoning, and cognitive flexibility, approaching dementia in 15 to 25% of NPSLE cases [12, 13].

Cognitive impairment in SLE is not consistently related to psychiatric manifestations and can be detected even in the absence of other current or past overt CNS manifestations [12, 224]. It appears to be selectively linked to aCL but not to anti-P antibodies [13]. History of persistently elevated aCL antibodies over a period of 1–5 years has been linked to greater and sustained cognitive impairment and may be responsible for the long-term subtle deterioration in cognitive function in SLE [62, 64]. Specifically, reduced psychomotor speed has been selectively linked to persistent IgG aCL positivity, and reduced conceptual reasoning and executive function to IgA aCL positivity [64]. Cognitive dysfunction in some studies appears to be a relatively stable feature of CNS involvement in SLE [225], although in others impairment waxes and wanes over time [62, 64]. This may be because aCL levels fluctuate in SLE patients, albeit not always with disease activity. Continued observation with serum samples every 3–4 months may help assess risk for cognitive impairment in the SLE patient and give insights into the nature of some of the patient's complaints.

Psychiatric Disorders

The range of mood disorders in SLE is wide (major depressive episode, mood disorder with depressive, manic, or mixed features) and is estimated to affect 69 to 75% of SLE patients, if standardized examination instruments are used [12, 13]. The prevalence of anxiety disorders ranges from 7 to 70% [12, 13]. Psychosis and depression are two specific psychiatric disorders affecting 5% to 40% of SLE cases, not always in the context of exacerbated disease activity [12, 13, 226].

Psychiatric disorders in SLE have been linked to anti-ribosomal P, but not aCL, antibodies [62, 224, 227, 228]. Anti-ribosomal P antibodies are specific to SLE and their prevalence varies across ethnic groups. Serum, but not CSF, anti-ribosomal P antibodies are strongly associated with the development of psychosis and depression in SLE, but are not implicated in coexisting cognitive dysfunction [18, 228].

It can be difficult to distinguish between the presence of acute confusional state, SLE-mediated psychosis, and corticosteroid-induced psychosis in some SLE patients. Acute confusional state is equivalent to "delirium" defined in Diagnostic and Statistical Manual IV (DSM-IV) as an observable state of impaired consciousness, cognition, mood, affect, and behavior [109]. The term organic brain syndrome is not recommended for general use as "acute confusional state" is more precise with a validated definition. Acute confusional states are often accompanied by cognitive deficits, however, if only cognitive deficits are present and not other features described previously, the syndrome should be diagnosed as "cognitive dysfunction."

Psychosis is defined as a severe disturbance in the perception of reality characterized by delusions and/or hallucinations. If psychosis occurs exclusively during times when an impaired level of consciousness is present, the syndrome should be diagnosed as "acute confusional state." If the onset of psychosis is accompanied by the presence of anti-ribosomal P antibodies or

other evidence of systemic SLE-related organ involvement, the syndrome is most likely related to a direct SLE-related CNS effect. If, however, psychosis occurs as corticosteroids are administered, or the corticosteroid dose is escalated for the treatment of SLE-related disease activity, psychosis as a side effect of corticosteroid therapy should be strongly suspected. This is particularly true if anti-ribosomal P antibody levels are also not elevated.

Risk Factors for Development of NPSLE Syndromes

A number of clinical and serologic parameters have been suggested as risk factors for CNS involvement in SLE. The presence of discoid and articular manifestations is thought to associate with a more benign course and less likelihood of NPSLE [20], while their absence at onset or during flares seems to bestow a higher risk of CNS involvement, primarily cerebrovascular disease and seizures, and also psychosis, acute confusional state, and isolated cognitive dysfunction [20]. Other associations with high likelihood of NPSLE are low serum levels of C_3, and especially C_4, high-titer aCL (IgG) antibodies, and cutaneous vasculitic lesions [20].

When age at disease onset is factored in, the predictive clinical value of cutaneous and articular features for NPSLE is less clear. There is a higher prevalence of discoid lesions, malar rash, cutaneous vasculitis, and neurological involvement at SLE onset and during the course of the disease in juvenile-onset SLE relative to adult-, and elderly-onset SLE [229–232]. Furthermore, hypocomplementemia (C_3 and C_4) and higher anti-DNA and aCL (IgG) antibody levels also are more prevalent in early/juvenile-onset SLE [229–231]. Hence, the risk for CNS involvement in SLE may be more related to a particular serologic profile at the onset of SLE and/or during the course of the disease.

A number of studies suggest that race/ethnicity plays a role in the clinical and serologic phenotype, thus the significance of some immunological and clinical markers for CNS involvement may vary depending on the ethnic composition of the SLE samples studied. SLE-related cutaneous symptoms are more common in Caucasians than in African-Americans, but discoid lupus is more common in African-Americans [233]. Similarly, the prevalence of malar rash and discoid rash is higher in Caucasian than Aborigine Australian SLE samples [234]. It remains to be empirically demonstrated that the risk of CNS involvement in these racial groups depends on their relative susceptibility to develop the articular and cutaneous manifestations in question.

The prevalence of aPL antibodies, including aCL and LAC, is lower in African American and Australian Aborigines than in American and Australian Caucasian SLE samples, respectively [234, 235]. This and the predominance of IgA isotype of both aCL and anti-β_2-GPI antibodies are thought to explain the low frequency of APL syndrome manifestations (e.g., cerebral ischemia) in African-Americans [236]. Given its strong association with aPL antibodies, especially aCL, it is not known whether these variations translate into differential racial prevalence of SLE-related cognitive dysfunction [62, 64]. The fact that specific types of cognitive dysfunction (psychomotor speed and executive function) are differentially affected by IgG aCL and IgA aCL [62] further complicates potential racial effects on cognition in SLE.

Similarly, the prevalence of anti-P antibodies appears to vary across different ethnic groups with SLE (Chinese, Mexican-American, Caucasian, African American, and Greek) [228]. Given the strong and specific association between anti-P antibodies and these psychiatric manifestations in SLE [11, 18, 19], the repercussions in differential prevalence of SLE-related psychosis and depression are not known. The fact that anti-P antibodies are strongly clustered with IgG aCL antibodies in lupus sera, even if they are independently elicited [237], just adds to the complexity of identifying risk factors for specific CNS manifestations in SLE.

NEURODIAGNOSTIC NPSLE

Imaging

There is no single diagnostic test sensitive and specific for NPSLE. The assessment of individual patients is based on clinical neurological and rheumatologic evaluation, immunoserologic testing, brain imaging, and psychiatric and neuropsychologic assessment. These examinations are used to support or refute the clinical diagnostic impression and rule out alternative explanations, and form the basis for prospective monitoring of clinical evolution and response to treatment interventions. Neuroimaging techniques have been used to help identify, locate, and hopefully differentiate the pathophysiological mechanisms that give rise to the wide range of clinical NPSLE manifestations.

Focal neurological symptoms of NPSLE correlate with structural (MRI) abnormalities, and in fact MRI is usually normal in NPSLE patients with diffuse psychiatric (depression, psychosis) and no other CNS manifestations [20, 21]. However, when diffuse manifestations are also accompanied by generalized seizures, MRI shows multiple small, high intensity lesions that often resolve with treatment [20, 21]. In general, diffuse

psychiatric manifestations are more easily detected with functional neuroimaging (SPECT, PET).

On structural MRI, the majority (40 to 80%) of abnormalities in NPSLE are small focal lesions concentrating in periventricular and subcortical white matter [21, 22]. Cortical atrophy, ventricular dilation, diffuse white matter, and gross infarctions are also common [21]. MRI reveals multiple discrete white matter lesions in periventricular, cortical/subcortical junction, and frontal lobe more commonly in patients with past NPSLE manifestations, than in SLE patients without history of NPSLE [20–22]. Focal NPSLE manifestations (stroke, chorea, focal seizures, migraine headaches) uniformly reveal large and small high-intensity MRI lesions in white and gray matter, which tend to persist despite treatment and correlate with elevated aPL antibodies (LAC and aCL), peripheral vasculitis, livedo reticularis, and the patients' focal manifestations [20].

More than 60% of subcortical white matter lesions detected by MRI are missed by FDG-PET during active major (stroke, seizure, psychosis) and minor (headache, dizziness, memory complaints) NPSLE episodes [238]. However, MRI has missed 50–100% of the posterior white matter abnormalities detected by FDG-PET during active minor and major NPSLE [239, 240]. When detected by MRI, these lesions are found mostly in patients with a long history of major NPSLE [240]. Nevertheless, functional methods are generally more sensitive, detecting brain abnormalities much earlier than MRI, even when no overt CNS manifestations are noted, in line with the vasculopathic nature of NPSLE pathogenesis [238, 239].

Visually analyzed FDG-PET consistently reveals abnormalities in prefrontal, parietal (inferior and superior), parieto-occipital, posterior temporal, and occipital gray and white matter regions in active and quiescent NPSLE [239, 240]. Prefrontal, anterior cingulate, and inferior parietal white matter abnormalities have been seen during acute NPSLE but not during quiescent NPSLE [241]. The metabolic disturbances in parieto-occipital (peritrigonal) white matter remain an intriguing finding. Approximately 60 to 80% of active minor and major NPSLE patients consistently show bilateral parieto-occipital white matter FDG-PET hypometabolism in the context of normal conventional MRI and no other PET abnormalities [239, 240].

More recently, magnetic resonance spectroscopy (MRS) has revealed neurometabolic abnormalities even in white and gray matter that appears normal on conventional MRI. Such abnormalities are thought to reflect neuronal injury or loss and demyelination and have been found during active as well as quiescent periods of NPSLE manifestations [242].

Electroencephalography

The EEG is commonly abnormal in any patient with a condition known to cause an encephalopathy. The abnormality is characterized by diffuse slowing (frequencies of less than 8 Hz) and can occur in the setting of many of the conditions that lower seizure threshold [218]. No study has specifically correlated focal or generalized EEG abnormalities with seizure type in SLE patients. However, about 75% of EEG's in SLE patients with seizures demonstrate diffuse abnormalities and 25% focal abnormalities [243].

Cerebrospinal Fluid Evaluation

Cerebrospinal fluid analysis is recommended in some cases of NPSLE manifestations. A lumbar puncture cannot be performed in the following situations: (a) anticoagulated patients, (b) patients with fewer than 20,000 platelets/mm^3, (c) patients with a focal mass lesion or edema that would increase risk for herniation. The CSF analysis is essential to the diagnosis of CNS infection, but in the absence of infection may also be helpful in suggesting a CNS SLE flare. When immune-mediated CNS damage is ongoing during an SLE flare, the CSF IgG index or synthesis rate is often elevated and an oligoclonal banding pattern is seen [11]. In many patients these abnormalities normalize when the flare resolves. Findings of pleocytosis, elevated protein, or hypoglycorrhachia are nonspecific and seen in only about one-third of patients [11].

Autoantibody Testing

Autoantibodies with association to NPSLE include anti-phospholipid (aPL), anti-ribosomal P (aP [psychosis, major depression]) [114], and anti-neuronal (aN [more frequent in NPSLE than non-NPSLE, most frequent in diffuse presentations like cognitive dysfunction and organic brain syndrome]) [114]. With the exception of aPL, they are of limited availability and applicability in the general clinical setting, but may help distinguish diffuse vs focal CNS involvement in active NPSLE [11, 114, 224, 244].

Prognosis of Patients with NPSLE Syndromes

As has been discussed, NPSLE syndromes are multiple and varied. The presence of these syndromes often comes and goes, sometimes without any other apparent SLE-related disease activity. The impact of specific NPSLE syndromes on the overall prognosis for a patient with SLE is not clear, except for the case of

TABLE 2 Treatment of Various Manifestations of Neuropsychiatric SLE

Neuropsychiatric Manifestations	Symptomatic treatment*	Immune-modulating treatment
Seizures	Antiepileptic therapy	High-dose corticosteroids or effective treatment of extraneural disease activity
Delirium	No specific symptomatic therapy	Effective treatment of extraneural disease activity
Psychosis	Antipsychotic medications	Effective treatment of extraneural disease activity
Cerebral vasculopathy	Anticoagulation or antiplatelet agents in selected cases	1. High-dose corticosteroids 2. Cytotoxic immunosuppressives 3. Combination of both
Stroke	1. Anticoagulation 2. Antiplatelet agents	Effective treatment of extraneural disease activity
Transverse myelopathy	No specific symptomatic therapy	1. High-dose corticosteroids 2. Cytotoxic immunosuppressives 3. Combination of both
Cognitive dysfunction	No specific symptomatic therapy	Effective treatment of extraneural disease activity
Anxiety and depression	1. Psychotherapy 2. Cognitive-behavior therapy 3. Supportive-type therapy 4. Biofeedback 5. Pain control 6. Antidepressive agents 7. Anxiolytics	Effective treatment of extraneural disease activity
Drug-induced aseptic meningitis	Withdrawal and avoidance of offending drugs	No specific immunomodulating therapy
Headaches	Migraine treatments Antiplatelet agents	Treatment of extraneural disease activity
Movement disorders	Dopamine antagonists	1. High-dose corticosteroids 2. Anticoagulation if related to anti-phospholipid antibodies
Thrombotic thrombocytopenic purpuras	No specific symptomatic therapy	1. High-dose corticosteroids 2. Cytotoxic immunosuppressives 3. Plasmapheresis
Idiopathic pseudotumor cerebri	1. Carbonic anhydrase inhibitors 2. Repeated lumbar punctures 3. Optic nerve decompression	High-dose corticosteroids

* Includes treatment of secondary causes such as drugs, infections, and metabolic problems related to kidney and liver dysfunction, and electrolyte disturbances.

peripheral neuropathy, discussed previously. All of the NPSLE syndromes have a short-term adverse effect on quality of life and disease morbidity. Studies addressing long-term effects reveal that up to 20% of SLE patients continue to show persisting clinical signs and/or exacerbations of NPSLE manifestations such as seizures, psychosis, and migraines, and thus need long-term therapeutic management [11, 245, 246].

The short-term prognosis is usually favorable in most NPSLE syndromes [113]. Exceptions include transverse myelitis and cerebrovascular disease that may result in significant long-term deficits [113]. However, NPSLE has been identified as a predictor of increased mortality both as a group (5 and 10 year survival) [247, 248], and by individual syndromes (organic brain syndrome [RR 2.92] [249], cranial neuropathy [RR 6.83] [249], and seizures [RR 1.77] (250). The impact on survival is only exceeded by infection [250] and renal disease [129].

Treatment of Patients with NPSLE Syndromes

The management of patients with NPSLE includes symptomatic and immunosuppressive therapies, but evidence for the efficacy of the treatment modalities commonly used is largely limited to uncontrolled clinical trials and anecdotal experience (Tables 2 and 3). An excellent review of general treatment issues in SLE can be found in a supplement to *Lupus* entitled "Special Issue: Old Treatment/New Treatment [251]." In addition, a review of treatment for NPSLE syndromes [252] has also been recently published. The key to treatment

TABLE 3 Immune-Modulating Therapy Commonly Used for NPSLE

Therapeutic agent	Indications
High-dose corticosteroids	Seizures in the setting of active SLE[a] Cerebral vasculopathy[a] Any CNS manifestation in the setting of active SLE
Intravenous pulse cyclophosphamide	Cerebral vasculopathy[a] Non-thrombotic focal neurological deficits in SLE[a] Transverse myelitis[a]
Hydroxychloroquine	Long-term control of active SLE Antiplatelet effect Cholesterol lowering effect
Intravenous immunoglobulin	Any CNS manifestation unresponsive to corticosteroid therapy, cytotoxic therapy, or the combination Concomitant thrombocytopenia
Plasmapheresis	Any CNS manifestation unresponsive to corticosteroid therapy, cytotoxic therapy, the combination, or to intravenous immunoglobulin

[a] May require the combination of both high-dose corticosteroid and intravenous cyclophosphamide.

is to first establish the correct diagnosis by carefully following the guidelines set forth for the diagnosis of NPSLE syndromes by the ACR. It is also important to remember that for many NPSLE syndromes, symptomatic treatment may also be needed in addition to immunomodulatory therapy. These are discussed along with suggested immunomodulatory medications in the following sections. As mentioned previously, very few controlled trials specifically addressing any treatment modality for a specific NPSLE syndrome exists.

Low endogenous levels of dehydroepiandrosterone (DHEA) and other androgens are typical for patients with autoimmune diseases such as SLE, and augmentation of androgen levels has been suggested as a treatment [253]. A number of small controlled and uncontrolled clinical trials with SLE patients suggest that DHEA contributes to amelioration of disease activity, decreasing corticosteroid requirements, diminished flares, and improved cognition [254]. A short-term (1 month) course of DHEA under double-blinded placebo-controlled conditions demonstrated significant therapeutic effects on cognitive dysfunction in mild to moderate SLE [255]. Attention/concentration and learning/memory deficits were the most responsive to DHEA and treatment benefits were unrelated to depression or SLE disease activity. There is no approved standardized therapeutic regime of DHEA for SLE, but DHEA is widely used by SLE patients as a food supplement. Unfortunately, the Federal Drug Administration did not approve the use of DHEA to treat active disease in patients with SLE. More double-blinded placebo-controlled clinical trials are needed to assess the long-term benefits of this agent and its potential risks, which include possible lowering of HDL cholesterol and hormonal dysregulation (with potential adverse consequences for coagulation, atherosclerosis, or development of neoplasms) [254].

Cyclophosphamide is a cytotoxic immunosuppressive treatment option with documented therapeutic benefits in the management of severe NPSLE manifestations unresponsive to other treatment modalities (e.g., cerebral vasculopathy), and is an effective adjunctive agent when used with glucocorticoids for the treatment of transverse myelopathy [246]. A randomized controlled clinical trial comparing long-term use of cyclophosphamide and methylprednisolone reported better overall therapeutic control of SLE-related neurological manifestations (refractory seizures, peripheral and cranial neuropathy, and optic neuritis) with monthly intravenous cyclophosphamide [256]. Larger numbers of improved cases (95 vs 63%) and lower longitudinal levels of disease activity were also observed under cyclophosphamide; the two medications showed relatively equal incidence of new infections (52 vs 63%). However, other studies with SLE report an increased dose-dependent risk of infection and sepsis (fatal in 18% of cases) with intravenous cyclophosphamide [257]. Some [258] have pointed out that the question of efficacy and safety of cyclophosphamide as an alternative to methylprednisolone remains largely unanswered, as it has not been thoroughly assessed by randomized controlled clinical trials.

High-dose chemotherapy combined with autologous hematopoietic stem cell transplantation showed remarkable therapeutic benefits in cases of severe, life-threatening SLE with neuropsychiatric manifestations (and other organ involvement) that had been unresponsive to multiple monthly cycles of intravenous cyclophosphamide [259]. Within months, stem cell transplantation resulted in clinical remission of SLE, significant decline in anti-dsDNA antibody titers, normalization of serum complement levels, reduction and even discontinuation of glucocorticoids, and normalization of the T-cell receptor repertoire [259].

Acute Inflammatory Demyelinating Polyradiculoneuropathy (Guillain-Barré Syndrome)

In regards to the treatment options, most data available is in primary GBS and CIDP. Glucocorticoids for acute inflammatory demyelinating polyradiculoneu-

ropathy were once considered a standard of care until a lack of benefit was demonstrated [260]. This is not the case in CIDP, where treatment failure of steroids is the exception, in which case cyclophosphamide, cyclosporine, interferon, total lymphoid irradiation, and mycophenolate mofetil have been proposed [261].

There is good quality data documenting the efficacy of both plasmapheresis and IVIg. These include double-blind, sham-controlled trials and plasmapheresis–IVIg combinations [262]. Despite their effectiveness in the acute setting, chronic cases require serial maintenance treatments that are costly and impractical, making other maintenance immunosupression more desirable. However, treatment should be tailored to individual patient needs.

The use of the previously mentioned modes of treatment in NPSLE-associated polyradiculoneuropathy has also been documented, yet not nearly as extensively as in their primary counterparts. In acute polyradiculoneuropathy, the response to steroids is inconstant, with about 50% failure rate described in the literature [110, 263]. The outcome in these cases of failure was either successful escalation of therapy or death [110]. Again, the opposite is true in the few cases of NPSLE-associated CIDP where all eight clinical episodes of CIDP treated with high-dose steroids or ACTH responded adequately [111, 112].

There is limited data on plasmapheresis and IVIg, and they are only on the acute form of the disorder. Three case reports of treatment with plasmapheresis resulted in two successes and one failure [263]. A single case report of a patient with severe polyradiculoneuropathy and need for ventilatory support was treated with 5 daily IVIg infusions resulted in steady improvement and sustained remission at 1 year with minimal sequelae [110]. There is one additional case report describing treatment with a combination of high-dose steroids, plasmapheresis, and cyclophosphamide that resulted in very slow improvement and significant sequelae.

Aseptic Meningitis

NPSLE-related aseptic meningitis is responsive to steroid therapy [114]. The clinical course is usually benign [117], but chronic cases have been described [118].

Autonomic Disorder

The management of cases of NPSLE-associated autonomic disorders reported in the literature includes symptomatic treatment like secretagogues and mineralocorticoids, with or without the use of corticosteroids, with variable outcomes [119]. The use of other immunosuppressive agents (namely azathioprine) has also been described [119]. It is known that disease activity alters autonomic responses, thus patients with autonomic dysfunction have a higher risk of life-threatening complications during an SLE exacerbation [123]. Although corticosteroids have been known to positively affect the course of autonomic dysfunction [122], their role has been questioned due to frequent treatment failures and evidence suggestive of interference with circadian blood pressure variation through alterations in the adrenal axis [121].

Cerebrovascular Disease

Anatomopathologic studies have shown the presence of multiple small infarcts in approximately 50% of all NPSLE patients. In this resides a clue to the clinical dilemma of therapy for stroke in SLE as the underlying vasculopathy is a chronic process. Treatment for ischemia (with some of the emerging neuroprotectant medications) does not alter the underlying blood vessel abnormality. Appropriate therapy for cerebrovascular SLE must include the prevention of vasculopathies as well as minimizing the ischemic damage when it occurs. Unless there is evidence of active inflammation, corticosteroids and immunosupression are rarely indicated. Anticoagulation in cerebral venous thrombosis is controversial, but at least two studies show potential benefit: a controlled of anticoagulation alone, and a case series with combination of anticoagulation and corticosteroids [126].

Therapy consists of limiting the extent of damage to the ischemic tissue. Acute therapy includes rest, antiplatelet agents, and appropriate blood pressure management avoiding tight blood pressure control that may result in hypoperfusion. Although numerous studies in animals show that antiadhesion molecules, cytokines or their antagonists, and antioxidants all reduce the extent of ischemic damage, their role in humans are less than clear. Some fibrinolytic procedures show promise in patients with atherosclerotic disease and thromboses. It is not known whether strokes in patents with a predisposition to bleed would respond in the same way. Because patients with strokes normally heal well with conservative therapy, the major issue is usually prevention of recurrent disease.

Demyelinating Syndrome

Lupoid Sclerosis

In patients with motor impairments, physical therapy should be considered at any stage of the patient's illness

[160]. No cases of the use of MS immunomodulating therapy (IF-β1 and 2, glatiramer acetate) in NPSLE-related demyelinating syndrome are available in the literature.

Optic Neuritis

Corticosteroids have been used in almost all cases of SLE with optic neuritis found in the literature. In a case series of 12 patients treated with oral prednisone, distinct favorable outcomes occurred in only three, with equivocal improvement in an additional two patients [175]. One criticism of this paper is the use of relatively low doses of prednisone [264]. Corticosteroid pulse therapy has been proposed as a faster, more effective alternative, with several instances of cases treated successfully [168].

The use of monthly pulse-dose intravenous cyclophosphamide in the optic neuritis of NPSLE has been described both in new onset, previously untreated cases [170], as well as in cases refractory to corticosteroids and oral immunosuppression [168]. They both show complete recovery in about 50% and partial response in 30–50% by visual acuity testing. Visual field testing [168] revealed somewhat less favorable numbers (40% complete response, 45% partial response, and no improvement in 15%).

Plasmapheresis may be a useful addition to corticosteroids in cases where cytotoxic agents may be contraindicated. One case of Devic's syndrome in a pregnant patient with SLE was successfully treated with plasmapheresis in combination with high-dose corticosteroids, resulting in a successful response [173].

Transverse Myelitis

The optimal treatment strategy in NPSLE-related TM is unknown, as there are no controlled trials [178]. Factors associated with favorable outcomes are early treatment and higher doses of corticosteroids, often in pulse doses and in combination with cyclophosphamide [176, 179, 181, 185, 265, 266], an approach currently favored by most clinicians. The association with aPL would imply a role for anticoagulation, but it has never been studied [179].

Several treatment approaches have been described in case reports of refractory cases. Plasmapheresis in different combinations resulted in varying but mostly unfavorable outcomes [179, 267]. Intrathecal dexamethasone and methotrexate was successful in a case refractory to 80 mg/day of prednisolone [268]. Autologous stem cell transplant was done in a case of catastrophic, refractory Devic's syndrome with near-resolution and prolonged remission at 18 months. Physical therapy is of utmost importance in the management of residual deficits at any point in the patient's illness. Although most sequelae consist of flaccid paralysis, residual spasticity may be much more disabling. Selective posterior rhizotomy has been described as an effective approach [269].

Headache

Effective therapy often depends on accurate diagnosis. Evaluation for abnormalities in the sinuses, dentition, and neck are important. Musculoskeletal abnormalities may respond well to nonsteroidal anti-inflammatory drugs, physical therapy, and appropriate exercise. The serotonin receptor agonist (such as sumatriptan) must be used with caution in patients with coronary artery or cerebrovascular disease. A study shows acetaminophen, aspirin, and caffeine to be an excellent therapy for migraine and may be appropriate in patients with SLE and migraine. Agents like beta- or calcium channel-blockers and anticonvulsants are useful in migraine prophylaxis and may be of benefit in patients with SLE. Headache from intracranial hypertension often responds to corticosteroids [270], which are undesirable for long-term management. Acetazolamide with or without furosemide, serial lumbar punctures, and lumboperitoneal shunt are escalating modes of treatment in persistently symptomatic patients.

Peripheral and Cranial Neuropathies

As in other manifestations of NPSLE, no controlled studies of the treatment of peripheral neuropathies exist. Mild symptoms are managed symptomatically. Tricyclic antidepressants and gabapentin have been used to manage neuropathic pain. Corticosteroids remain the first line agent whenever treatment is indicated. However, in cases of severe symptoms if an acute vasculitic process is suspected (i.e., mononeuritis multiplex), cyclophosphamide and pulse-dose corticosteroids are recommended by most. In most case reports and case series, varying doses of oral corticosteroid ranging 10 to 80 mg/day resulted in resolution of the symptoms [149, 155–157].

Movement Disorders

As in other NPSLE manifestations, treatment protocols have not been developed due to the lack of controlled trials. Chorea has a good prognosis, and most treatment approaches result in success, illustrated in most of the literature. In a review of 50 cases of chorea in SLE or aPS [198], over 50% received corticosteroids in varying doses. Haloperidol was the next most

common treatment. Anticoagulation and antiplatelet therapy alone or in combination was also successful. Combination therapy and/or cytotoxic agents were successfully used in treatment failures. Other cases resolved spontaneously or on discontinuation of contraceptives. The chosen treatment approach should match the associated features of the disease: active SLE and chorea would preferentially be treated with corticosteroids, whereas haloperidol would be favored in the absence of active disease; aCL/aPS with thrombotic complications would make anticoagulation the better option [198, 201, 202, 206, 271, 272]. Tremor failed usual treatment for essential tremor and resolved with corticosteroids [200].

Myasthenia Gravis

No randomized controlled trials of the treatment of SLE-related MG exist. Treatments used are the same as those for primary MG with an important exception. Treatment options in primary MG include oral anticholinesterase inhibitors, immunosupression with corticosteroids, cytotoxic agents, plasmapheresis or IVIg, and thymectomy [273]. While most of these therapies have been used in SLE-related MG, there are concerns that thymectomy, commonly recommended in MG, may induce the appearance of SLE [210, 217] and may even exacerbate established cases.

It is known that thymectomy is of no benefit in the treatment of SLE and may induce postoperative flares [212, 274]. Animal studies of thymectomy in autoimmune and normal mice have shown enhanced autoimmunity [213, 275]. A prospective case control study looked at the frequency and occurrence of autoimmune phenomena in recent and distant thymectomy and controls. Results showed an increasing tendency to autoimmunity over time, as evidenced by higher frequencies and titers of ANA, anti-DNA, RF, and aCL in distant vs. recent thymectomies and controls that was further enhanced at follow-up 3 years later. Also, SLE and MCTD in one case each were seen at follow-up only in the distant thymectomy group [276].

Seizures

The decision whether to initiate anticonvulsant therapy after a first seizure is controversial in any patient, including those with SLE. If the seizure is known to be due to a condition that lowers seizure threshold, anticonvulsant treatment is not begun. If a generalized seizure occurs in the setting of an SLE flare, acute high dose corticosteroid therapy is usually used. If the diagnosis of epilepsy is made, the anticonvulsant drugs used in SLE patients are the same as those used in epilepsy patients without SLE. Although some of the anticonvulsant drugs are known to cause a drug-induced SLE syndrome, they have not been implicated in worsening the disease course in patients with SLE.

Cognitive Dysfunction

Unfortunately, there is no specific treatment for the cognitive dysfunction that commonly occurs in patients with SLE at this time. It is hoped that with ongoing research efforts into the underlying pathophysiology of SLE-related cognitive dysfunction, a better understanding of this NPSLE manifestation will be available, leading to new therapeutic strategies.

Psychiatric Disease

In the case of SLE-related psychosis that is not due to corticosteroid side effects, the current treatment recommendations are high-dose corticosteroid therapy along with antipsychotic medications needed to control the patient's symptoms. No specific immunomodulatory therapy is currently recommended for other psychiatric manifestations. Symptomatic treatment such as antianxiety and antidepressant medications and group or individual therapy should be considered.

References

1. American College of Rheumatology (ACR) ad hoc committee on neuropsychiatric lupus. (1999). The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndrome. *Arthritis Rheum*. **42**, 599–608.
2. Drenkard, C., Villa, A. R., Reyes, E., *et al*. (1997). Vasculitis in systemic lupus erythematosus. *Lupus* **6**, 235–242.
3. Liem, M. D., Gzesh, D. J., and Flanders, A. E. (1996). MRI and angiographic diagnosis of lupus cerebral vasculitis. *Neuroradiology* **38**, 134–136.
4. Moore, P. M. (1998). Immune-mediated vasculopathies of the central nervous system. In "*Prognosis in Neurology*" (J. M. Gilchrist, Ed.), pp. 117–122. Butterworth-Heinemann, Melbourne.
5. De Marcaida, J. A., and Reik, L. (1999). Central nervous system infections: Disorders that mimic central nervous system infections. *Neurol. Clin.* **17**, 901–915.
6. Sibbitt, W. L., Sibbitt, R. R., and Brooks, W. M. (1999). Neuroimaging in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* **42**, 2026–2038.
7. Rivest, C., Lew, R., Welsing, P., *et al*. (2000). Association between clinical factors, socioeconomic status, and organ damage in recent onset systemic lupus erythematosus. *J. Rheumatol*. **27**, 680–684.
8. Futrell, N., Schultz, L. R., and Millikan, C. (1992) Central nervous system disease in patients with systemic lupus erythematosus. *Neurology.* **42**, 1649–1657.

9. Kaell, A. T., Shetty, M., Lee, B. C. P., and Lockshin, M. D. (1986). The diversity of neurologic events in systemic lupus erythematosus. *Arch. Neurol.* **43**, 273–276.
10. McNicholl, J., Glynn, D., Mongey, A., Hutchinson, M., and Bresnihan, B. (1994). A prospective study of neurophysiologic, neurologic and immunologic abnormalities in systemic lupus erythematosus. *J. Rheumatol.* **21**, 1061–1066.
11. West, S. G., Emlen, W., Wener, M. H., and Kotzin, B. L. (1995). Neuropsychiatric lupus erythematosus: A 10-year prospective study on the value of diagnostic tests. *Am. J. Med.* **99**, 153–163.
12. Ainiala, H., Loukkola, J., Peltola, J., Korpela, M., and Hietaharju, A. (2001). prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. *Neurology* **57**, 496–499.
13. Bry, R. L., Holliday, S. L., Saklad, A. R., Navarrete, M. G., Hermosillo-Romo, D., Stallworth, G. L., *et al*. (2002). Neuropsychiatric syndromes in SLE: Prevalence using standardized definitions in the San Antonio Study of Neuropsychiatric Disease Cohort. *Neurology* **58**, 1214–1220.
14. Costallat, L., Bertolo, M., and Appenzeller, S. (2001). The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes: Analysis of 527 patients. *Lupus* **10**, S32.
15. Hanly, J. G. (2001). Neuropsychiatric lupus. *Curr. Rheumatol. Rep.* **3**, 205–212.
16. Hanly, J. G., Walsh, N. M. G., and Sangalang, V. (1992). Brain pathology in systemic lupus erythematosus. *J. Rheumatol.* **19**, 732–741.
17. Belmont, H. M., Abramson, S. B., and Lie, J. T. (1996). Pathology and pathogenesis of vascular injury in systemic lupus erythematosus: Interactions of inflammatory cells and activated endothelium. *Arthritis Rheum.* **39**, 9–22.
18. Isshi, K., and Hirohata, S. (1998). Differential roles of the anti-ribosomal P antibody and antineuronal antibody in the pathogenesis of central nervous system involvement in systemic lupus erythematosus. *Arthritis Rheum.* **41**, 1819–1827.
19. Isshi, K., and Hirihata, S. (1996). Association of anti-ribosomal P protein antibodies with neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* **39**, 1483–1490.
20. Karassa, F., Ioannidis, J. P., Boki, K., *et al*. (2000). Predictors of clinical outcome and radiologic progression in patients with neuropsychiatric manifestations of systemic lupus erythematosus. *Am. J. Med.* **109**, 628–634.
21. Sibbitt, W. L., Sibbitt, R. R., and Brooks, W. M. (1999). Neuroimaging in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* **42**, 2026–2038.
22. Gonzalez-Crespo, M. R., Blanco, F. J., Ramos, A., *et al*. (1995). Magnetic resonance of the brain in systemic lupus erythematosus. *Br. J. Rheumatol*. **34**, 1055–1060.
23. Kelley, V. R., and Wuthrich, R. P. (1999). Cytokines in the pathogenesis of systemic lupus erythematosus. *Semin. Nephrol.* **19**, 57–66.
24. Kirou, K. A., and Crow, N. K. (1999). New pieces to the SLE cytokine puzzle. *Clin. Immunol.* **91**, 1–5.
25. Jara, L. J., Irigoyen, L., Ortiz, M. J. Zazueta, B., Bravo, G., and Espinoza, L. R. (1998). Prolactin and interleukin 6 in neuropsychiatric lupus erythematosus. *Clin. Rheumatol.* **17**, 110–114.
26. Alcocer-Varela, J., Aleman-Hoey, D., and Alarcon-Segovia, D. (1992). Interleukin-1 and interleukin 6 activities are increased in the cerebrospinal fluid of patients with CNS lupus erythematosus and correlate with local late T-cell activation markers. *Lupus* **1**, 111–117.
27. Isshi, K., Hirohata, S., Hashimoto, T., and Miyashita, H. (1994). Systemic lupus erythematosus presenting with diffuse low density lesions in the cerebral white matter on computed axial tomography scans: Its implication in the pathogenesis of diffuse central nervous system lupus. *J. Rheumato.* **21**, 1758–1762.
28. Kozora, E., Laudenslager, M., Lemieux, A., and West, S. G. (2001). Inflammatory and hormonal measures predict neuropsychological functioning in systemic lupus erythematosus and rheumatoid arthritis patients. *J. Int. Neuropsychol. Soc.* **7**, 745–754.
29. Viedt, C., Hansch, G. M., Brandes, R. P., Kubler, W., and Kreuzer, J. (2000). The terminal complement complex C5b-9 stimulates interleukin-6 production in human smooth muscle cells through activation of transcription factors NF-κB and AP-1. *FASEB J.* **14**, 2370–2372.
30. Shikano, M., Sobajima, H., Yoshikawa, H., Toba, T., Kushimoto, H., Katsumata, H., *et al*. (2000). Usefulness of a highly sensitive urinary and serum IL-6 assay in patients with diabetic nephropathy. *Nephron* **85**, 81–85.
31. Kako, S., Nagase, T., and Nagata, N. (1999). Circulating levels of interleukin-6, its soluble receptor and interleukin-6/interleukin-6 receptor complexes in patients with type 2 diabetes mellitus. *Acta Diabetol.* **36**, 67–72.
32. van Aken, B. E., Jansen, J., van Deventer, S. J., and Reitsma, P. H. (2000). Elevated levels of homocysteine increase IL-6 production in monocytic Mono Mac 6 cells. *Blood Coagul. Fibrinolysis* **11**, 159–164.
33. Yudkin, J. S., Kumari, M., Humphries, S. E., and Mohamed-Ali, V. (2000). Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? *Atherosclerosis* **148**, 209–214.
34. Young, D. G., Skibinski, G., Mason, J. I., and James, K. (1999). The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGF-betal) levels in normal healthy blood donors. *Clin. Exp. Immunol.* **117**, 476–481.
35. Straub, R. H., Konecna, L., Hrach, S., Rothe, G., Kreutz, M., Scholmerich, J., *et al*. (1998). Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: Possible link between endocrinosenescence and immunosenescence. *J. Clin. Endocrinol. Metab.* **83**, 2012–2017.
36. Maier, S. F., Goehler, L. E., Fleshner, M., and Watkins, L. R. (1998). The role of the vagus nerve in cytokine-

to-brain communication. *Ann. N. Y. Acad. Sci.* **840**, 289–300.
37. Hickey, W. F. (1991). T-lymphocyte entry into the central nervous system. *J. Neurosci. Res.* **28**, 254–260.
38. Raine, C. S., Cannella, B., Duijvestijn, A. M., and Cross, A. H. (1990). Homing to the central nervous system vasculature by antigen-specific lymphocytes. II lymphocyte/endothelial cell adhesion during the initial stages of autoimmune demyelination. *Lab. Invest.* **63**, 476–489.
39. Rossler, K., Neuchrist, C., Kitz, O., Kraft, D., and Lassmann, H. (1992). Expression of leukocyte adhesion molecules at the human blood-bran-barrier. *J. Neurosci. Res.* **31**, 365–374.
40. Savage, C. O. S., Cooke, S. P. (1993). The role of the endotheliumin system vasculitis. *J. Autoimmunity* **6**, 237–249.
41. Zabry, Z., Waldschmidt, M. M., Hendrickson, D., Keiner, J., Love-Homan, L., Takei, F., *et al*. (1992). Adhesion molecules on muring brain microvascular endothelial cells: Expression and regulation of ICAM-1 and Lgp 55. *J. Neuroimmunol.* **36**, 1–11.
42. Dopp, J. M., Breneman, S. M., and Oschowka, J. A. (1994). Expression of ICAM-1, VCAM-1, L-selectin, and leukosialin in the mouse central nervous system during the induction and remission stages of experimental allergic encephalomyelitis. *J. Neuroimmunol.* **54**, 129–144.
43. Wong, D., and Dorovino-Zis, K. (1992). Upregulation of intercellular adhesion moleculte-1 (ICAM-1) expression in primary cultures of human brain microvascular endothelial cells by cytokines and lipopolysaccharide. *J. Neuroimmunol.* **39**, 11–22.
44. Belmont, H. M., Buyoh, J., Giorno, R., and Abramson, S. (1994). Upregulation of endothelial cell adhesion molecules characterized disease activity in systemic lupus erythematosus. *Arthritis Rheum.* **37**, 376–383.
45. Sharief, M. K., Noori, M. A., Ciardi, M., Cirelli, A., and Thompson, E. J. (1993). Increased levels of circulating ICAM-1 in serum and cerebrospinal fluid of patients with active multiple sclerosis. Correlation with TNF-alpha and blood-brain barrier damage. *J. Neuroimmunol.* **43**, 15–21.
46. Janssen, B. A., Luqmani, R. A., Gordon, C., Hemingway, I. H., Bacon, P. A., Gearing, A. J., *et al*. (1994). Correlation of blood levels of soluble vascular cell adhesion molecule-1 with disease activity in systemic lupus erythematosus and vasculitis. *Br. J. Rheumatol.* **33**, 1112–1116.
47. Horwitz, D. A., Gray, J. D., Behrendsen, S. C., *et al*. (1998). Decreased production of interleukin-12 and other Th1-type cytokines in patients with recent-onset systemic lupus erythematosus. *Arthritis Rheum*. **41**, 838–844.
48. Barcellini, W., Rizzardi, G. P., Borghi, M. O., *et al*. (1996). In vitro type-1 and type-2 cytokine production in systemic lupus erythematosus: Lack of relationship with clinical disease activity. *Lupus* **5**, 139–145.
49. Dinarello, C. A. (1999). IL-18: A Th1-inducing, proinflammatory cytokine and new member of the IL-1 family. *J. Allergy Clin. Immunol.* **103**, 11–24.
50. Sfikakis, P. P., Charalambopoulos, D., Vayoipoulos, G., Oglesby, R. P. S., and Tsokos, G. C. (1994). Increased levels of intercellular adhesion molecule-1 in the serum of patients with SLE. *Clin. Exp. Rheumatol.* **12**, 5–9.
51. Matsuda, J., Gohchi, K., Gotoh, M., Tsukamoto, M., and Saitoh, N. (1994). Circulation intercellular adhesion molecule-1 and soluble interleukin 2-receptor in patients with systemic lupus erythematosus. *Eur. J. Haematol.* **52**, 302–303.
52. Wellicome, S. M., Kapahi, P., Mason, J. C., Lebranchu, Y., Yarwood, H., and Haskard, D. O. (1993). Detection of a circulting form of vascular cell adhesion molecule-1: Raised levels in rheumatoid arthritis and systemic lupus erythematosus. *Clin. Exp. Immunol.* **92**, 412–418.
53. Spronk, P. E., Bootsma, H., Huitema, M. G., *et al*. (1994). Levels of soluble VCAM-1, ICAM-1 and E-Selectin during disease exacerbations in patients with SLE. *Clin. Exp. Immunol.* **97**, 439–444.
54. Machold, K. P., Kiener, H. P., Graninger, W., *et al*. (1993). Soluble ICAM-1 in patients with rheumatoid arthritis and SLE. *Clin. Immunol. Immunopathol.* **68**, 74–78.
55. Wong, C. K., Ho, C. Y., Li, E. K., and Lam, C. W. K. (2000). Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4α) concentrations in patients with systemic lupus erythematosus. *Lupus* **9**, 589–593.
56. Fehniger, T. A., Shah, M. H., Turner, M. J., *et al*. (1999). Differential cytokine and chemokine gene expression by human NK cells following activation with IL-18 or IL-15 in combination with IL-12: Implications for the innate immune response. *J. Immunol.* **162**, 4511–4520.
57. Dao, T., Ohashi, K., Kayano, T., *et al*. (1997). Interferon γ-inducing factor, a novel cytokine, enhances Fas ligand-mediated cytotoxicity of muring T helper cells. *Cell Immunol.* **173**, 230–235.
58. Egerer, K., Feist, E., Rohr, U., Pruss, A., Burmester, G.-R., and Dörner, T. (2000). Increased serum soluble CD14, ICAM-1 and E-selectin correlate with disease activity and prognosis in systemic lupus erythematosus. *Lupus* **9**, 614–621.
59. Roubey, R. A. (1996). Immunology of the antiphospholipid antibody syndrome. *Arthritis Rheum.* **39**, 1444–1454.
60. Giaradi, G., Pierangeli, S., Holers, V. M., Espinola, R., Lui, X., Harris, N., and Salmon, J. E. (2001). Complement activation is required for antiphospholipid antibody induced thrombosis. *Arthritis Rheum.* **44**, S162.
61. Brey, R. L., and Escalante, A. (1998). Neurological manifestations of antiphospholipid antibody syndrome. *Lupus* **7**, (Suppl. 2), S67–S74.
62. Hanly, J. G., Hong, C., Smith, S., and Fisk, J. D. (1999). A prospective analysis of cognitive function and anticardiolipin antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 728–734.
63. Denburg, S. D., Carbott, R. M., Ginsberg, J. S., and Denburg, J. A. (1997). The relationship of antiphospholipid antibodies to cognitive function in patients with systemic lupus erythematosus. *J. Int. Neuropsychol. Soc.* **3**, 377–386.

64. Menon, S., Jameson-Shortall, E., Newman, S. P., Hall-Craggs, M. R., Chinn, R., and Isenberg, D. A. (1999). A longitudinal study of anticardiolipin antibody levels and cognitive functioning in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 735–741.
65. Lai, N. S., and Lan, J. L. (2000). Evaluation of cerebrospinal anticardiolipin antibodies in lupus patients with neuropsychiatric manifestations. *Lupus* **9**, 353–357.
66. Maeshima, E., Maeshima, S., Yamada, Y., and Yukawa, S. (1993). [Antiphospholipid antibodies and regional cerebral blood flow in systemic lupus erythematosus]. *Ryumachi* **33**, 125–130.
67. Kao, C. H., Lan, J. L. ChangLai, S. P. Liao, K. K., Yen, R. F., and Chieng, P. U. (1999). The role of FDG-PET, HMPAO-SPET and MRI in the detection of brain involvement in patients with systemic lupus erythematosus. *Eur. J. Nucl. med.* **26**, 129–134.
68. Mitchell, S. R., Nguyen, P. O., and Katz, P. (1990). Increased risk of neisserial infections in systemic lupus erythematosus. *Semin. Arthritis Rheum*. **20**, 174–184.
69. Iliopoulos, A. G., and Tsokos, G. C. (1996). Immunopathogenesis and spectrum of infections in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **25**, 318–336.
70. Wysenbeek, A. J., Leibovici, L., and Zoldan, J. (1990). Acute central nervous system complications after pulse steroid therapy in patients with systemic lupus erythematosus. *J. Rheumatol.* **17**, 1695–1696.
71. Wolkowitz, O. M. (1994). Prospective controlled studies of the behavioral and biological effects of exogenous corticosteroids. *Psychoneuroendocrinology* **19**, 233–255.
72. Wolkowitz, O. M., Weingartner, H., Rubinow, D. R., *et al*. (1993). Steroid modulation of human memory: Biochemical correlates. *Biol. Psychiatry* **33**, 744–746.
73. Newcomer, J. W., Craft, S., Hershey, T., Askins, K., and Bardgett, M. E. (1994). Glucocorticoid-induced impairment in declarative memory performance in adult humans. *J. Neurosci.* **14**, 2047–2053.
74. Shak, A. K. (1996). Reversible MRI lesions (letter). *Neurology* **46**, 1188–1189.
75. Yaffe, K., Ferriero, D., Barkovich, A. J., and Rowley, H. (1995). Reversible MRI abnormalities following seizures. *Neurology* **45**, 104–108.
76. Theofilopoulos, A. N. (1992). Murine models of lupus. In "Systemic Lupus Erythematosus" (R. G. Lahita, Ed), pp. 121–194. Churchill Livingstone, New York.
77. Klinman, D. M., and Steinberg, A. D. (1995). Inquiry into murine and human lupus. *Immunol. Rev.* **144**, 157–193.
78. Shoenfeld, Y., Blank, M., Aharoni, R., Teitelbaum, D., and Arnon, R. (1993). Manipulation of autoimmune disease with T-suppressor cells: Lesions from experimental SLE and EAE. (Letter) *Immunology* **36**, 109–116.
79. Pierangeli, S. S., and Harris, E. N. (1996). *In vivo* models of thrombosis for the antiphospholipid syndrome. *Lupus* **5**, 451–455.
80. Krause, I., Blank, M., and Shoenfeld, Y. (1996). Immunointerventions in experimental model for antiphospholipid syndrome. *Ann. Intern. Med.* **147**, 50–53.
81. Merino, R., Iwamoto, M., Fossati, L., Muniesa, P., Araki, K., Takahashi, S., *et al*. (1993). Prevention of systemic lupus Erythematosus in autoimmune BXSB mice by a transgene encoding I-E α-chain. *J. Exp. Med.* **178**, 1189.
82. Wantanabe-Fukunga, R., Brannan, C. I., Copeland, N. G., Jenkins, N. A., and Nagata, S. (1992). Lymphoproliferation disorder in mice explained by defects in fast antigen that mediates apoptosis. *Nature* **356**, 314–317.
83. Mozes, E., Kohn, L. D., Hakim, F., and Singer, D. S. (1993). Resistance of MHC class I-deficient mice to experimental systemic lupus Erythematosus. *Science* **261**, 91–93.
84. Lampert, P., and Oldstone, M. B. (1973). Host immunoglobulin IgG and complement deposits in the choroids plexus during spontaneous immune complex disease. *Science* **180**, 408–410.
85. Alexander, E. L., Murphy, E. D., Roths, J. B., and Alexander, G. E. (1983). Congenic autoimmune murine models of central nervous system disease in connective tissue disorders. *Ann. Neurol.* **14**, 242–248.
86. Rudick, R. A., and Eskin, T. A. (1983). Neuropathological features of a lupus-like disorder in autoimmune mice. *Ann. Neurol.* **14**, 325–332.
87. Hoffman, S. A., Arbogast, D. N., Ford, P. M., Shucard, D. W., and Harbeck, R. J. (1987). Brain-reactive autoantibody levels in the sera of ageing autoimmune mice. *Clin. Exp. Immunol.* **70**, 74–83.
88. Nandy, K., Lal, H., Bennett, M., and Bennett, D. (1983). Correlation between a learning disorder and elevated brain-reactive antibodies in aged C57BL/6 and young NZB mice. *Life Sci.* **33**, 1499–1503.
89. Moore, P. M. (1990). Immunoglobulin binding to neuronal cell surface epitopes in murine systemic lupus erythematosus. *J. Neuroimmunol.* **30**, 101–109.
90. Moore, P. M. (1992). Evidence for bound antineuronal antibodies in brain of NZB/W mice. *J. Neuroimmunol.* **38**, 147–154.
91. Khin, N. A., and Hoffman, S. T. (1993). Brain reactive monoclonal auto-antibodies: Production and characterization. *J. Neuroimmunol.* **44**, 137–148.
92. Hess, D. C., Taormina, M., Thompson, J., Sethi, K. D., Diamond, B., Rao, R., and Feldman, D. S. (1993). Cognitive and neurologic deficits in the MRL/lpr mouse: A clinicopathologic study. *J. Rheumatol.* **20**, 610–617.
93. Brey, R. L., Cote, S., and Teale, J. M. (1993). Neurological dysfunction and autoantibody producing B Cells from brain in a lupus-prone mouse strain. *Neurology* **43**, 420.
94. Schrott, L. M., Morrison, L., Wimer, R., Wimer, C., Behan, P. O., and Denenberg, V. H. (1994). Autoimmunity and avoidance learning in NXRF recombinant inbred strains. *Brain Behav. Immunol.* **8**, 100–110.
95. Forester, M. J., Retz, K. C., and Lal, H. (1988). Learning and memory deficits associated with autoimmunity: Significance in aging and Alzheimers' disease. *Drug Dev. Res.* **15**, 253–273.
96. Sakic, B., Szechtman, H., Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1993). Brain-reactive antibodies and behavior of autoimmune MRL-lpr mice. *Physiol. Behav.* **54**, 1025–1029.

97. Keir, A. B. (1990). Clinical neurology and brain histopathology I NZB/NZW F1 lupus mice. *J. Comp. Pathol.* **102**, 165–177.
98. Vogelweid, C. M., Hohnson, G. C., Besch-Williford, C. L., Basler, J., and Walker, S. E. (1991). Inflammatory central nervous system disease in lupus-prone MRL/lpr mice: Comparative histologic and immunohistochemical findings. *J. Neuroimmunol.* **35**, 89–99.
99. Brey, R. L., Cote, S., and Teale, J. M. (1993). Neurological dysfunction and autoantibody producing B cells from brain in a lupus-prone mouse strain. *Neurology* **43**, 420.
100. Sakic, B., Szechtman, H., Keffer, M., Talangbayan, H., Stead, R., and Denburg, J. A. (1992). A behavioral profile of autoimmune lupus-prone MRL mice. *Brain Behav. Immunol.* **6**, 265–285.
101. Sakic, B., Szechtman, H., Talangbayan, H., Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1994). Behaviour and immune status of MRL mice in the postweaning period. *Brain Behav. Immunol.* **8**, 1–13.
102. Sakic, B., Szechtman, H., Stead, R., and Denburg, J. A. (1996). Joint pathology and behavioral performance in autoimmune MRL-lpr mice. *Physiol. Behav.* **60**, 901–905.
103. Sakic, B., Szechtman, H., Talangbayan, H., Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1994). Disturbed emotionality in autoimmune MRL-lpr mice. *Physiol. Behav.* **56**, 609–617.
104. Sakic, B., Denburg, J. A., Denburg, S. D., and Szechtman, H. (1996). Blunted sensitivity to sucrose reward in autoimmune MRL-lpr mice: A curve-shift study. *Brain Res. Bull*. **41**, 305–311.
105. Crnic, L. S., and Schrott, L. M. (1996). Increased anxiety behaviors in autoimmune mice. *Behav. Neurosci.* **110**, 492–502.
106. Ziporen, L., Eilam, D., Goldberg, I., Afek, A., Polak, S., Bardea, A., Mike, A., Kopoloivc, Y., Korzcyn, A. D., and Shoenfeld, Y. (1996). Neurological dysfunctions associated with antiphospholipid antibodies: Animal model. *Lupus* **5**, 533.
107. Aron, A. L., Cuellar, M. L., Brey, R. L., Gharavi, A. E., and Shoenfeld, Y. (1995). Early onset of autoimmunity in MRL/++ mice following immunization with beta-2-glycoprotein 1. *Clin. Exp. Rheum. Immunol.* **101**, 78–81.
108. Brey, R. L., Cote, S. A., Zhongren, L., and Teale, J. (1995). Central nervous system dysfunction is accelerated in autoimmune-prone mice immunized with neuronal surface proteins. *Neurology* **45**, 205.
109. Appendix http://www.rheumatology.org/ar/1999/aprilappendix.html
110. Lesprit, P., Mouloud, F., Bierling, P., Schaffer, A., Cesaro, P., Brun-Buisson, C., and Godeau, B. (1996). Prolonged remission of SLE-associated polyradiculoneuropathy after a single course of intravenous immunoglobulin. *Scand. J. Rheumatol.* **25**, 177–179.
111. Rechthand, E., Cornblath, D. R., Stern, B. J., and Meyerhoff, J. O. (1984). Chronic demyelinating polyneuropathy in systemic lupus erythematosus. *Neurology* **34**, 1375–1377.
112. Millette, T. J., Subramony, S. H., Wee, A. S., and Harisdangkul, V. (1986). Systemic lupus erythematosus presenting with recurrent acute demyelinating polyneuropathy. *Eur. Neurol.* **25**, 397–402.
113. Kovacs, J. A. J., Urowitz, M. B., and Gladman, D. D. (1993). Dilemmas in neuropsychiatric lupus. *Rheum. Dis. Clin. North. Am.* **19**, 795–813.
114. West, S. G. (1994). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am.* **20**, 129–158.
115. Mok, C. C., Lau, C. S., and Wong, R. W. S. (2001). Neuropsychiatric manifestations and their clinical associations in southern Chinese patients with systemic lupus erythematosus. *J. Rheumatol.* **28**, 766–771.
116. Canoso, J. J., and Cohen, A. S. (1975). Aspectic meningitis in systemic lupus erythematosus. Report of three cases. *Arthritis Rheum.* **18**, 369–374.
117. Sands, M. L., Ryczak, M., and Brown, R. B. (1988). Recurrent aseptic meningitis followed by transverse myelitis as a presentation of systemic lupus erythematosus. *J. Rheumatol.* **15**, 862–864.
118. Lancman, M. E., Mesropian, H., and Granillo, R. J. (1989). Chronic aseptic meningitis in a patient with systemic lupus erythematosus. *Can. J. Neurol. Sci.* **16**, 354–356.
119. Hoyle, C., Ewing, D. J., and Parker, A. C. (1985). Acute autonomic neuropathy in association with systemic lupus erythematosus. *Ann. Rheum. Dis.* **44**, 420–424.
120. Gamez-Nava, J. I., González-Lopez, L., Ramos-Remus, C., Fonseca-Gomez, M. M., Cardona-Muñoz, E. G., and Suárez-Almazor, M. E. (1998). Autonomic dysfunction in patients with systemic lupus erythematosus. *J. Rheumatol.* **25**, 1092–1096.
121. Liote, F., and Osterland, C. K. (1994). Autonomic neuropathy in systemic lupus erythematosus: Cardiovascular autonomic function assessment. *Ann. Rheum. Dis.* **53**, 671–674.
122. Altomonte, L., Mirone, L., Zoli, A., and Magaro, M. (1997). Autonomic nerve dysfunction in systemic lupus erythematosus: Evidence for a mild involvement. *Lupus* **6**, 441–444.
123. Straub, R. H., Zeuner, M., Lock, G., Rath, H., Hein, R., Scholmerich, J., and Lang, B. (1996). Autonomic and sensorimotor neuropathy in patients with systemic lupus erythematosus and systemic sclerosis. *J. Rheumatol.* **23**, 87–92.
124. Mathias, C. J. (1997). Autonomic disorders and their recognition. *N. Engl. J. Med.* **336**, 721–724.
125. Haas, L. F. (1982). Stroke as an early manifestation of systemic lupus erythematosus. *J. Neurol. Neurosurg. Psychiatry.* **45**, 554–556.
126. Vidailhet, M., Piette, J. C., Wechsler, B., Bousser, M. G., and Brunet, P. (1990). Cerebral venous thrombosis in systemic lupus erythematosus. *Stroke* **21**, 1226–1231.
127. Harris, E. N., Gharavi, A. E., Asherson, R. A., Boey, M. L., and Hughes, G. R. (1984). Cerebral infarction in systemic lupus: Association with anticardiolipin antibodies. *Clin. Exp. Rheumatol.* **2**, 47–51.
128. Brey, R. L., Gharavi, A. E., and Lockshin, M. D. (1990). Neurologic complications of antiphospholipid antibodies. *Rheum. Dis. Clin. North Am.* **19**, 833–850.
129. Tsokos, G. C., Tsokos, M., leRiche, N. G. H., and Klippel, J. H. (1986). A clinical and pathologic study of cere-

brovascular disease in patients with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **16**, 70–78.
130. Sivri, A., Hasçelik, Z., Çeliker, R., and Başgöze, O. (1995). Early detection of neurological involvement in systemic lupus erythematosus. *Electromyogr. Clin. Neurophysiol.* **35**, 195–199.
131. Omdal, R., Henriksen, O. A., Mellgren, S. I., and Husby, G. (1991). Peripheral neuropathy in systemic lupus erythematosus. *Neurology* **41**, 808–811.
132. Huynh, C., Ho, S. L., Fong, K. Y., Cheung, R. T. F., Mok, C. C., and Lau, C. S. (1999). Peripheral neuropathy in systemic lupus erythematosus. *J. Clin. Neurophysiol.* **16**, 164–168.
133. Hughes, R. A. C., Cameron, J. S., Hall, S. M., Heaton, J., Payan, J., and Teoh, R. (1982). Multiple mononeuropathy as the initial presentation of systemic lupus erythematosus—nerve biopsy and response to plasma exchange. *J. Neurol.* **228**, 239–247.
134. Bloch, S. L., Jarrett, M. P., Swerdlow, M., and Grayzel, A. I. (1979). Brachial plexus neuropathy as the initial presentation of systemic lupus erythematosus. *Neurology* **29**, 1633–1634.
135. Omdal, R., Løseth, S., Torbergsen, T., Koldingsnes, W., Husby, G., and Mellgren, S. I. (2001). Peripheral neuropathy in systemic lupus erythematosus—a longitudinal study. *Acta Neurol. Scand.* **103**, 386–391.
136. Harel, L., Mukamel, M., Brik, R., Blau, H., and Straussberg, R. (2002). Peripheral neuropathy in pediatric systemic lupus erythematosus. *Pediatr. Neurol.* **27**, 53–56.
137. Matsuki, Y., Hidaka, T., Matsumoto, M., *et al*. (1999). Systemic lupus erythematosus demonstrating serum anti-GM1 antibody with sudden onset of drop foot as the initial presentation. *Intern. Med.* **38**, 729–732.
138. Fierro, B., Brighina, F., Amico, L., *et al*. (1999). Evoked potential study and radiological findings in patients with systemic lupus erythematosus. *Electromyogr. Clin. Neurophysiol.* **39**, 305–513.
139. Sabbadini, M. G., Manfredi, A. A., Bozzolo. E., *et al*. (1999). Central nervous system involvement in systemic lupus erythematosus patients without overt neuropsychiatric manifestations. *Lupus* **8**, 11–19.
140. Omdal, R., Mellgren, S. I., Gøransson, L., Skjesol, A., Lindal, S., Koldingsnes, W., and Husby, G. (2002). Small nerve fiber involvement in systemic lupus erythematosus. A controlled study. *Arthritis Rheum.* **46**, 1228–1232.
141. Omdal, R., Mellgren, S. I., Husby, G., Salvesen, R., Henriksen, O. A., and Torbergsen, T. (1993). A controlled study of peripheral neuropathy in systemic lupus erythematosus. *Acta Neurol. Scand.* **88**, 41–46.
142. McCombe, P. A., McLeod, J. G., Pollard, J. D., Guo. Y.-P., and Ingall, T. J. (1987). Peripheral sensorimotor and autonomic neuropathy associated with systemic lupus erythematosus. *Brain* **110**, 533–549.
143. Scheinberg, L. (1956). Polyneuritis in systemic lupus erythematosus. Review of the literature and report of a case. *N. Engl. J. Med.* **255**, 416–421.
144. Bódi, I., Váradi, P., Pokorny, G., Engelhardt, J., Dibó G., Vécsei, L., and Miko, T. L. (1998). Polyneuropathy with endoneurial immune complex deposition as the first manifestation of systemic lupus erythematosus. *Acta Neuropathol.* **96**, 297–300.
145. Genevay, S., Hayem, G., Hamza, S., Palazzo, E., Meyer, O., and Kahn, M. F. (2002). Oculomotor palsy in six patients with systemic lupus erythematosus. A possible role of antiphospholipid syndrome. *Lupus* **11**, 313–316.
146. Lee, J. S., Roh, Y. B., Oum, B. S., and Kwak, I. S. (2000). Isolated oculomotor nerve palsy with pupillary abnormality in systemic lupus erythematosus. *J. Pediatr. Ophthalmol. Strabismus* **37**, 241–243.
147. Sedwick, L. A., and Burde, R. M. (1983). Isolated sixth nerve palsy as initial manifestation of systemic lupus erythematosus. A case report. *J. Clin. Neuro-Ophthalmol.* **3**, 109–110.
148. Rosenstein, E. D., Sobelman, J., and Kramer, N. (1989). Isolated, pupil-sparing third nerve palsy as initial manifestation of systemic lupus erythematosus. *J. Clin. Neuro-Ophthalmol.* **9**, 285–288.
149. Friedman, A. S., Folkert, V., and Khan, G. A. (1995). Recurrence of systemic lupus erythematosus in a hemodialysis patient presenting as a unilateral abducens nerve palsy. *Clin. Nephrol.* **44**, 338–339.
150. Keane, J. R. (1995). Eye movement abnormalities in systemic lupus erythematosus. *Arch. Neurol.* **52**, 1145–1149.
151. Blaustein, D. A., and Blaustein, S. A. (1998). Antinuclear antibody negative systemic lupus erythematosus presenting as bilateral facial paralysis. *J. Rheumatol.* **25**, 798–800.
152. Lundberg, P. O., and Werner, I. (1972). Trigeminal sensory neuropathy in systemic lupus erythematosus. *Acta Neurol. Scand.* **48**, 330–340.
153. Vyse, T., Luxon, L. M., and Walport, M. J. (1994). Audiovestibular manifestations of the antiphospholipid syndrome. *J. Laryngol. Otol.* **108**, 57–59.
154. Andonopoulos, A. P., Naxakis, S., Goumas, P., and Lygatsikas, C. (1995). Sensorineural hearing disorders in systemic lupus erythematosus. A controlled study. *Clin. Exp. Rheumatol.* **13**, 137–141.
155. Vaile, J. H., and Davis, P. (1998). Isolated unilateral vagus nerve palsy in systemic lupus erythematosus. (Letter) *J. Rheumatol.* **25**, 2287–2288.
156. Saluja, S., Singh, R. R., Misra, A., Gairola, A. K., Prasad, K., Ahuja, G. K., and Malaviya, A. N. (1989). Bilateral recurrent laryngeal nerve palsy in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **7**, 81–83.
157. Gordon, T., and Dunn, E. C. (1990). Systemic lupus erythematosus and right recurrent laryngeal nerve palsy. *Br. J. Rheumatol.* **29**, 308–309.
158. Chan, C. N., Li, E., Lai, F. M., and Pang, J. A. (1989). An unusual case of systemic lupus erythematosus with isolated hypoglossal nerve palsy, fulminant acute pneumonitis, and pulmonary amyloidosis. *Ann. Rheum. Dis.* **48**, 236–239.
159. Sloan, J. B., Berk, M. A., Gebel, H. M., and Fretzin, D. F. (1987). Multiple sclerosis and systemic lupus erythematosus: Occurrence in two generations of the same family. *Arch. Intern. Med.* **147**, 1317–1320.
160. Kaplan, P. E., and Betts, H. B. (1977). Lupoid sclerosis: Evaluation and treatment. *Arch. Phys. Med. Rehabil.* **58**, 24–28.

161. Kinnunen, E., Muller, K., Keto, P., Ketonen, L., Helve, T., and Sepponen, R. (1993). Cerbrospinal fluid and MRI findings in three patients with multiple sclerosis and systemic lupus erythematosus. *Acta Neurol. Scand.* **78**, 356–360.
162. McCombe, P. A., Chalk, J. B., and Pender, M. P. (1990). Familial occurrence of multiple sclerosis, thyroid disease and systemic lupus erythematosus. *J. Neurol. Sci.* **97**, 163–171.
163. Scott, T. F., Hess, D., and Brillman. J. (1994). Antiphospholipid antibody syndrome mimicking multiple sclerosis and systemic lupus erythematosus clinically and by magnetic resonance imaging. *Arch. Intern. Med.* **154**, 917–920.
164. Rolak, L. A. (1996). The diagnosis of multilple sclerosis. *Neurol. Clin.* **14**, 27–43.
165. Giorgi, D., and Balacco Gabrieli, C. (1999). Optic neuropathy in systemic lupus erythematosus and antiphospholipid syndrome (APS): Clinical features, pathogenesis, review of the literature and proposed ophthalmological criteria for APS diagnosis. *Clin. Rheumatol.* **18**, 124–131.
166. Levine, S. R., Crofts, J. W., Lesser, G. R., Floberg, J., and Welch, K. M. (1988). Visual symptoms associated with the presence of a lupus anticoagulant. *Ophthalmology* **95**, 686–692.
167. Oppenheimer, S., and Hoffbrand, B. I. (1986). Optic neuritis and myelopathy in systemic lupus erythematosus. *Can. J. Neurol. Sci.* **13**, 129–132.
168. Galindo-Rodriguez, G., Avina-Zubieta, J. A., Pizarro, S., Diaz de Leon, V., Saucedo, N., Fuentes, M., and Lavalle, C. (1999). Cyclophosphamide pulse therapy in optic neuritis due to systemic lupus erythematosus: An open trial. *Am. J. Med.* **106**, 65–69.
169. April, R. S., and Vansonnenberg, E. (1976). A case of neuromyelitis optica (Devic's syndrome) in systemic lupus erythematosus. Clinopathologic report and review of the literature. *Neurology* **26**, 1066–1070.
170. Rosenbaum, J. T., Simpson, J., and Neuwelt, C. M. (1997). Successful treatment of optic neuropathy in association with systemic lupus erythematosus using intravenous cyclophosphamide. *Br. J. Ophthalmol.* **81**, 130–132.
171. Giorgi, D., Balacco Gabrieli, C., and Bonomo, L. (1999). The association of optic neuropathy with transverse myelitis in systemic lupus erythematosus. (Letter) *Rheumatology* **38**, 191–192.
172. Jabs, D. A., Miller, N. R., Newman, S. A., Johnson, M. A., and Stevens, M. B. (1986). Optic neuropathy in systemic lupus Erythematosus. *Arch. Ophthalmol.* **104**, 564–568.
173. Bonnet, F., Mercie, P., Morlat, P., Hocke, C., Vergnes, C., Ellie, E., Viallard, J. F., Faure, I., Pellegrin, J. L., Beylot, J., and Leng, B. (1999). Devic's neuromyelitis optica during pregnancy in a patient with systemic lupus erythematosus. *Lupus* **8**, 244–247.
174. Cinefro, R. J., and Frenkel, M. (1978). Systemic lupus erythematosus presenting as optic neuritis. *Ann. Ophthalmol.* **10**, 559–563.
175. Smith, C. A., and Pinals, R. S. (1982). Optic neuritis in systemic lupus erythematosus. *J. Rheumatol.* **9**, 963–966.
176. Andrianakos, A. A., Duffy, J., Suzuki, M., and Sharp, J. T. (1975). Transverse myelopathy in systemic lupus erythematosus. Report of three cases and review of the literature. *Ann. Intern. Med.* **83**, 616–624.
177. Hachen, H. J., and Chantraine, A. (1979). Spinal cord involvement in systemic lupus erythematosus. *Paraplegia* **17**, 337–346.
178. Mok, C. C., Lau, C. S., Chan, E. Y., and Wong, R. W. (1998). Acute transverse myelopathy in systemic lupus erythematosus: Clinical presentation, treatment, and outcome. *J. Rheumatol.* **25**, 467–473.
179. Kovacs, B., Lafferty, T. L., Brent, L. H., and DeHoratius, R. J. (2000). Transverse myelopathy in systemic lupus erythematosus: An analysis of 14 cases and review of the literature. *Ann. Rheum. Dis.* **59**, 120–124.
180. Schantz, V., Oestergaard, L. L., and Junker, P. (1998). Shrinking spinal cord following transverse myelopathy in a patient with systemic lupus erythematosus and the phospholipid antibody syndrome. *J. Rheumatol.* **25**, 1425–1428.
181. Barile, L., and Lavalle, C. (1992). Transverse myelitis in systemic lupus erythematosus—the effect of IV pulse methylprednisolone and cyclophosphamide. *J. Rheumatol.* **19**, 370–372.
182. Warren, R. W., and Kredich, D. W. (1984). Transverse myelitis and acute central nervous system manifestations of systemic lupus erythematosus. *Arthritis Rheum.* **27**, 1058–1060.
183. Marabani, M., Zoma, A., Hadley, D., and Sturrock, R. D. (1989). Transverse myelitis occurring during pregnancy in a patient with systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**, 160–162.
184. Provenzale, J., and Bouldin, T. W. (1992). Lupus-related myelopathy: Report of three cases and review of the literature. *J. Neurol. Neurosurg. Psychiatry* **55**, 830–835.
185. Propper, D. J., and Bucknall, R. C. (1989). Acute transverse myelopathy complicating systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**, 512–515.
186. Provenzale, J. M., Barboriak, D. P., Gaensler, E. H. L., Robertson, R. L., and Mercer, B. (1994). Lupus-related myelitis: Serial MR findings. *Am. J. Neuroradiol.* **15**, 1911–1917.
187. Sfikakis, P. P., Mitsikostas, D. D., Manoussakis, M. N., Foukaneli, D., and Moutsopoulos, H. M. (1998). Headache in systemic lupus erythematosus: A controlled study. *Br. J. Rheumatol.* **37**, 300–303.
188. Fernández-Nebro, A., Palacios-Muñoz, R., Gordillo, J., Abarca-Costalago, M., De Haro-Liger, M., Rodríguez-Andreu, J., and González-Santos, P. (1999). Chronic or recurrent headache in patients with systemic lupus erythematosus: A case control study. *Lupus* **8**, 151–156.
189. Glanz, B. I., Venkatesan, A., Schur, P. H., Lew, R. A., and Khoshbin, S. (2001). Prevalence of migraine in patients with systemic lupus erythematosus. *Headache* **41**, 285–289.
190. Atkinson, R. A., and Appenzeller, O. (1975). Headache in small vessel disease of the brain: A study of patients with systemic lupus erythematosus. *Headache* **15**, 198–201.

191. Isenberg, D. A., Meyrick-Thomas, D., Snaith, M. L., McKeran, R. O., and Royston, J. P. (1982). A study of migraine in systemic lupus erythematosus. *Ann. Rheum. Dis.* **41**, 30–32.
192. Vázquez-Cruz, J., Traboulssi, H., Rodriquez-De la Serna, A., Geli, C., Roig, C., and Díaz, C. (1990). A prospective study of chronic or recurrent headache in systemic lupus erythematosus. *Headache* **30**, 232–235.
193. Rozell, C. L., Sibbitt, Jr, W. L., and Brooks, W. M. (1998). Structural and neurochemical markers of brain injury in the migraine diathesis of systemic lupus erythematosus. *Cephalalgia* **18**, 209–215.
194. Markus, H. S., and Hopkinson, N. (1992). Migraine and headache in systemic lupus erythematosus and their relationship with antibodies against phospholipids. *J. Neurol.* **239**, 39–42.
195. Brandt, K. D., and Lessell, S. (1978). Migrainous phenomena in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 7–16.
196. Montalban, J., Cervera, R., Font, J., Ordi, J., Vianna, J., Haga, H. J., Tintore, M., Khamashta, M. A., and Hughes, G. R. V. (1992). Lack of association between anticardiolipin antibodies and migraine in systemic lupus erythematosus. *Neurology* **42**, 681–682.
197. Omdal, R., Waterloo, K., Koldingsnes, W., Husby, G., and Mellgren, S. I. (2001). Somatic and psychological features of headache in systemic lupus erythematosus. *J. Rheumatol.* **28**, 772–779.
198. Cervera, R., Asherson, R. A., Font, J., Tikly, M., Pallares, L., Chamorro, A., and Ingelmo, M. (1997). Chorea in the antiphospholipid syndrome: Clinical, radiologic, and immunologic characteristics of 50 patients from our clinics and the recent literature. *Medicine* **76**, 203–212.
199. Khamashta, M. A., Gil, A., Anciones, B., Lavilla, P., Valencia, M. E., Pintado, V., and Vázquez, J. J. (1988). Chorea in systemic lupus erythematosus: Association with antiphospholipid antibodies. *Ann. Rheum. Dis.* **47**, 681–683.
200. Venegoni, E., Biasioli, R., Lamperti, E., Rinaldi, E., Salmaggi, A., and Novi, C. (1994). Tremor as an early manifestation of systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **12**, 199–201.
201. Wolf, R. E., and McBeath, J. G. (1985). Chorea gravidarum in systemic lupus erythematosus. *J. Rheumatol.* **12**, 992–993.
202. Asherson, R. A., Derksen, R. H. W. M., Harris, E. N., Bouma, B. N., Gharavi, A. E., Kater, L., and Hughes, G. R. V. (1987). Chorea in systemic lupus erythematosus and "lupus-like" disease: Association with antiphospholipid antibodies. *Semin. Arthritis Rheum.* **16**, 253–259.
203. Hatron, P. Y., Bouchez, B., Wattel, A., Arnott, G., and Devulder, B. (1986). Chorea, systemic lupus, circulating lupus anticoagulant. (Letter) *J. Rheumatol.* **13**, 991–993.
204. Asherson, R. A., Harris, N. E., Gharavi, A. E., and Hughes, G. R. V. (1986). Systemic lupus erythematosus, antiphospholipid antibodies, chorea, and oral contraceptives. (Letter) *Arthritis Rheum.* **29**, 1536–1539.
205. Cervera, R., Khamashta, M. A., Font, J., Sebastiani, G. D., Gil, A., Lavilla, P., Domenech, I., Aydintug, A. O., Jedryka-Goral, A., de Ramon, E., *et al*. (1993). Systemic lupus erythematosus: Clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. *Medicine* **72**, 113–124.
206. Lusins, J. O., and Szilagyi, P. A. (1975). Clinical features of chorea associated with systemic lupus erythematosus. *Am. J. Med.* **58**, 857–861.
207. Olsen, J. E. (1968). Chorea minor associated with systemic lupus erythematosus. Report of a case. *Acta. Med. Scand.* **183**, 127–129.
208. Bryun, G. W., and Padberg, G. (1984). Chorea and systemic lupus erythematosus. A critical review. *Eur. Neurol.* **23**, 278–290.
209. Wolf, S. M., and Barrows, H. S. (1966). Myasthenia gravis and systemic lupus erythematosus. *Arch. Neurol.* **14**, 254–258.
210. Petersen, P., and Lund, J. (1969). Systemic lupus erythematosus following thymectomy for myasthenia gravis. *Danish Med. Bull.* **16**, 179–181.
211. Abbruzzese, G., Abbruzzese, M., Bacigalupo, A., and Ratto, S. (1979). Systemic lupus erythematosus after thymectomy in a patient with myasthenia gravis. (Letter) *Neurology* **29**, 1436–1437.
212. Chan, M. K. L., and Britton, M. (1980). Comparative clinical features in patients with myasthenia gravis with systemic lupus erythematosus. *J. Rheumatol.* **7**, 838–842.
213. Killian, P. J., and Hoffman, G. S. (1980). Coexistence of systemic lupus erythematosus and myasthenia gravis. *South. Med. J.* **73**, 244–246.
214. Vaiopoulos, G., Sfikakis, P. P., Kapsimali, V., Boki, K., Panayiotidis, P., Aessopos, A., Tsokos, G. C., and Kaklamanis, P. (1994). The association of systemic lupus erythematosus and myasthenia gravis. *Postgrad. Med. J.* **70**, 741–745.
215. Meloni, F., Gueli, N., Villatico Campbell, S., Di Maio, F., Di Bernardo, M. G., Di Nuzzo, M., and Carmenini, G. (1996). A case of systemic lupus erythematosus (SLE) and myasthenia gravis (MG). *Allergy* **51**, 362–363.
216. Barbosa, R. E., Cordova, S., and Cajigas, J. C. (2000). Coexistence of systemic lupus erythematosus and myasthenia gravis. (Letter) *Lupus* **9**, 156–162.
217. Mevorach, D., Perrot, S., Buchanan, N. M., Khamashta, M., Laoussadi, S., Hughes, G. R., and Menkes, C. J. (1995). Appearance of systemic lupus erythematosus after thymectomy: Four case reports and review of the literature. *Lupus* **4**, 33–37.
218. Scheuer, M. L., and Pedley, T. A. (1990). The evaluation and treatment of seizures. *N. Engl. J. Med.* **323**, 1468–1472.
219. Herranz, M. T., Rivier, G., Khanashta, M. A., Blaser, K. U., and Hughes, G. R. V. (1994). Association between antiphospholipid antibodies and epilepsy in patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 568–571.
220. van Dam, A. P. (1991). Diagnosis and pathogenesis of CNS lupus. *Rheumatol. Int.* **11**, 1–11.
221. Mackworth-Young, C. G., and Hughes, G. R. V. (1985). Epilepsy: An early symptom of systemic lupus erythe-

matosus. *J. Neurol. Neurosurg. Psychiatry* **48**, 185–192.
222. Feinglass, R. J., Arnett, F. C., Dorsch, C. A., Zizic, T. M., and Stevens, M. B. (1997). Neurological manifestations of systemic lupus erythematosus: Diagnosis, clinical spectrum, and relationship to other features of the disease. *Medicine* **55**, 323–339.
223. McCure, W. J., and Globus, J. (1988). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am.* **14**, 149–167.
224. Tzioufas, A. G., Tzortzakis, N. G., Panou-Pomonis, E., *et al*. (2000). The clinical relevance of antibodies to ribosomal-P common epitope in two targeted systemic lupus erythematosus populations: A large cohort of consecutive patients and patients with active central nervous system disease. *Ann. Rheum. Dis.* **59**, 99–104.
225. Waterloo, K., Omdal, R., Husby, G., *et al*. (2001). Neuropsychological function in SLE: A five year longitudinal study. *Lupus* **10**, (Suppl. 1), 35.
226. Sabbadini, M. G., Manfredi, A. A., Bozzolo, E., *et al*. (1999). Central nervous system involvement in SLE patients without overt neuropsychiatric manifestations. *Lupus* **8**, 11–19.
227. Yalaoui, S., Gorgi, Y., Hajri, R., Goucha, R., Chaabouni, L., Kooli, C., and Ayed, K. (2002). Autoantibodies to ribosomal P proteins in systemic lupus erythematosus. *Joint, Bone, Spine: Revue du Rhumatisme* **69**, 173–176.
228. Arnett, F. C., Reveille, J. D., Moutsopoulos, H. M., *et al*. (1996). Ribosomal P antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **39**, 1833–1839.
229. Formiga, F., Moga, I., Pac, M., *et al*. (1999). Mild presentation of systemic lupus erythematosus in elderly patients assessed by SLEDAI. *Lupus* **8**, 462–465.
230. Carreño, L., Lopez-Longo, F. J., Monteagudo, I., *et al*. (1999). Immunological and clinical differences between juvenile and adult onset of systemic lupus erythematosus. *Lupus* **8**, 287–292.
231. Font, J., Cervera, R., Espinosa, G., *et al*. (1998). Systemic lupus erythematosus in childhood: Analysis of clinical and immunological findings in 34 patients and comparison with SLE characteristics in adults. *Ann. Rheum. Dis.* **57**, 456–459.
232. Antolin, J., Amerigo, M. J., Cantabrana, A., *et al*. (1995). Systemic lupus erythematosus: Clinical manifestations and immunological parameters in 194 patients. Subgroup classification of SLE. *Clin. Rheumatol.* **14**, 678–685.
233. Duriseti, R. S. (2001). Lupus. *Medicine* **2**, 1–12.
234. Segasothy, M., and Phillips, P. (2001). Systemic lupus erythematosus in Aborigines and Caucasians in Central Australia: A comparative study. *Lupus* **10**, 439–444.
235. Petri, M. (1998). The effect of race on the presentation and course of SLE in the United States. *Lupus* **7**, S22.
236. Cucurull, E., Gharavi, A. E., Diri, E., *et al*. (1999). IgA anticardiolipin and anti-β_2-glycoprotein I are the most prevalent isotypes in African American patients with systemic lupus erythematosus. *Am. J. Med. Sci.* **318**, 55–60.
237. Ghirardello, A., Doria, A., Zampieri, S., *et al*. (2000). Anti-ribosomal P protein antibodies detected by immunoblotting in patients with connective tissue diseases: Their specificity for SLE and association with IgG anticardiolipin antibodies. *Ann. Rheum. Dis.* **59**, 975–981.
238. Kao, C. H., Ho, Y. J., Lan, J. L., Changlai, S. P., Liao, K. K., and Chieng, P. U. (1999). Discrepancy between regional cerebral blood flow and glucose metabolism of the brain in systemic lupus erythematosus patients with normal brain magnetic resonance imaging findings. *Arthritis Rheum.* **42**, 61–68.
239. Weiner, S. M., Otte, A., Schumacher, M., Klein, R., Gutfleisch, J., Brink, I., *et al*. (2000). Diagnosis and monitoring of central nervous system involvement in systemic lupus erythematosus: Value of F-18 fluorodeoxyglucose PET. *Ann. Rheum. Dis.* **59**, 377–385.
240. Otte, A., Weiner, S. M., Peter, H. H., Mueller-Brand, J., Goetze, M., Moser, E., *et al*. (1997). Brain glucose utilization in systemic lupus erythematosus with neuropsychiatric symptoms: A controlled positron emission tomography study. *Eur. J. Nucl. Med.* **24**, 787–791.
241. Komatsu, N., Kodama, K., Yamanouchi, N., Okada, S., Noda, S., Nawata, Y., *et al*. (1999). Decreased regional metabolic rate for glucose in systemic lupus erythematosus patients with psychiatric symptoms. *Eur. Neurol.* **42**, 41–48.
242. Chinn, R. J. S., Wilkinson, I. D., Hall-Craggs, M. A., *et al*. (1997). Magnetic resonance imaging of the brain and cerebral proton spectroscopy in patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 36–46.
243. Omdal, R., Selseth, B., Klow, N. E., Husby, G., and Mellgren, S. I. (1989). Clinical neurological, electrophysiological and cerebral CT scans findings in systemic lupus erythematosus. *Scand. J. Rheumatol.* **18**, 283–289.
244. Ward, M. M., Pyun, E., and Studenski, S. (1996). Mortality risks associated with specific clinical manifestations of systemic lupus erythematosus. *Arch. Intern. Med.* **156**, 1337–1344.
245. Swaak, A. J., van den Brink, H. G., Smeenk, R. J., *et al*. (1999). Systemic lupus erythematosus: Clinical features in patients with a disease duration of over 10 years, first evaluation. *Rheumatology* **38**, 953–958.
246. Swaak, A. J., van den Brink, H. G., Smeenk, R. J., *et al*. (2001). Systemic lupus erythematosus. Disease outcome in patients with disease duration of at least 10 years: Second evaluation. *Lupus* **10**, 51–58.
247. Abel, T., Gladmen, D. D., and Urowitz, M. B. (1980). Neuropsychiatric lupus. *J. Rheumatol.* **7**, 325–333.
248. Gibson, T., and Myers, A. R. (1976). Nervous system involvement in systemic lupus erythematosus. *Ann. Rheum. Dis.* **35**, 398–406.
249. Cook, R. J., Gladman, D. D., Pericak, D., and Urowitz, M. B. (2000). Prediction of short term mortality in systemic lupus erythematosus with time dependent measures of disease activity. *J. Rheumatol.* **27**, 1892–1895.
250. Ward, M. M., Pyun, E., and Studenski, S. (1996). Mortality risks associated with specific clinical manifestations of systemic lupus erythematosus. *Arch. Intern. Med.* **156**, 1337–1344.
251. Shoenfeld, Y., and Khamashta, M., guest editors. (2001). Special Issue: Old treatment/new treatment. *Lupus* **3**, 1–248.

252. Navarrete, G., and Brey, R. L. (2000). Neuropsychiatric systemic lupus erythematosus. *Curr. Treat. Options Neurol.* **2**, 473–485.
253. Strand, V. (2000). New therapies for systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **26**, 1–16.
254. van Vollenhoven, R. F. (2000). Dehydroepiandrosterone in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **26**, 135–145.
255. van Vollenhoven, R. F., Elliot, D., Powell, M., *et al*. (2001). Dehydroepiandrosterone improves cognitive function in patients with systemic lupus erythematosus: Results of a double-blinded, placebo controlled pilot study. *Lupus* **10**, (Suppl. 1), S51.
256. Barile, L., Olguin, L., Ariza, R., *et al*. (2001). Controlled clinical trial of cyclophosphamide, vs. methylprednisolone in severe neurologic manifestations in SLE. *Lupus* **10**, S34.
257. Bellomio, V., Spindler, A., and Cunio, S. (2001). Cyclophosphamide in systemic lupus erythematosus: Cumulative dose and adverse events. *Lupus* **10**, S104.
258. Trevisani, V. F., Castro, A. A., Neves-Neto, J. F., and Atallah, A. N. (2000). Cyclophosphamide versus methylprednisolone for the treatment of neuropsychiatric involvement in systemic lupus erythematosus. *Cochrane Database Syst. Rev.* **3**, CD002265.
259. Traynor, A. E., Schroeder, J., Rosa, R., *et al*. (2000). Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and hematopoietic stem-cell transplantation: A phase I study. *Lancet* **356**, 701–707.
260. Hughes, R. D. (1991). Ineffectiveness of high-dose intravenous methylprednisolone in Guillain-Barre syndrome. (Letter) *Lancet* **338**, 1142.
261. Brannagan, T. H., Pradhan, A., Heimann-Patterson, T., Winkelman, A. C., Styler, M. J., Topolsky, D. L., Crilley, P. A., Schwartzman, R. J., Brodsky, I., and Gladstone, D. E. (2002). High-dose cyclophosphamide without stem-cell rescue for refractory CIDP. *Neurology* **58**, 1856–1858.
262. Clark, W. F., Rock, G. A., Buskard, N., Shumak, K. H., LeBlond, P., Anderson, D., and Sutton, D. M. (1999). Therapeutic plasma exchange: An update from the Canadian apheresis group. *Ann. Intern. Med.* **131**, 453–462.
263. Robson, M. G., Walport, M. J., and Davies, K. A. (1994). Systemic lupus erythematosus and acute demyelinating polyneuropathy. *Br. J. Rheumatol.* **33**, 1074–1077.
264. Fleet, W. S., and Watson, R. T. (1986). Autoimmune optic neuritis: A potentially treatable form of visual loss. *Ann. Ophthalmol.* **18**, 144–146.
265. Sherer, Y., Hassin, S., Shoenfeld, Y., Levy, Y., Livneh, A., Ohry, A., and Langevitz, P. (2002). Transverse myelitis in patients with antiphospholipid antibodies—the importance of early diagnosis and treatment. *Clin. Rheumatol.* **21**, 207–210.
266. Harisdangkul, V., Doorenbos, D., and Subramony, S. H. (1995). Lupus transverse myelopathy: Better outcome with early recognition and aggressive high-dose intravenous corticosteroid pulse treatment. *J. Neurol.* **242**, 326–331.
267. Slovick, D. I. (1986). Treatment of acute myelopathy in systemic lupus erythematosus with plasma exchange and immunosuppression. (Letter) *J. Neurol. Neurosurg. Psychiatry* **49**, 103–105.
268. Valesini, G., Priori, R., Francia, A., Balestrieri, G., Tincani, A., Airo, P., Cattaneo, R., Zambruni, A., Troianello, B., Chofflon, M., and Miescher, P. A. (1994). Central nervous system involvement in systemic lupus Erythematosus: A new therapeutic approach with intrathecal dexamethasone and methotrexate. *Springer Semin. Immunopathol.* **16**, 313–321.
269. Yang, T. F., Lee, S. S., Lin, P. H., Chen, H., and Chan, R. C. (2002). Effect of selective posterior rhizotomy on transverse myelitis in a patient with systemic lupus erythematosus. *Am. J. Phys. Med. Rehabil.* **81**, 467–468.
270. Padeh, S., and Passwell, J. H. (1996). Systemic lupus erythematosus presenting as idiopathic intracranial hypertension. *J. Rheumatol.* **23**, 1266–1268.
271. Heilman, K. M., Kohler, W. C., and LeMaster, P. C. (1971). Haloperidol treatment of chorea associated with systemic lupus erythematosus. *Neurology* **21**, 963–965.
272. Thomas, D., Byrne, P. D., and Travers, R. L. (1979). Systemic lupus erythematosus presenting as post-partum chorea. *Aust. N. Z. J. Med.* **9**, 568–570.
273. Drachman, D. B., Jones, R. J., and Brodski, R. A., (2003). Treatment of refractory myasthenia: "Rebooting" with high-dose cyclophosphamide. *Ann. Neurol.* **53**, 29–34.
274. Mackay, I. R., and Smalley, M. (1966). Results of thymectomy in systemic lupus erythematosus: Observations on clinical course and serological reactions. *Clin. Exp. Immunol.* **1**, 129–138.
275. Grinlinton, F. M., Lynch, N. M., and Hart, H. H. (1991). A pair of monozygotic twins who are concordant for myasthenia gravis but became discordant for systemic lupus erythematosus post-thymectomy. *Arthritis Rheum.* **34**, 916–919.
276. Gerli, R., Paganelli, R., Cossarizza, A., Muscat, C., Piccolo, G., Barbieri, D., Mariotti, S., Monti, D., Bistoni, O., Raiola, E., Venenzi, F. M., Bertotto, A., and Franceschi, C. (1999). Long-term immunologic effects of thymectomy in patients with myasthenia gravis. *J. Allergy Clin. Immunol.* **103**, (5 Part 1), 865–872.

27

PSYCHIATRIC ASPECTS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Wendy S. Cohen
W. Neal Roberts, Jr.
James L. Levenson

INTRODUCTION

In a disease, systemic lupus erythematosus (SLE), that afflicts children and young adults, flares without warning, and attacks multiple organ systems so indiscriminately, it seems particularly unjust that it also targets the brain—the core of reason, emotion, and the essence of self. Neuropsychiatric symptoms of SLE were first reported in 1872 by Kaposi, who described two lupus patients with delirium [1]. Depending on the diagnostic methodology utilized, neuropsychiatric manifestations have a prevalence of up to 75–90% [2–4]. They encompass a wide variety of conditions, ranging from stroke, seizures, headaches, neuropathy, transverse myelitis, and movement disorders, to cognitive deficits, depression, mania, anxiety, psychosis, and delirium (acute confusional state). Central nervous system (CNS) involvement is a major cause of morbidity in SLE, and is second only to renal failure and accelerated atherosclerosis as a cause of mortality. The pathogenesis of neuropsychiatric syndromes in SLE is multifaceted and complex, including direct CNS involvement, secondary effects of infection (CNS and systemic), uremia, electrolyte disturbances, hypertension, and other systemic diseases, medication side effects, and psychological reactions to chronic illness. While neurologic disorders in SLE are discussed in Chapter 26, and cognitive impairment in Chapter 28, the present chapter focuses on psychiatric manifestations of SLE, including their prevalence, pathogenesis, classification, diagnosis, and treatment.

Neuropsychiatric SLE syndromes that are not attributable to a localized brain lesion are sometimes classified as "diffuse," and include psychosis, "organic" mood and anxiety disorders, delirium, coma, headaches, generalized seizures, and cognitive dysfunction. "Focal" neurologic SLE syndromes include stroke, cerebrovascular hemorrhage, transverse myelitis, motor disorders, neuropathy, and focal seizures. The validity of the diffuse vs focal categorization of neuropsychiatric SLE is still debated. Classifying neuropsychiatric SLE syndromes as reversible vs irreversible may be useful, since it may have greater implications regarding pathogenesis and treatment.

PATHOGENESIS OF PSYCHIATRIC MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Psychiatric syndromes in SLE can be due to (a) direct CNS involvement (primary neuropsychiatric SLE), (b) infection, other systemic illness, or drug-induced side effects (secondary neuropsychiatric SLE), (c) reaction to chronic illness, and (d) comorbid primary psychiatric illness.

Direct Pathophysiologic CNS Effects of SLE (Primary Neuropsychiatric SLE)

Autopsy Studies

Studies of brain pathology at autopsy have provided clues to the pathogenesis of CNS lupus. In a classic study by Johnson and Richardson in 1968 [5], the most prevalent finding was a noninflammatory small vessel vasculopathy with microinfarcts, whereas vasculitis was rare. Subsequently, a larger study [6] corroborated these findings, and revealed a high prevalence of CNS infection associated with corticosteroid use. Nevertheless, autopsy studies have inherent limitations, since they reveal only anatomical changes, and are performed only on patients with fatal disease. Autopsy studies also cannot exclude whether CNS pathology has been affected by confounding factors such as medication, infection, other systemic illness, or elapsed time (i.e., between neuropsychiatric symptoms and autopsy). In addition, the correlation between brain pathology and neuropsychiatric lupus symptoms is poor. Approximately 20% of brains were histologically normal [5], suggesting disturbances in neurophysiology that are either transient, or not associated with structural abnormalities.

Neuropathogenesis

In a review of the pathogenesis of neuropsychiatric SLE, two major mechanisms of CNS injury have been proposed: (1) antibody-mediated neuronal injury, and (2) antibody-mediated microvasculopathy [7]. Autoantibodies may directly damage neurons either by causing cell death, or by transiently and reversibly impairing neuronal function. Increased *apoptosis*, coupled with impaired clearance of apoptotic cells [8, 9], may result in prolonged exposure of the immune system to DNA (and other intracellular fragments), thereby promoting production of anti-DNA and other autoantibodies [7]. *Complement* factors, particularly C1q, are crucial in the phagocyte-mediated clearance of apoptotic cells. Since a third of SLE patients have antibodies to C1q [10], the resultant C1q deficiency may cause decreased apoptotic cell clearance, and thus enhanced autoantibody production [7]. Antibody-mediated microvasculopathy seems to involve two processes: (a) endothelial damage due to anti-cardiolipin or anti-endothelial antibodies binding to the vessel wall [11], and to a lesser extent, immune complex deposition, and (b) coagulation disturbances due to the prothrombotic effects of anti-cardiolipin antibodies [12], both culminating in ischemia or infarction. These two pathogenic mechanisms may be mutually reinforcing, perpetuating the disease process. Microvascular endothelial injury in the CNS may increase the permeability of the blood–brain barrier, leading to influx of autoantibodies, and further CNS damage.

Factors implicated in the pathogenesis of primary CNS SLE include autoimmune antibodies (anti-neuronal, anti-lymphocyte, anti-ribosomal, and anti-phospholipid antibodies), alterations in cytokines (interferon α, interleukins [IL-1, IL-2, IL-6, IL-8, IL-10], and tumor necrosis factor α [TNF-α]), and complement factors C1q and complement-derived anaphylatoxins C3a and C5a [7, 13, 14].

Autoimmune antibodies that cross-react with neuronal membrane antigens, ribosomal proteins, or phospholipids on platelet or endothelial surfaces seem to play a much larger role in direct CNS involvement than does classic immune complex deposition affecting microvasculature [7, 15, 16]. Serum *anti-ribosomal-P antibodies* have been associated with psychosis [17–22] and severe depression [18, 20], albeit not consistently [23, 24]. In a study by Arnett, the presence of anti-ribosomal-P antibodies conferred a markedly increased risk of psychosis (odds ratio 4.4) and depression (odds ratio 10.5) [20]. As psychosis remits, the antiribosomal-P antibody titer declines [18–21], suggesting that this autoantibody may have pathogenic significance (although the effects of corticosteroid treatment could not be excluded). *Anti-neuronal antibodies*, and, perhaps less commonly, circulating cytokine effects directly on neurons [25], have been associated with neuropsychiatric manifestations that involve global impairment of higher integrative functions [26], including psychosis, depression, delirium, coma, and cognitive dysfunction. In contrast, *anti-phospholipid antibodies* (e.g., anti-cardiolipin), causing discrete episodes of inappropriate clotting [27, 28], and vasculitis involving immune complexes, may account for the less common focal neurologic syndromes. In addition to their association with stroke [29–31], anti-cardiolipin antibodies are also strongly associated with cognitive dysfunction [26, 32, 33]. Accordingly, anti-phospholipid antibodies of different precise specificities may be acting via two different mechanisms to produce focal stroke in a few cases and cognitive impairment in a larger number. *In vitro* evidence suggests that anti-phospholipid antibodies may directly depolarize synaptic terminals. This mechanism may account for some neuropsychiatric symptoms of the antiphospholipid syndrome not attributable to ischemia [34].

Cytokines appear to be involved in the pathogenesis of neuropsychiatric SLE. Although their role remains unclear, they may modulate the autoimmune response, indirectly enhance autoantibody production [35], exacerbate vasculopathy [7, 36], or cause CNS neurotoxicity via increased nitric oxide production [14]. In

neuropsychiatric SLE, interleukins IL-6, IL-8, and IL-10 are markedly elevated in the cerebrospinal fluid (CSF), without concomitant increase in the serum IL-6 and IL-8, suggesting intrathecal synthesis of these cytokines. Resolution of symptoms with cytotoxic therapy is accompanied by a decline in CSF IL-6 levels, although this covariance may be attributable to the treatment itself [37]. The association between neuropsychiatric lupus and elevated interleukin-6 in the CSF has been corroborated in several other studies [27, 38, 39], and psychosis has been linked to increased CSF interferon α [25]. Interestingly, when interferon α is administered therapeutically (e.g., for malignancies, hepatitis C. or amyotrophic lateral sclerosis), it produces neuropsychiatric side effects, including depression, suicidal ideation, delirium, cognitive impairment, and psychosis [40–42]. The neuropsychiatric morbidity associated with interferon α and other cytokines [40] is consistent with, but not proof of, the involvement of cytokine dysregulation in the pathogenesis of CNS lupus. Increased systemic production of TNF-α, a proinflammatory cytokine, correlates with CSF metabolites of nitric oxide, an inflammatory mediator, as well as with the severity of neuropsychiatric SLE symptoms [14]. In patients with neuropsychiatric lupus, kinin levels are increased in plasma and CSF, with disproportionate elevation of tissue kallikrein activity in the CSF relative to plasma, suggesting intrathecal synthesis. Kinin-mediated induction of proinflammatory cytokines, such as IL-1 β, IL-6, and TNF-α, suggests a possible pathogenic role for kinins and cytokines in neuropsychiatric SLE [43]. Nevertheless, since alterations in cytokine levels occur in infection and other inflammatory processes, these fluctuations are not specific to CNS lupus. (See Chapter 26 for an in-depth discussion of the pathogenesis of CNS SLE.)

Reversible vs Irreversible Neuropsychiatric SLE Syndromes: Implications Regarding Pathogenesis

With the possible exception of cognitive dysfunction, all the major psychiatric manifestations of SLE, namely psychosis, depression, mania, anxiety, and delirium, exhibit a degree of reversibility, as does coma. Even the cognitive deficits are sometimes corticosteroid-responsive when cognition is assessed formally [44, 45]. Since psychiatric syndromes tend to resolve within 2 to 3 weeks with corticosteroid treatment [44], they are probably due to reversible or transient mechanisms rather than irreversible neuronal death. Such mechanisms include transient permeability defects in endothelium that permit autoantibodies to traverse the blood–brain barrier. Other potentially reversible mechanisms are antigen–antibody binding, antibody production by B cells, cytokine–receptor binding, and increased cytokine levels.

The reversibility of psychiatric dysfunction stands in contrast to most focal neurologic events, which often have no more reversibility than atherosclerotic stroke, and are associated with fixed lesions on neuroimaging. Similarly, in a subset of patients, the progressive nature of cognitive impairment [33, 45], often with cerebral atrophy, suggests cumulative irreversible CNS damage. This irreversibility, both clinically and neuroanatomically, implies neuronal death, as would be expected from ischemia. Ischemia may be attributable to anticardiolipin antibodies, causing focal clotting, with resultant micro- or macroinfarcts. Less common causes of ischemia include emboli or vasculitis, which involves immune-complex deposition (culminating in thrombosis, sometimes with hemorrhage) immune-complex deposition (causing microinfarcts), or emboli. Yet neuronal cell death at a slow pace may be more common than what may be surmised by the low frequency of overt stroke, and may be at least partly attributable to excitotoxicity-induced apoptosis. Newly described anti-NMDA (*N*-methyl-D-aspartate) receptor antibodies, a subset of anti-DNA antibodies that cross react with NR2 glutamate receptor, can cause cell death by accelerated apoptosis [46]. Because the NR2 glutamate receptor is widespread on neuronal membranes, autoantibodies to it could constitute a possible mechanism leading to generalized cerebral atrophy [47]. Table 1 pairs autoantibodies and cytokines with the putatively associated primary neuropsychiatric SLE syndrome.

Risk factors for direct CNS involvement in SLE include cutaneous vasculitis (odds ratio 33) and antiphospholipid syndrome and its manifestations, especially arterial thromboses (odds ratio 13), as well as recurrent fetal demise, livedo reticularis, and high titers of IgG anti-cardiolipin antibodies [48]. Patients with mainly articular manifestations or discoid rash have a much lower risk of overt neuropsychiatric lupus, as do those few who are ANA-negative, and those with drug-induced SLE. Anti-phospholipid antibodies (anticardiolipin antibodies and lupus anticoagulant) may be the single strongest marker of CNS risk in SLE, given their association with stroke [29–31], cognitive dysfunction [26, 28, 33], and epilepsy [29, 49–51]. A history of previous neuropsychiatric episodes predicts future CNS involvement; each event confers a greater than threefold increased risk of new or recurrent CNS episodes [52]. Preliminary genetic research suggests a possible linkage between locus SLEB3 and lupus with neuropsychiatric manifestations [53]. Hypoandrogenism may also increase the risk of neuropsychiatric SLE [54].

TABLE 1 Autoantibodies and Cytokines Associated with Neuropsychiatric SLE Syndromes

Autoantibody or cytokine	Most likely neuropsychiatric presentation	Other associated neuropsychiatric features	Putative mechanism or neuropathology
Anti-neuronal and anti-lymphocyte antibodies (cross-react with neuronal membrane) [22, 47a, 85, 86]	Cognitive deficits Psychosis		Reversible neuronal dysfunction, but not cell death
Anti-neurofilament antibodies (serum) [47b, 156]	Delirium Cognitive deficits	Seizures	
Anti-ribosomal P protein antibodies (serum) [17, 18, 20, 21]	Psychosis	Severe depression	?Autoreactive T cells
Anti-NMDA receptor antibodies [46]	Impaired learning Other cognitive deficits	Psychosis	Apoptotic cell death accelerated by overstimulation of neuronal NMDA receptors Cerebral atrophy
Anti-phospholipid (including anti-cardiolipin) antibodies (serum) [28–33, 49–51]	Cognitive deficits	Stroke Seizures (common)	Microinfarcts (3 mm size) Noninflammatory (bland) vasculopathy Macroinfarcts (rare)
Interleukin 6 [27]	?Depression	?Psychosis (rare)	
Interferon-alpha (serum, CSF) [25]	Psychosis		
Immune complexes	Stroke		Vasculitis Thrombosis, hemorrhage

Secondary Causes of Neuropsychiatric Symptoms in SLE: Infection, Other CNS or Systemic Illness, or Drug-Induced Side Effects

Although neuropsychiatric symptoms can be attributable to CNS lupus, they are often secondary to complications of SLE or its treatment—especially infection. Since lupus patients have underlying immune dysregulation, and are often immunosuppressed due to corticosteroid or cytotoxic therapy, they are predisposed to CNS and systemic infections, including opportunistic infections, which can simulate neuropsychiatric lupus [2, 55]. Such CNS infections include cryptococcal, tubercular, meningococcal, and Listeria meningitis, herpes encephalitis, neurosyphilis, CNS nocardiosis, toxoplasmosis, brain abscesses, and progressive multifocal leukoencephalopathy. Other etiologies of neuropsychiatric manifestations in SLE include uremia, hypertensive encephalopathy, cerebral lymphoma, and medication side effects, particularly corticosteroids and hydroxychloroquine. See Tables 2, 5, and 6 (latter two tables are on medication side effects).

TABLE 2 Secondary Medical and Psychiatric Causes of Neuropsychiatric Symptoms in SLE

- Secondary medical causes not attributable to CNS lupus
  - CNS infections
    - Meningitis/encephalitis
    - CNS abscess
    - Leukoencephalopathy
  - Systemic infections
  - Renal failure (due to lupus nephritis)
  - Fluid/electrolyte disturbance
  - Hypertensive encephalopathy
  - Hypoxemia (due to lupus pneumonitis, congestive heart failure (secondary to lupus-induced myocarditis, endocarditis, coronary artery disease, or pulmonary hypertension))
  - Fever
  - CNS tumor (e.g., cerebral lymphoma due to immunosuppression, cytotoxic therapy)
  - Medication side effects (especially corticosteroids, hydroxychloroquine)
  - Comorbid medical illness not directly due to SLE or its treatment
    - Hypothyroidism or hyperthyroidism
    - Thrombotic thrombocytopenic purpura
    - Fibromyalgia
- Psychiatric symptoms in reaction to chronic illness
- Comorbid psychiatric illness

PSYCHOLOGICAL IMPACT OF ILLNESS

Stressful Aspects of SLE

Although coping with any debilitating illness is difficult, certain aspects of SLE make it particularly stressful. SLE is a multisystem disease that can be severe, chronic, and unpredictable. It requires major adjustments by the patient and family, and can lead to adaptive or maladaptive responses.

Multisystem Involvement

Since SLE can present in a myriad of ways involving almost any organ system, or with vague systemic symptoms, the diagnosis is often elusive. Patients typically go from one specialist to another over the course of several years, and undergo extensive diagnostic workups before being diagnosed with lupus. One patient commented, "I knew I was ill, but I could get no one [to listen]. . . . It [my constellation of symptoms] was never anybody's department" [56]. The inability of physicians to make a diagnosis may erode the patient's confidence in the medical system. Furthermore, when no etiology can be found for the symptoms, the physician may deem the illness psychogenic, and recommend psychotherapy. Such referrals can engender resentment, since they invalidate the patient's conviction that she is physically ill. The patient may become alienated from the mental health field, and may not be amenable to psychiatric treatment when it may truly be needed in the future [56].

After being diagnosed with SLE, the patient initially may feel relieved that there is a defined illness causing her bewildering array of symptoms. This relief, or "honeymoon period," may abruptly terminate when the symptoms persist or recur despite treatment, or the treatment itself causes serious side effects.

Since SLE can affect many organ systems, the patient may worry that the illness pervades her entire body, even when the disease is limited. The diffuse nature of SLE distinguishes it from most other chronic, recurrent diseases, such as asthma or inflammatory bowel disease, which are primarily limited to one organ. In SLE, the sick portion cannot be cordoned off, for it encompasses the entire self [57].

Because of the multisystem involvement in SLE, and the increasing specialization in medicine, SLE patients are often under the care of an entourage of specialists. Accordingly, the role of the primary care physician may be subordinated, or lost entirely [57]. The patient may then feel there is a specialist for every organ system, but no one taking care of her as a whole.

Severity and Chronicity

The severity and chronicity of SLE result in multiple losses. Patients lose their sense of physical well-being, and may become debilitated or incapacitated. Fatigue alone can be very disabling. As the illness worsens, there is increased dependence on caregivers, and loss of autonomy. Patients also lose the pleasure of participating in certain activities, either because of physical limitations or medical restrictions. Some patients, especially young women, lament having to forgo sunbathing and engaging in outdoor sports. The risk of miscarriage or provocation of a lupus flare may result in giving up childbearing. Patients may at times experience a "loss of self" (or loss of mind) due to the neuropsychiatric manifestations of SLE, especially psychosis, delirium, and cognitive impairment. The CNS involvement and physical debility may result in loss of employment or career. Marital relationships are often strained by the stress of coping with SLE. If a patient feels God has punished her by making her ill, or forsaken her by not healing her, her faith may be shaken. Finally, though less frequently than in past decades, SLE results in premature loss of life.

Unpredictability

One of the most stressful aspects of SLE is its unpredictable course, with sudden exacerbations, remissions, and variable prognosis. The unpredictability of SLE results in a profound loss of control, as well as a loss of ability to plan for the future. One patient commented,

"When I had even a few good days in a row, my hopes would begin to rise, and I would think of remission. Then I would feel worse, and those hopes would be crushed. I felt as if I were trapped on a wild seesaw; I was helplessly fixed on one end, the disease on the other. The illness was in complete control, and all I could do was try to hold on. My family and close friends rode the seesaw with me, equally powerless to stop the mad ride" [58].

Psychological Reactions to Having SLE

In response to the stresses and losses associated with SLE, patients may experience a variety of reactions, which become maladaptive only if they impair functioning or interfere with treatment. Perhaps the most common reactions are grief and depression. Grieving over losses, both actual and anticipated, associated with lupus is a normal reaction to chronic illness. In contrast to depression, grief is an intense but nonsustained sadness, and is generally not accompanied by significant functional impairment. It is also not associated with psy-

chomotor retardation, guilty rumination, worthlessness, hopelessness, preoccupation with death, or suicidal ideation. Depression is characterized by a more sustained, pervasive dysphoria, and/or anhedonia, that is, the loss of interest or pleasure in previously enjoyable activities. (See Classification of Psychiatric Disorders in Systemic Lupus Erythematosus for an in-depth discussion.) When confronted with the unpredictability and chronicity of SLE, some patients feel utterly powerless, and stop making any effort to impact their situation. This resignation, termed learned helplessness [59], is often a sign of depression, and may present as apathy.

Lupus patients may regress into the "sick role," and become increasingly dependent on others for their care. Spouses or other family members may aggravate this tendency by being overly protective of the patient [56]. Regression may also be exacerbated by the disparity between appearing well, yet feeling ill, and having to demonstrate that one is ill to elicit support. Alternatively, to achieve a semblance of normalcy, at times patients minimize their symptoms, which ironically then reduces the support they receive from others, and intensifies their sense of aloneness. This feeling of aloneness is also reinforced by public ignorance and lack of understanding of lupus. Consequently, in addition to having to divulge their illness, patients also may have to explain it to others. SLE patients may become socially withdrawn, especially if they are self-conscious about their appearance. Women with a malar rash or discoid lesions may feel branded as if by the "scarlet letter" [57]. Patients, particularly adolescents, may avoid socializing because of self-consciousness about corticosteroid-induced side effects such as weight gain, "moon facies," increased facial hair, and easy bruising [56].

Anxiety is also an exceedingly common reaction to having SLE, and is often associated with depression and insomnia. In a study by Liang, the most prevalent fears of SLE patients were worsening disease, disability, and death [59]. In particular, patients may worry about developing cognitive impairment, or becoming paralyzed or unable to speak due to strokes. Such neurologic deficits may be especially frightening to contemplate since they may result in debilitation, loss of autonomy, and inability to care for oneself. Similarly, patients may worry about developing worsening renal failure necessitating dialysis. A major concern is the fear of becoming a burden to their families physically, emotionally, and also financially. Some patients with SLE may become incessantly preoccupied with their own mortality [60].

In response to having SLE, some patients feel victimized, and express their anger and frustration. They may become irritable, short-tempered, or hostile, especially toward their spouse and other family members. Other patients react to having SLE by denying their illness. Believing they are well, they are poorly adherent to medical treatment and follow-up.

The unpredictable nature of SLE may cause patients to scale down their own activities and aspirations, and thus avoid being devastated by setbacks. When expectations are set low enough, the vicissitudes of the illness may become more tolerable. Endeavoring to make sense out of their illness, patients may retrospectively misattribute the cause of their SLE flares. Consequently, patients may progressively curtail their activities, social encounters, or even their thought patterns to minimize the risk of relapse. They may also engage in superstitious or ritualistic behavior to prevent recurrence. Patients may blame themselves for having SLE. Such self-blame may be reinforced by the concept of SLE as an autoimmune disease—and the perception that the body is literally waging war against itself [57].

Although depression, denial, and other negative reactions to having SLE are common, at least 50% of patients experience positive reactions at some point during their illness [60]. Patients may sometimes perceive their illness as "a gift." Having such a serious illness can lead patients to reorient their priorities toward spirituality and relationships with family and friends. Patients may also develop a greater appreciation of their remaining abilities, the simple pleasures of life, and the value of time. A primary goal of psychotherapy with SLE patients is to help them reframe their illness in a more positive light, and thereby come to terms with its impact on their lives. Well-adjusted patients are able to find a balance between conflicting needs: the need for empathy and understanding, which requires that patients acknowledge being ill, and the need to appear healthy and move on with their lives, despite being ill [56].

PSYCHOLOGICAL FACTORS AFFECTING ONSET AND COURSE OF SYSTEMIC LUPUS ERYTHEMATOSUS, AND ADHERENCE TO TREATMENT

Stress: Its Role in the Onset and Exacerbations of SLE

Although many patients and clinicians believe lupus may be precipitated or exacerbated by stress, studying this issue is complex, and fraught with methodological difficulties. First, stress is difficult to define, let alone quantify. Events that might be perceived as exceedingly stressful to one patient may leave another unfazed.

Second, negative stressful events, such as divorce, job termination, or death of a child, differ from positive ones, such as marriage or a promotion. Third, most studies of stress-induced illness are retrospective, and therefore vulnerable to biased recall. Patients may be more likely to remember flares and stressful events that are temporally associated with each other, than stresses not associated with flares, or flares occurring in the absence of apparent stress. Fourth, it is important to distinguish between stress intensifying the perception of symptoms, vs etiologically inducing symptoms. In addition, confounding factors must be excluded that could be misdiagnosed as a stress-related lupus flare. For example, stress can predispose to infection, and can exacerbate joint pain from fibromyalgia, a comorbid condition in many SLE patients. Corticosteroid withdrawal can cause arthralgias and mood lability, mimicking a stress-induced flare [61]. Finally, in studying stress-induced illness, it is difficult to distinguish cause and effect. While stress may cause a lupus flare, it is also likely, if not inevitable, that lupus flares cause stress.

Stress-Induced Immune Dysregulation in SLE

If stress precipitates SLE flares, the pathogenesis probably involves immune dysregulation rather than immunosuppression, since theoretically, the latter would decrease disease activity. Several studies have provided support for stress-induced immune dysregulation in SLE. Hinrichsen has shown that in response to acoustic, psychological, and exercise-induced stress, the normal increase in B- and T-supppressor/cytotoxic lymphocytes, and decrease in T-helper lymphocytes are blunted in SLE patients, relative to healthy controls, patients with sarcoidosis, and others receiving corticosteroids [62, 63]. In a study by Jacobs, stress (due to public speaking) was associated with a transient increase in interleukin (IL)-4 producing cells in SLE patients, but not in healthy controls [64]. Since IL-4 producing cells cause proliferation and expansion of activated B cells, and consequently, increased autoantibody production, the stress-induced increased IL-4 levels could precipitate a lupus flare. Furthermore, unlike controls, SLE patients under stress did not develop increased natural killer cell activity or increased levels of interleukin-10, an immunosuppressive cytokine. (Note: Although SLE patients were not receiving major immunosuppressive agents, some patients were on hydroxychloroquine or low-dose methotrexate [64].) In a 6-month prospective study of 41 patients with SLE, daily stress correlated positively with anti-nuclear and anti-double-stranded DNA antibodies [65].

Stress and the Onset of SLE

In a literature review by Herrmann [66], only two studies [67, 68] investigated stress as an etiologic factor in the onset of SLE, and only one was controlled. Otto and Mackay reported that 20 patients hospitalized for SLE had significantly greater stress prior to the onset of their illness than did the seriously ill hospitalized controls [67].

Stress and Exacerbations of SLE

Several studies have supported the contention that stress can exacerbate SLE. Ropes reported that in his cohort of SLE patients, 41 of 45 lupus flares occurred after physical or emotional stress [69]. In another retrospective study, 12 of 19 patients reported experiencing flares of SLE after stressful periods, including the Christmas holiday [70]. A longitudinal study by Wekking revealed that relative to patients with rheumatoid arthritis, those with SLE had a stronger correlation between daily stressors and anxiety, depression, and physical symptoms, although these did not necessarily parallel immunologic parameters [71]. Accordingly, stress, psychiatric symptoms, and the patient's perception of the severity of her lupus are intricately linked [72].

Nevertheless, there is some conflicting evidence regarding whether stress impacts the course of SLE. In a naturalistic study after a severe earthquake in Los Angeles, none of the rheumatology patients studied (10 with SLE and 13 with rheumatoid arthritis) residing near the epicenter developed a significant flare of illness [73]. Among lupus patients followed prospectively, depression and anxiety scores correlated directly with the patients' perception, but not with objective measures, of SLE activity [74].

Individuals vary in their vulnerability to stress-induced lupus symptoms. While some patients seem to have symptoms precipitated by stress, others seem relatively impervious. However, even among stress-responders, the worsening symptoms were associated with distress, but not always with increased disease activity [75].

Premorbid Personality Styles and Response to Having SLE

An astute physician once observed that "it is more important what kind of patient has the illness, than what kind of illness the patient has." Underlying personality traits are often accentuated during times of stress, including illness. Since personality style influences how a patient copes with a chronic, relapsing disease such as

SLE, optimizing the outcome requires that the physician tailor the treatment approach to that individual. In a classic article, Kahana and Bibring delineated several personality styles and the corresponding medical approach that is most therapeutic [76]. Patients with *obsessive* personality traits tend to be rigid, rational, and detail-oriented, and use their intellect to bring order and gain a sense of mastery in stressful situations. For such patients, illness represents a loss of control, and lupus can be particularly frightening since it can affect that which they value most, their minds. To regain control, such patients become even more compulsive, extensively documenting their own symptoms and researching their illness. These patients respond best to a detailed explanation of SLE and its treatment. Patients with *dependent* traits appear needy, clingy, and helpless; their pleas for care seem insatiable. In response to illness, they regress even further, but are terrified of abandonment. The staff should provide them with consistent, nurturing care, while being nonjudgmental in curtailing unreasonable expectations. Patients who are *self-sacrificing* have often experienced many adversities in life, and subordinate their own needs to those of others. For such patients, illness is a means of receiving love and care via increased suffering. Giving reassurance to such patients is counterproductive; it tends to intensify their distress and physical complaints. These patients respond best when the physician acknowledges their suffering, and presents treatment as another hardship they must endure for the sake of those they love. *Histrionic* patients tend to be melodramatic, emotional, and attention-seeking, and are easily overwhelmed. Such patients may perceive illness as a blemish or flaw that makes them unappealing. While focusing on changes in their appearance, they may seem oblivious to more serious manifestations of SLE, yet tend to catastrophize (i.e., imagine the worst). Their focus on more superficial, less dangerous aspects of SLE, (e.g., facial rash) may be a way of defending against more frightening possibilities. The physician should employ a gentle supportive approach with histrionic patients to decrease their apprehensiveness. It is best to provide them with necessary information about SLE and its treatment, but in less detail than for a patient with obsessive traits. Patients with *narcissistic* traits are arrogant and condescending, and have a sense of entitlement, often demanding to be treated by the premier specialist and expecting unlimited access. Nevertheless, their haughtiness is a veneer for fragile self-esteem and feelings of inadequacy. In such patients, illness violates their sense of superiority and invincibility. They respond best when the physician validates them for their achievements, without devaluing his own skills and expertise, for example, "I am impressed that you have been able to be so productive in your work despite your illness; many other patients have been unable to do so." Patients who have *paranoid* traits are suspicious and wary that others might take advantage of them, and tend to harbor resentment. For such patients, illness is perceived as harm inflicted by others, and it exacerbates their suspiciousness and hostility. These patients, who often develop adversarial relationships with their physicians, should be informed as early as possible of any planned diagnostic workup or treatment. In interacting with such patients, physical and emotional closeness should be avoided. Finally, *aloof* patients tend to be reclusive and emotionally withdrawn, and prefer solitary activities. To such patients, illness signifies a threat to their inner sanctum. In working with them, the physician should have minimal expectations regarding their interaction and participation, while not allowing them to become too isolated. [76]

CLASSIFICATION OF PSYCHIATRIC DISORDERS IN SYSTEMIC LUPUS ERYTHEMATOSUS

> Nomenclature's shifting, semantically drifting,
> A term's definition gets muddled.
> Each author will bend it to what he intended,
> And leave the poor reader befuddled. [77]

The literature on neuropsychiatric SLE has been plagued by problems with nomenclature, particularly the lack of standardized terminology. Many investigators have defined terms idiosyncratically or not at all, confounding interpretation. At times, immersing oneself in the literature on neuropsychiatric SLE is utterly bewildering—the language seems to make sense but the meaning is elusive. The terminology has also varied depending on the specialty of the examiner. In a patient with disorientation and a fluctuating level of consciousness, a neurologist may diagnose "encephalopathy," a psychiatrist, "delirium," and a rheumatologist, "acute confusional state." Furthermore, the terminology has been inconsistent over the years, thereby making it difficult to compare studies retrospectively. Terms such as lupoid sclerosis have become obsolete, only to be supplanted by new terms that overlap but are not quite synonymous. More importantly, since there is no gold standard for the diagnosis of neuropsychiatric SLE, ascertaining which conditions are direct CNS manifestations of SLE, vs a reaction to illness, has been controversial. Debate has ensued regarding whether or not headache, depression, anxiety, and other "minor" conditions should be conceptualized as neuropsychiatric manifestations of lupus (see later). Interestingly, in

the 1982 American Rheumatism Association/American College of Rheumatology (ARA/ACR) revised criteria for SLE, neuropsychiatric involvement was defined narrowly, including only seizures and psychosis [78]. Finally, terms such as lupus cerebritis have obfuscated our understanding, since they imply a pathogenesis (inflammation) that remains unproven and is probably incorrect.

To rectify these problems, the American College of Rheumatology (ACR) convened a committee to devise standardized nomenclature for neuropsychiatric SLE, and published guidelines in 1999 [79]. The committee eschewed any terminology that suggested an underlying pathogenesis unless the latter had been well-established (e.g., stroke). It defined neuropsychiatric lupus as "the neurologic syndromes of the central, peripheral, and autonomic nervous system, and the psychiatric syndromes observed in patients with SLE in which other causes have been excluded." The committee proposed "case definitions" for 19 neuropsychiatric syndromes by specifying diagnostic and exclusionary criteria, and "methods of ascertainment" (laboratory workup, neuropsychiatric testing, and neuroimaging). Psychiatric disorders included psychosis, acute confusional state, cognitive dysfunction, anxiety disorder, and mood disorders. Mood disorders were subdivided into (a) major depressive-like episode, (b) mood disorder with depressive features not meeting criteria for major depressive episode, (c) mood disorder with manic features, and (d) mood disorder with mixed features—that is, major depression, minor depression, mania, and mixed episode, respectively. When feasible, terminology from the Diagnostic and Statistical Manual IV (DSM IV) was utilized. A reactive psychological disturbance must be excluded in diagnosing psychosis, anxiety, and mood disorders. If the psychiatric disorder is felt to be a direct CNS manifestation of lupus but occurred in the context of significant psychosocial stress, the latter was to be listed as an association. According to the ACR committee report, the nomenclature was developed to classify neuropsychiatric syndromes and promote research, not to diagnose individual patients. Overall, the ACR criteria significantly broadened the spectrum of syndromes that can be considered neuropsychiatric SLE [79].

Some investigators have criticized the ACR nomenclature for neuropsychiatric SLE for its low specificity. In a cross-sectional population-based study, Ainaila assessed the validity of the ACR nomenclature by comparing the prevalence of neuropsychiatric symptoms in SLE patients and healthy controls. Using the ACR criteria, the sensitivity was 91%; however, the specificity was only 46%. The ACR criteria could not distinguish SLE patients from either neuropsychiatric SLE patients or healthy controls. To enhance the specificity and clinical utility of the criteria, the authors excluded conditions such as headache, anxiety, mild cognitive dysfunction, and mild depression that are not known to be associated with underlying neurologic injury. Accordingly, psychiatric SLE was limited to acute confusional state, psychosis, moderate or severe cognitive dysfunction, and severe depression. Relative to the ACR criteria, their revised criteria had a much higher specificity, 93%, and a slightly higher correlation with the neuropsychiatric SLICC damage index, but a lower sensitivity—46% [80]. Given the reciprocal relationship between sensitivity and specificity, the ACR criteria may be better suited for identifying all possible cases of neuropsychiatric SLE. In contrast, Ainiala's revised diagnostic criteria may be preferable in selecting SLE patients most likely to have direct CNS involvement, thereby minimizing the risk of administering potentially toxic medication unnecessarily [81].

A more fundamental problem with the ACR classification system is that it is difficult to apply clinically. To diagnose neuropsychiatric SLE as the cause of the psychiatric symptoms, one must exclude a primary psychiatric disorder (as well as substance use or a reaction to illness). In contrast, in DSM IV, a primary psychiatric disorder cannot be diagnosed unless medical disorders and substance use are ruled out as etiologies. An inherent problem with the ACR approach is that whereas medical disorders and substance use can be easily excluded, there are no clinical criteria or laboratory tests for excluding primary psychiatric disorders. For example, if a patient with SLE becomes depressed, it is unclear how to rule out primary depression as the cause.

Cognitive Dysfunction

Cognitive dysfunction is the most common neuropsychiatric disorder in patients with SLE, occurring in up to 80% of patients [3, 4]. Cognitive deficits include decreased attention and concentration, impaired memory, difficulty word-finding and problem-solving, and decreased psychomotor speed. On detailed neuropsychological testing, even patients who have never had overt neuropsychiatric symptoms are often found to have cognitive impairment. The cognitive dysfunction cannot be explained by corticosteroids, depression, emotional distress, or systemic disease activity [82–84]. Patients with anti-cardiolipin antibodies are at significantly increased risk of stroke [29–31], and have a three- to fourfold increased risk of cognitive impairment, which is often progressive [28, 33, 45]. Cognitive impairment may be associated with serum lymphocytotoxic antibodies [85], CSF anti-neuronal antibodies [86], and pathological findings such as microinfarcts and cor-

tical atrophy. While cognitive dysfunction often fluctuates and is reversible [45, 87], presumably when attributable to edema and inflammation, it tends to be irreversible when secondary to multiple infarcts, and may culminate in dementia [88].

Perhaps with the exception of schizophrenia and severe depression ("pseudodementia"), primary psychiatric disorders do not typically cause cognitive dysfunction. Accordingly, *in SLE patients with psychiatric symptoms, the presence of cognitive dysfunction, particularly delirium, should prompt a thorough workup for medical etiologies*. Hypothyroidism should also be considered as a possible contributing factor in patients with cognitive impairment. (See Chapter 26 for a detailed discussion of cognitive dysfunction in SLE.)

Depression

Depression is characterized by sad or dysphoric mood, and/or anhedonia (loss of interest or pleasure in formerly enjoyable activities). "Neurovegetative symptoms" of depression include decreased or increased sleep, appetite, and weight, often with decreased libido, decreased energy, and psychomotor agitation or retardation. In addition to neurovegetative symptoms, depression is also characterized by poor concentration, indecisiveness, guilty rumination, feelings of helplessness, worthlessness, or hopelessness, preoccupation with death, and suicidal ideation. Typically, depressed patients appear sad or tearful and have a negative or pessimistic view of themselves, the world, and the future [89]. When depression is severe, it is sometimes accompanied by psychotic symptoms.

Depression seems to be second only to cognitive dysfunction as the most common neuropsychiatric disorder in SLE. The reported prevalence of depression has varied widely, depending on the diagnostic criteria utilized, the patient population (inpatient vs outpatient), and prospective vs retrospective analysis. In research using structured interviews, the prevalence of depression in SLE has been approximately 50% [90]. Since depression is very common in the general population, especially in women, a portion of the depression in lupus patients reflects background prevalence rates.

In SLE, the pathogenesis of depression is multifactorial. Depression may be a preexisting primary psychiatric disorder, an iatrogenically induced illness, particularly from corticosteroids, a reaction to having a chronic disease, and possibly, a direct CNS manifestation of lupus.

The question of whether depression is a direct manifestation of CNS SLE, or a reaction to the stress and multiple losses associated with a chronic debilitating illness, remains unresolved. Evidence that depression is a primary CNS manifestation of SLE includes the possible association of severe depression with antiribosomal P antibodies [18, 20], although others [23, 24, 91] have not corroborated this finding. Utset reported a strong correlation between depression and CNS lupus (defined as stroke, seizures, psychosis, aseptic meningitis, or organic brain syndrome), but not with systemic organ involvement. Depression in SLE patients was also strongly associated with secondary Sjögren's syndrome, a condition that can affect the CNS [92]. In contrast, many investigators have contended that depression in SLE is a reaction to chronic illness. Supporting evidence includes several studies [60, 93], but not all [90], showing a comparable incidence of depression in SLE and rheumatoid arthritis, a disease that is often treated with corticosteroids but does not appear to involve the CNS. Also, there is a lack of association between depression and neurologic symptoms [94] or systemic disease activity [95, 96]. Finally, many studies have found a significant correlation between psychosocial stressors and depression in SLE [72, 95–98].

The ACR classification system for neuropsychiatric SLE divides depression into two subtypes. The first, "major depressive-like episode," is virtually identical to the DSM IV entity, major depression. The second subtype, "mood disorder with depressive features," is a milder depression, most analogous to the DSM IV entity, "depressive disorder not otherwise specified." To diagnose depression as a neuropsychiatric manifestation of SLE, one must exclude depression due to (a) the stress of the illness (adjustment disorder), (b) substance use, or (c) a primary psychiatric disorder. Unfortunately, as previously mentioned, there are no clinical criteria or laboratory tests for excluding a primary depression. Also, in contrast to DSM IV, in the ACR classification system, a depression that is severe enough to meet criteria for major depression, but that occurs in reaction to stress, is not considered a major depressive-like episode, and is excluded as a neuropsychiatric manifestation of SLE.

Diagnosing depression in SLE is confounded by the overlap between depressive symptoms and those of SLE or its treatment. In particular, neurovegetative symptoms and poor concentration are difficult to interpret since they are often attributable to SLE itself and/or medication. Reliable indicators of severe depression in the medically ill include crying, indecisiveness, suicidal ideation, sense of failure, feelings of punishment, and loss of interest in people [99]. If the patient does not feel depressed and is still able to enjoy herself, the likelihood of depression is exceedingly low. Screening questionnaires such as the Beck Depression Inventory can be used to diagnose depression, quantitate its severity, and monitor response to treatment [89]. Such

questionnaires may overdiagnose depression somewhat since they do not distinguish between neurovegetative and cognitive symptoms due to SLE or its treatment, vs those due to depression. Depression must also be distinguished from grief, which is a normal response to the losses associated with chronic illness (see Psychological Reactions to Having SLE).

In SLE patients who present with depressive symptoms, certain medical conditions should be considered in the differential diagnosis. *Hypothyroidism* should be ruled out, since it can mimic depression, and is more common in SLE than in the general population [100]. *Fatigue* due to SLE can be incapacitating and may simulate, as well as precipitate depression. Interestingly, fatigue in SLE patients does not appear to be associated with changes in serum cytokine levels, anti-phospholipid antibodies, or other markers of disease activity, routine lab parameters, or medication treatment, although abnormal CNS cytokine levels could not be excluded [101]. Fatigue due to deconditioning appears to account for as much as half of the persisting fatigue reported by patients with low or suppressed SLE activity. In patients who have become sedentary and thus physically deconditioned after multiple lupus flares, exercise may be beneficial in alleviating both fatigue [102] and depression.

Mild reactive depression is generally treated with psychotherapy alone. In more severe depression, patients respond best to psychotherapy, particularly cognitive behavioral therapy, in combination with antidepressant medication (or occasionally ECT).

Anxiety

Anxiety is quite common in SLE patients, often as a reaction to chronic illness (see Maladaptive Reactions). It may be constant and free-floating (generalized anxiety), intensifying in response to life stressors. Anxiety may also occur suddenly in discrete episodes (panic attacks) with physical symptoms (such as tachycardia, dyspnea, dizziness, lightheadness, nausea, diaphoretic palms), and the urge to flee or a sense of impending doom. SLE patients may also present with social phobia (avoiding interacting with people due to self-consciousness, feelings of inadequacy, or fear of being judged or scrutinized), or specific phobias (including needle phobia, a common cause of noncompliance in medically ill patients). Although the ACR criteria for anxiety exclude anxiety reactions to the stress of having SLE, patients may nevertheless experience hospitalization, medical procedures, interventions, or other aspects of their illness as traumatic, and may have posttraumatic stress-like symptoms that impact on their compliance with treatment and follow-up. Such symptoms include reexperiencing the trauma (in flashbacks, nightmares, and intrusive memories), avoidance of feelings (detachment, emotional numbing) or situations reminiscent of the trauma, and hyperarousal (insomnia, hypervigilance, increased startle response). Anxiety may also manifest as obsessions (recurrent, intrusive, senseless thoughts that engender anxiety) and compulsions (repetitive, senseless acts to alleviate anxiety or prevent a certain event from occurring). In response to the unpredictability of SLE, some patients become superstitious, and develop idiosyncratic beliefs or engage in odd rituals to prevent relapse.

The question of whether anxiety is attributable to direct CNS involvement in SLE, or simply a reaction to chronic illness, remains controversial. Arguments for and against anxiety as a direct CNS manifestation of lupus are analogous to those detailed previously for depression.

The ACR criteria for anxiety exclude not only anxiety induced by the stress of having SLE, but also anxiety occurring during the course of delirium, psychosis, mood disorders, and substance use. Substance withdrawal, particularly alcohol and benzodiazepines, and excessive caffeine use should be considered in any patient complaining of anxiety. Corticosteroids, usually at doses greater than 20 mg/day of prednisone, may cause anxiety. Hyperthyroidism also can cause significant anxiety, usually of the generalized type.

Anxiety is treated with psychotherapy, often in conjunction with antidepressants (such as selective serotonin reuptake inhibitors (SSRIs)), and benzodiazepines. Patient education regarding SLE and its treatment can significantly ameliorate anxiety.

Mania

Mania is characterized by euphoria, irritability, or even dysphoria, with mood lability, grandiosity or inflated self-esteem, and speeding up of thoughts (racing thoughts, flight of ideas), speech (rapid, pressured), and activity level (increased goal-directed activity and decreased need for sleep). Hypomania, a milder form of mania, has the same symptoms but does not impair the patient's overall functioning. Manic patients feel they don't require much sleep, in contrast to patients with insomnia, who complain of lack of sleep. During manic episodes, patients often engage in spending sprees, promiscuity, and other risky or thrill-seeking behavior. When severe, mania may be accompanied by psychotic symptoms, such as paranoia and delusions of grandeur. Since manic episodes can closely resemble cocaine, amphetamine, or alcohol intoxication, substance abuse must be ruled out. Antidepressants can precipitate mania in patients with a bipolar diathesis or

undiagnosed bipolar disorder (manic-depression). Nevertheless, in patients with SLE, the most common cause of mania is corticosteroid therapy. Like depression and anxiety, mania is a clinical diagnosis. Treatment includes discontinuing offending drugs, such as antidepressants, and if possible, tapering corticosteroids. Mood stabilizers (e.g., lithium, divalproex, and carbamazepine), benzodiazepines, antipsychotic drugs, and occasionally ECT are used in treating acute mania. Mood stabilizers are also employed in the prophylaxis of recurrent manic and depressive episodes.

Psychosis

Psychosis is defined as an impairment in reality testing, manifested by delusions (fixed false beliefs) or hallucinations (auditory, visual, olfactory, gustatory, or tactile sensations in the absence of a stimulus). In psychosis due to primary CNS lupus, hallucinations are more commonly visual than auditory, in contrast to schizophrenia. Thought disorder (i.e., tangential or disorganized thinking), and disorganized behavior are included in the DSM description of some psychotic disorders, but not in the ACR criteria. Psychosis in SLE patients can be a manifestation of direct CNS involvement, and in some [17–22] but not all [23, 24] studies, has been linked to serum anti-ribosomal P antibodies. Interestingly, corticosteroids appear to be an uncommon cause of psychosis in SLE patients. Nevertheless, distinguishing psychosis due to CNS lupus from corticosteroid-induced psychosis presents a major diagnostic challenge (see later). Psychosis secondary to substance abuse, particularly cocaine, amphetamines, phencyclidine, and hallucinogens, or alcohol abuse or withdrawal, must also be considered. Psychosis is diagnosed clinically, and treated with antipsychotic drugs, such as haloperidol, risperidone, olanzapine, quetiapine, and ziprasidone.

Acute Confusional State (Delirium)

Acute confusional state, or delirium, was formerly termed organic brain syndrome. It is defined as an impairment of consciousness with impaired ability to focus, sustain, or shift attention. Typically, it has an abrupt onset, and is marked by fluctuations in level of consciousness throughout the day. The degree of disorientation also varies, and may be interspersed with periods of relative lucidity. Sleep–wake cycle is disrupted. Patients sometimes have mood lability and may experience visual illusions or hallucinations and paranoia. There are two subtypes of delirium, a hyperactive and a hypoactive form. *Hyperactive delirium* is characterized by agitation, hypervigilance, and hyperarousal. The patient may be thrashing around in bed and pulling out IV lines. Because of the marked agitation, hyperactive delirium is often misdiagnosed as psychosis or personality disorder ("acting out"). In contrast, *hypoactive delirium* is characterized by amotivation, apathy, somnolence, and quiet confusion. It frequently goes undetected, or is misdiagnosed as depression [103]. The ACR criteria for acute confusional state excludes that due to metabolic disturbances, cerebral infections, substance abuse or withdrawal, or primary neuropsychiatric disorders not related to SLE. Patients presenting with an acute confusional state require a diagnostic workup to rule out underlying medical etiologies, particularly infection, CNS disease (including seizures), metabolic disturbances, and medication-induced effects. Where possible, treatment is based on the underlying etiology. Antipsychotic drugs, sometimes in conjunction with low-dose benzodiazepines, are helpful in alleviating agitation and psychotic symptoms (see Table 9).

Personality Change

Although not included in the ACR classification of neuropsychiatric SLE, personality change is important to recognize since it may signify underlying brain involvement. Personality changes have been reported in SLE patients whose disease has damaged the frontal or temporal lobes. Presentations are typical of those due to any process, such as stroke or traumatic brain injury, affecting that region of the brain. Frontal lobe syndrome has two general patterns: dorsolateral and orbitofrontal [104]. The dorsolateral type is characterized by apathy, abulia (lack of will), and amotivation, and may mimic depression or hypoactive delirium. In contrast, the orbitofrontal type is manifested by impulsivity, disinhibition, promiscuity, mood lability, and impaired judgment, and may resemble mania. Some patients with complex partial seizures have interictal traits such as profound emotions, religious, ethical, and philosophical preoccupation, humorlessness, loquaciousness, hypergraphia, viscosity (social clinginess), hyposexuality, and aggression [105].

PREVALENCE

Estimates of the prevalence of neuropsychiatric disorders in SLE have been exceedingly variable, ranging from 17 to 91% [2–4, 72, 106–108]. This variation is due to multiple factors: (a) lack of standardized terminology, (b) changing terminology over time, (c) differences in diagnostic methods, (d) variations in which disease entities are considered direct manifestations of CNS SLE vs a reaction to a chronic unpredictable illness, (e) differences in study population (inpatient vs outpatient, active neuropsychiatric SLE vs history of neuropsychiatric SLE, (f) differences in specialty of investigator

(rheumatologist, neurologist, psychiatrist, or psychologist), (g) inclusion or exclusion of mild psychiatric symptoms, and (h) inclusion or exclusion of particular CNS neurologic disorders, and the arbitrariness of separating them from psychiatric disorders.

Using the American College of Rheumatology nomenclature, two studies, a cross-sectional Finnish population-based study [3] and a cohort study of predominantly Mexican-Americans [4], examined the prevalence of neuropsychiatric syndromes in outpatients with SLE. Overall, 80–91% of patients had at least one neuropsychiatric disorder, and the prevalence rates in the two studies were similar for individual neuropsychiatric syndromes. Cognitive dysfunction was the most common neuropsychiatric condition, occurring in 79–80%; however, less than a third of those patients had moderate to severe impairment. Major depression occurred in 28–39%, mania or mixed episodes in 3–4%, anxiety in 13–24%, and psychosis in 0–5%. Acute confusional state occurred in 7% of the Finnish patients with SLE, and was not reported in the Mexican-American cohort. Although comparable prevalence studies have not been reported for acutely ill inpatients with neuropsychiatric SLE, the incidence of stroke, seizures, psychosis, and particularly acute confusional state, is likely to be substantially higher.

CORTICOSTEROID-INDUCED PSYCHIATRIC SYMPTOMS

Corticosteroids have been shown to cause a variety of psychiatric syndromes, including mania, depression, mixed states, psychosis, anxiety, insomnia, and delirium. Evidence for an etiologic relationship has come from several studies. In a study by Cade, 55 patients with lupus nephritis were randomized to four treatment groups: prednisone alone, azathioprine alone, prednisone and azathioprine, or heparin and azathioprine [109]. The dosage of prednisone was 60–100 mg/day. The incidence of "psychosis" was 9/28 (32%) in the two groups treated with prednisone, and 0/27 (0%) in the two groups not receiving prednisone [109]. The Boston Collaborative Drug Surveillance Program study demonstrated that in various disorders treated with corticosteroids, the incidence of psychiatric symptoms was dose-related. Psychiatric symptoms occurred in 1.3% of patients receiving ≤40 mg/day of prednisone, 4.6% of patients receiving 41–80 mg/day, and 18.4% of patients receiving >80 mg/day. The mean prednisone dosage in patients who developed psychiatric reactions was 60 mg/day [110]. This dose-relationship has been confirmed in subsequent studies [111]. In a study of asthma patients receiving prednisone, elevations in mood were not correlated with alleviation of asthma symptoms [112], again supporting a causal relationship between corticosteroids and mood changes.

Females are at greater risk for corticosteroid-induced psychiatric symptoms [111, 113]. In addition, a history of mental illness may not increase the risk for corticosteroid-induced psychiatric symptoms [113].

In a majority of patients, the onset of psychiatric symptoms is within the first 2 weeks of corticosteroid treatment [113]. A review of the literature revealed that approximately 90% of corticosteroid-induced psychiatric symptoms occurred within the first 6 weeks of treatment [111]. The dosage of corticosteroids does not appear to affect the time of onset [114]. Nevertheless, rapid onset of psychiatric symptoms has been reported with intravenous pulse methylprednisolone [115].

The incidence of various types of corticosteroid-induced psychiatric reactions in medically ill patients has been reviewed by Lewis and Smith (Table 3) [111, table adapted to include their data].

Mild psychiatric side effects from corticosteroids include insomnia, hyperexcitability, mood lability, mild euphoria, irritability, anxiety, agitation, and racing thoughts. These symptoms may occur in isolation, or as a prodrome to more serious psychiatric reactions. Mood disorders are the most common type of psychiatric reaction to corticosteroids [111, 113]. In most studies, mania, hypomania (mild mania), and mixed states, occur slightly more frequently than depression [112, 113, 116]. Mood lability may be marked, and patients may experience both mania and depression during a single course of corticosteroid therapy [117].

Alternate-day corticosteroid therapy has precipitated mania on treatment days and depression on nontreatment days, simulating rapid-cycling bipolar disorder [118]. Mania and depression are often accompanied by psychotic symptoms such as hallucinations or delusions. In general, the psychiatric symptoms induced by corticosteroids closely resemble those of bipolar disorder [119]. Delirium and psychosis (without mood

TABLE 3 Corticosteroid-Induced Psychiatric Syndromes

	Psychotic features		
	Yes	**No**	**Total**
Depression	19	14	33 (35%)
Mania	21	8	29 (31%)
Mixed (manic/depressed)	6	0	6 (7%)
Psychosis	13	—	13 (14%)
Delirium	6	6	12 (13%)
Total (%)	65 (70%)	28 (30%)	93 (100%)

TABLE 4 Differentiating CNS Lupus Flare from Corticosteroid-Induced Psychiatric Reactions

	Active primary CNS lupus	**Corticosteroid-induced psychiatric reaction**
Onset	After ↓ corticosteroid dosage or ongoing low-dose Rx	Generally ≤2 wks after ↑ corticosteroid dosage (~ 90% within 6 weeks)
Corticosteroid dosage (mg/day of prednisone)	variable	Rare if <40 mg/day Common if ≥60 mg/day
Psychiatric symptoms	*Psychosis, delirium* Mood disorders Cognitive impairment (new onset)	*Mania, mixed states, or depression* (often with psychotic features) Delirium, psychosis
Systemic SLE symptoms	Often present *May coincide with onset of psychiatric symptoms*	Often present, but *precede onset of psychiatric symptoms* (prompting ↑ corticosteroid dose)
Labs	↑ Indices of inflammation (support dx if present but neither sensitive nor specific):	No specific lab findings
Quantitative brain MRI	White matter changes, microinfarcts, cortical atrophy	No specific findings (however, abnormalities may be present from previous episodes of primary CNS lupus)
Response to corticosteroid trial (or ↑ dose)	*Improvement*	*Exacerbation or persistence of symptoms*
Response to ↓ corticosteroid dose	Exacerbation	Improvement

symptoms) are less common, each occurring in approximately 13–14% of patients. Overall, 70% of patients with significant corticosteroid-induced psychiatric reactions have psychotic symptoms [111].

Corticosteroids cause cortical atrophy and loss of hippocampal neurons. Supporting evidence comes from brain magnetic resonance imaging [MRI] studies in patients with Cushing's disease, who have excess endogenous production of cortisol. In a more recent study, Zanardi compared the prevalence and severity of cerebral atrophy using head computed tomography (CT) in SLE patients, non-SLE patients receiving chronic steroids, and healthy controls [47]. Cerebral atrophy occurred at an equivalently higher frequency in SLE and non-SLE patients treated with corticosteroids, relative to controls. The degree of atrophy was more severe in SLE patients, and was not associated with steroid dose, or duration of disease. In SLE patients, the presence of cerebral atrophy was not associated with disease activity or the presence of neuropsychiatric manifestations [47]. In healthy controls, corticosteroids have been demonstrated to cause cognitive impairment, particularly errors of commission [120]. In medically ill patients, corticosteroids have been associated with reversible dementia [121]. Nevertheless, Denburg has reported that low-dose corticosteroids lead to cognitive improvement in some SLE patients [44], presumably because of subclinical CNS lupus.

Although corticosteroids can cause a variety of psychiatric symptoms, evidence summarized by Baker suggests that such symptoms in SLE patients are usually not attributable to corticosteroids [122]. First, severe psychiatric syndromes were reported historically in SLE prior to the introduction of corticosteroids, and occur in SLE patients who have not received corticosteroids. Second, psychiatric symptoms in SLE are more frequent and severe than in other disorders treated with comparable doses of corticosteroids. Third, psychiatric symptoms in SLE are often ameliorated, not worsened, by an increase in corticosteroid dosage or maintenance of high-dose corticosteroids. In contrast, a reduction in steroid dosage often does not alleviate psychiatric symptoms, and may exacerbate them. Finally, in SLE patients who have had a previous psychotic episode while on corticosteroids, retreatment with steroids usually does not precipitate a recurrence of the psychosis [122, 123].

Distinguishing corticosteroid-induced psychiatric reactions from a flare of CNS lupus is one of the most challenging aspects of treating SLE. This clinical dilemma is also difficult to study due to the lack of a gold standard in diagnosing neuropsychiatric SLE. Helpful distinguishing features are summarized in Table 4 [124]. Given the risk of untreated CNS lupus, and the likelihood that corticosteroids will alleviate such flares and only prolong or temporarily exacerbate

TABLE 5 Psychiatric Side Effects of Medications Used in Treating SLE

Medication	Psychiatric side effects
Corticosteroids	**Mood lability**, **euphoria, irritability**, **anxiety, insomnia**, **mania, depression, psychosis, delirium**,[a] cognitive disturbance
Immunosuppressants/immunomodulators	
Azathioprine	Delirium
Mycophenolate mofetil	(Rare) anxiety, depression, sedation
Cyclophosphamide	(Rare) delirium (at high doses)
Methotrexate	(Rare) delirium (at high doses)
Cyclosporine	Anxiety, delirium, visual hallucinations
Tacrolimus	Anxiety, delirium, insomnia, restlessness
Immunoglobulin (intravenous)	Delirium, agitation
LJP-394 (B-cell toleragen–anti-'anti-ds-DNA')	(None reported)
NSAIDS	Depression, anxiety, paranoia, hallucinations, hostility, confusion, delirium, ↓ concentration
Hydroxychloroquine	**Confusion, psychosis, mania, depression, nightmares, anxiety, aggression, delirium**

[a] Common reactions are in boldface type.

corticosteroid-induced psychiatric reactions, an empiric trial of corticosteroids is often the most prudent intervention. Denburg has endorsed a 2-week empiric corticosteroid trial [44]. In a slight variation, McClune has suggested that pending diagnostic test results, the dose of corticosteroids be doubled for 3 days [125]. If the psychiatric symptoms improve, then a CNS lupus flare is the probable diagnosis, warranting ongoing treatment with the higher dose. However, if the patient has an exacerbation of psychiatric symptoms, a diagnosis of CNS lupus becomes somewhat less tenable than a corticosteroid-induced psychiatric reaction. Accordingly, corticosteroids should be tapered to half of the original dosage [125].

The preferred treatment for corticosteroid-induced psychiatric reactions is tapering of corticosteroids, if possible. Such treatment alone results in a greater than 90% response rate. Corticosteroid-induced delirium often resolves within 5 to 10 days, whereas mood and psychotic disorders usually remit within 3 to 4 weeks [111]. Adjunctive treatment with antipsychotic drugs can help alleviate delirium, mania, and psychotic symptoms (with or without mood disorder). In addition, ECT appears to be effective in depressed patients who do not respond to corticosteroid taper or discontinuation [126]. Although the data are anecdotal, selective serotonin reuptake inhibitors such as citalopram or sertraline may also be beneficial for depression. Lithium was prophylactic against corticotropin-induced mania or depression with psychotic features [127]. Consequently, lithium, and perhaps other mood stabilizers, may be helpful in patients who have had previous corticosteroid-induced psychiatric reactions, and who require courses of high-dose steroids.

Corticosteroid Withdrawal Syndrome

Rapid tapering or discontinuation of corticosteroids can also induce psychiatric reactions by precipitating SLE flares, iatrogenic adrenal insufficiency, or possibly corticosteroid withdrawal syndrome. Corticosteroid withdrawal syndrome is manifested by flulike symptoms, such as headache, fever, myalgias, and arthralgias, and symptoms overlapping with those of adrenal insufficiency: weakness, anorexia, nausea, weight loss, orthostatic hypotension, and sometimes, psychiatric symptoms such as depression, anxiety, agitation, or psychosis [113, 128, 129]. Although it may be difficult to distinguish corticosteroid withdrawal syndrome from iatrogenic adrenal insufficiency or flares of CNS lupus, it is often unnecessary clinically, since all respond to an increase in corticosteroid dosage.

PEDIATRIC AND ADOLESCENT SYSTEMIC LUPUS ERYTHEMATOSUS: PSYCHIATRIC ASPECTS

Primary neuropsychiatric lupus occurs quite commonly in children and adolescents with SLE, usually within the first year of diagnosis [135]. Reported prevalence rates have been variable, ranging from 29 to 95% [135–139], most likely due to differences in diagnostic criteria and patient population. In a prospective study, when only serious neuropsychiatric syndromes were included (i.e., stroke, seizures, chorea, psychosis, delirium, major depression, and significant cognitive impairment), the prevalence of CNS lupus was 76%. Overall, using the 1999 American College of Rheuma-

TABLE 6 Psychiatric Side Effects of Medications Used in Treating Complications of SLE

Medication	Psychiatric side effects
Anticonvulsants	**Sedation, confusion, cognitive disturbance**, agitation, delirium, psychosis, depression, mania, nightmares
Antihypertensive agents, other cardiovascular drugs	
β-blockers	Depression, insomnia, nightmares, ↓ **libido**, (rare) delirium, hallucinations, psychosis
Ca^{2+} channel blockers	(Rare) depression
ACE inhibitors (e.g., captopril)	(Rare) mania, depression, delirium, anxiety, hallucinations
Digoxin	Delirium, visual hallucinations, psychosis, mania, depression, ↓ libido
Thiazides	Depression
HMG CoA reductase inhibitors	(Rare) anxiety, depression, obsessions, delusions
Antibiotics, other antimicrobials	
Fluoroquinolones (e.g., ciprofloxacin)	**Delirium, visual hallucinations, paranoia, agitation**, **anxiety cognitive impairment**, mania, depression, Tourette's-like syndrome
Cephalosporins	Euphoria, delusions, illusions, depersonalization
Trimethoprim-sulfamethoxazole, other sulfonamides	Confusion, delirium, psychosis, hallucinations, depression, euphoria
Clarithromycin	Delirium
Amphotericin B	**Depression**, delirium, psychosis
Metronidazole	Depression, crying spells, agitation, delirium, hallucinations
Opioids	**Delirium** (especially with meperidine), **visual hallucinations,** nightmares, anxiety, **euphoria, depression, sedation**, paranoia, agitation

[a] From [88] Sibbitt, 1999, p. 202; [130] Medical Letter, 1998; [131–134] Stoudemire, 2000, pp. 725–726, 867, 897–898, and 1058.
[b] Common reactions are in boldface type.

tology case definitions, 95% of patients developed neuropsychiatric SLE during a 6-year follow-up period. Mood disorders occurred in 57%, cognitive impairment in 55%, delirium in 35%, anxiety in 21%, and psychosis in 12% [139].

Interestingly, in a study of 140 children with chronic malaise, headaches, abdominal discomfort, and low-grade fever, who were felt to be avoiding school, 52% had a positive ANA, in contrast to 6% of controls. Nearly half of those with positive ANAs had titers greater than 1:160, suggesting underlying autoimmune fatigue [140].

Systemic lupus erythematosus can have particularly devastating ramifications during *adolescence*. Given the preoccupation with appearance and body image, coupled with the intense need for acceptance by peers, adolescents tend to be extremely self-conscious about overt manifestations of lupus and its treatment, such as a malar rash, discoid lesions, alopecia, and corticosteroid-induced moon facies and weight gain. This self-consciousness may culminate in depression and social isolation, or self-adjusting of corticosteroids [56, 141, 142]. Adolescents may also become despondent and anxious about the chronic, unpredictable nature of SLE. Since adolescents often have a sense of invincibility and immortality, they are predisposed to denial of their illness. Denial may be especially prominent in patients with hypertension or renal disease who are relatively asymptomatic. Denial may be exemplified by nonadherence with treatment via discontinuing medication, failing to show for appointments, or dropping out of treatment entirely [143]. *Denial of illness*, coupled with *socioeconomic disadvantage* and *limited education*, is a particularly poor prognostic triad. In response to having a serious illness, adolescents may exhibit irritability or anger, which is directed primarily against their parents, and to a lesser extent, the medical staff [142, 143]. The adolescent's struggle for autonomy and independence may manifest as defiance against treatment recommendations.

Systemic lupus erythematosus in childhood and adolescence also has repercussions for the patient's *parents and siblings*, who may become depressed, anxious, and overprotective of the child, or conversely, may engage in denial and unduly pressure her to be more active. With chronically ill adolescents, parents may have difficulty balancing respect for their child's struggle for autonomy vs the need for ongoing care. Parents' anger at the situation is typically directed at the medical staff, and often involves legitimate frustrations regarding poor communication among multiple caregivers, and the bureaucracy of the health care system. Siblings

may secretly resent the child for being ill, and for requiring disproportionate attention, and may act out or develop disruptive behavior. Consciously or unconsciously, siblings may also harbor guilt that they are healthy [143].

Diagnostic workup in children and adolescents with suspected CNS lupus is similar to that for adults. In pediatric patients, single photon emission computed tomography (SPECT) scan of the brain appears useful, and possibly more sensitive than brain MRI in diagnosing CNS lupus [135, 144]. Serum anti-neuronal antibodies correlate with the course of CNS illness; they are often positive at the onset of symptoms, and normalize as the symptoms resolve [144]. Serial cognitive screening is important in children with SLE, given not only the prevalence of cognitive impairment, but also the difficulty in detecting subtle cognitive deficits or failure to acquire new cognitive skills (i.e., in children, unchanged cognitive ability is abnormal) [138].

Several *interventions* are helpful in working with adolescents with SLE. As with all patients, education is crucial so that the adolescent understands the nature of the illness and rationale for treatment. The patient should be engaged as an active partner in her treatment, especially in decision-making [145]. Identifying the causes of treatment noncompliance, and addressing them in a nonjudgmental manner, is crucial, and is discussed in detail later in this chapter. Clear explanations should be given regarding restrictions on exposure to the sun. In general, patients should be encouraged to be as active as tolerated, and to have as normal a lifestyle as possible. The physician should ask about the patient's sexual activity, and discuss prophylaxis against pregnancy and sexually transmitted diseases, and the impact of SLE on childbearing capacity. Obstacles to care such as illiteracy, additional childcare responsibilities, parents' inability to get time off from work, and lack of transportation, insurance, or prescription coverage, are seemingly mundane nonmedical matters that are often overlooked. Nevertheless, assisting parents with these practical issues can help strengthen the therapeutic bond, and ensure ongoing patient follow-up [143].

Pharmacologic treatment for serious CNS manifestations is similar to that for adults, and includes corticosteroids, pulse cyclophosphamide, and plasmapheresis [136]. Although psychotropic medication use in children with SLE has not been formally studied, the overall approach is similar to that for adults, but with downward dosage adjustment. Except in stroke and rare cases of fulminant cerebral edema, the prognosis for pediatric CNS lupus is generally favorable [135, 136, 142]. (See Chapter 18 for a more detailed discussion of pediatric aspects of SLE.)

RELATIONSHIPS, SEXUAL INTIMACY, AND PREGNANCY IN SYSTEMIC LUPUS ERYTHEMATOSUS: PSYCHIATRIC ASPECTS

Systemic lupus erythematosus can have profound effects on a patient's marital and family relationships, sexuality, and childbearing capacity.

Marriage and Family Relationships

Spouses, other relatives, and friends often have difficulty coping with the patient's illness. Some may be overprotective and unduly limit the patient's independence and autonomy. Conversely, others may minimize the patient's illness and exert too much pressure on her to be active, particularly when she has no overt manifestations of SLE. Frequently, relatives and friends will vacillate between these responses. In response to the patient's illness, children may adopt a caretaker or "parentified" role, or may seek attention by "acting out," or complaining of physical ailments. Depression and anxiety are common in family members of the chronically ill, especially the primary caretakers. Like the patient, family members must grapple with the vicissitudes and unpredictability of the illness, and may need to adjust plans and life goals. Like physicians, they may also have the burden of bearing witness to suffering they are helpless to prevent.

Meeting with the patient and spouse (or partner) together is a beneficial step in educating them about SLE. Couples should be informed that the course and prognosis of SLE are variable and unpredictable. In such a discussion, it is important to be candid yet not pessimistic. The physician should emphasize the need for couples to be flexible in making lifestyle adjustments to cope not only with fatigue and SLE flares, but also remissions. The spouse (or partner) and other family members should also be informed that some symptoms, especially fatigue, may occur without overt signs of lupus.

Sexuality

Decreased libido is common, due to fatigue and debilitation, depression, and medication side effects (especially antihypertensives). Alopecia, malar rash, weight gain, or other changes in body image may also lead to perceived unattractiveness, and adversely affect sexual intimacy.

Pregnancy

Pregnancy tends to be an anxiety-provoking issue for couples, who must decide whether childbearing

warrants the maternal and fetal risks associated with SLE. Women may be at increased risk of SLE flares (usually mild) during pregnancy, especially the second and third trimester, and the postpartum period [146, 147], although this issue is controversial [148]. Some patients with antiphospholipid syndrome have been advised to forego reproduction due to the risk of thrombosis, preeclampsia, and fetal demise. This loss of childbearing capacity compounds the other losses and debilitation due to SLE, and may contribute to depression. Marital and family tension may escalate when a patient or her partner want her to risk jeopardizing her health by becoming pregnant, despite admonitions about possible worsening renal or heart failure, hypertension, or stroke.

Throughout pregnancy, patients may be apprehensive about possible miscarriage or premature delivery, or teratogenic effects from medications. Patients may also be concerned about "transmitting" lupus to their babies. Such concerns can be allayed by close prenatal monitoring, counseling, and patient education. If there is an adverse outcome, the patient may harbor guilt and self-blame. SLE patients, especially those with active nephritis or antiphospholipid syndrome, are more likely to have fetal loss, and may experience grief or become clinically depressed over recurrent miscarriages and stillbirths. Such patients may derive solace from bereavement counseling. (For a more detailed discussion of SLE and pregnancy, see Chapter 22.)

DIAGNOSIS OF PSYCHIATRIC SYNDROMES

Although many clinical signs and symptoms, and lab tests are suggestive of neuropsychiatric SLE, none is diagnostic. Diagnosis is primarily based on a constellation of clinical findings, lab tests, and neuroimaging that are corroboratory, and that exclude comorbid conditions and secondary causes of neuropsychiatric symptoms [88].

Mental Status Exam and Neuropsychiatric Testing

The *mental status exam* is essential in diagnosing psychiatric disturbances in SLE, particularly psychosis, delirium, and cognitive impairment. Not only is it inexpensive and portable, it can also be used serially to detect changes in mental state. The most important elements are assessment of orientation, attention, memory, and visuospatial perception, all of which are encompassed in the Mini-Mental Status Exam [149].

Neuropsychiatric testing provides an objective, more detailed, and more sensitive assessment of cognitive function. It is useful in establishing baseline cognitive status, and then can be repeated to assess any cognitive deterioration, or response to treatment. It also documents specific deficits that may enable patients to qualify for disability [88]. Since the testing is often not adjusted for cultural norms, it tends to overestimate cognitive deficits in minorities such as African-Americans [150]. Unfortunately, neuropsychiatric testing is time-consuming and expensive, and may not be feasible when patients are acutely ill. If no prior testing is available for comparison, it cannot always distinguish new from preexisting cognitive deficits. Even in the absence of active SLE (CNS or systemic flare), or previous documented CNS lupus, neuropsychiatric testing often reveals subtle cognitive abnormalities; consequently, such findings are not necessarily diagnostic of active neuropsychiatric lupus [83]. (See Chapter 28 for a more extensive discussion of cognitive assessment in SLE.)

Laboratory Workup

No laboratory test, or set of tests, is considered diagnostic of neuropsychiatric SLE. Nevertheless, in evaluating a patient for suspected CNS lupus, laboratory studies serve two purposes. First and most importantly, lab tests rule out infection, as well as other complications of SLE or its treatment that can present with neuropsychiatric symptoms. Second, positive laboratory studies confirm the presence of systemic lupus disease activity. While neuropsychiatric lupus often pursues a clinical course somewhat independent of flares in other organs, high disease activity increases the likelihood of primary CNS involvement, since the pathogenesis is shared between the CNS and other target organs. For example, CNS vasculitis might be more likely when the patient is experiencing cutaneous vasculitis than when the dermatologic exam and laboratory values are normal. However, *absence of systemic disease activity does not preclude CNS lupus. Moreover, systemic disease activity seems to have little, if any correlation with certain neuropsychiatric symptoms, particularly fatigue and cognitive dysfunction* [83, 151].

Laboratory tests can also be useful in evaluating psychiatric symptoms in lupus patients with a history of a primary psychiatric illness such as schizophrenia or major depression. Primary psychiatric disorders do not cause elevations in erythrocyte sedimentation rate (ESR) or other markers of inflammation. Accordingly, if the psychiatric symptoms temporally coincide with a markedly increased ESR, medical etiologies, particularly infection and CNS lupus flare, must be strongly considered.

Serum Labs

In evaluating lupus patients with neuropsychiatric symptoms, routine serum labs should include electrolytes, BUN, creatinine, transaminases, liver function tests, complete blood count, thyroid stimulating hormone, and coagulation tests (prothrombin time, international normalized ratio (INR), and partial thromboplastin time). Serologic tests to assess systemic disease activity include ESR, C-reactive protein, complement levels (C3, C4, CH50), and anti-DNA antibodies, all of which are elevated in systemic lupus flares [26]. Serum ANA titers need not be obtained, since they do not seem to correlate with systemic or CNS lupus activity.

Testing serum for *anti-phospholipid antibodies* (including lupus anti-coagulant and anti-cardiolipin) is crucial, particularly in patients with focal symptoms, since the results may determine treatment and prognosis. Failure to diagnose antiphospholipid syndrome in SLE can lead to stroke and other thrombotic complications, seizures, and progressive cognitive impairment [12, 28, 33, 45]. Unlike anti-phospholipid antibody-negative patients, those with antiphospholipid syndrome are treated primarily with anticoagulation rather than corticosteroid or cytotoxic therapy.

Serum anti-ribosomal-P antibodies have been linked to psychosis [17–22] and depression [18, 20]. These antibodies are positive in half of lupus patients who have psychiatric symptoms, but who test negative for antiphospholipid antibodies [152]. Titers parallel the course of illness. Despite this correlation, their usefulness as a diagnostic test in neuropsychiatric lupus is limited by their low positive predictive value: 13–16% for psychosis and depression [20], and for other reasons discussed later. Other serum autoantibodies, including anti-neuronal, anti-neurofilament, and anti-ganglioside antibodies, have not proven to be diagnostic markers for CNS lupus [13, 15, 26, 88]. Other proposed markers, such as serum S100B, an astrocytic calcium binding protein [153], have not been adequately studied.

CSF Analysis

When patients with SLE have neuropsychiatric symptoms, lumbar puncture is indicated (1) to rule out CNS infection, and (2) to assess the degree of lupus activity in the CNS. Routine CSF analysis, including cell count, differential, total protein, glucose, cultures, and cryptococcal antigen, should be obtained. In the absence of CNS infection, CSF pleiocytosis and increased CSF protein are suggestive of CNS lupus [2].

In addition, CSF studies should include oligoclonal bands and CSF IgG, both used in the diagnostic workup of multiple sclerosis. Both positive oligoclonal bands and elevated immunoglobulin index indicate increased local production of immunoglobulin within the CNS (by B-cells marginated within the meninges), even when white cells are not detected in the CSF. Additionally, positive oligoclonal banding in CSF has a narrow differential diagnosis, including neurosyphilis, Lyme disease, multiple sclerosis, Sjögren's syndrome, and CNS SLE. An elevated CSF albumin/serum albumin ratio suggests increased permeability of the blood–brain barrier, a finding suggestive, but not diagnostic of CNS lupus. Increased CSF anti-neuronal antibodies [22, 86], and cytokines interleukin-6 [27, 38] and interferon α [25] have been associated with neuropsychiatric SLE, but are not used diagnostically, for reasons discussed later.

Electroencephalogram (EEG)

In lupus patients with neuropsychiatric symptoms, the EEG is often abnormal, but seldom useful [154]. Most commonly, it reveals diffuse slowing, a nonspecific finding. EEG cannot distinguish primary CNS lupus from other conditions affecting the CNS, nor active from preexisting CNS lupus. Nevertheless, the EEG is helpful in two situations: (1) diagnosing subclinical seizure activity, and (2) differentiating hypoactive delirium (diffuse slowing on EEG) from depression (normal EEG).

Neuroimaging

Neuroimaging is an integral part of the diagnostic evaluation of patients with possible CNS lupus. Neuroimaging modalities can be subdivided into (a) structural studies, including computerized tomography (CT) and magnetic resonance imaging (MRI) of the brain, and (b) functional studies, such as magnetic resonance spectroscopy (MRS), functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and single photon emission computerized tomography (SPECT).

Cranial CT is frequently normal in neuropsychiatric lupus, but commonly reveals cerebral atrophy. Given its lower resolution, and lower sensitivity for detecting white matter lesions, it has generally been supplanted by brain MRI in the evaluation of neuropsychiatric lupus. Nevertheless, CT is useful for diagnosing intracranial hemorrhage and large strokes, and for ruling out brain abscesses or masses [154]. Otherwise, it is indicated only when MRI is not feasible.

Brain MRI is the preferred imaging technique for evaluating neuropsychiatric disease in patients with

SLE [155]. MRI is more sensitive than CT in detecting small focal lesions and white matter changes, edema, inflammation, and acute infarcts, and in quantifying the degree of atrophy. In CNS lupus, the most common finding on MRI scan is small discrete white matter lesions, especially in the subcortical areas, signifying microinfarcts. The brightness of these lesions on T2-weighted images reflects increased water signal, and by implication, increased permeability of the blood–brain barrier. Although such white matter lesions are found in over a third of SLE patients in general, they occur in over half of those with major psychiatric disturbances or neurologic deficits [156]. Other abnormalities on MRI include cerebral cortical atrophy, periventricular or diffuse white matter changes, and macroinfarcts. MRI abnormalities are found in 15–78% of SLE patients with active or previous neuropsychiatric SLE [155]. Among patients with psychiatric symptoms but no focal neurologic findings, less than 50% have MRI abnormalities [2]. Macroinfarcts are found in only one in 20 SLE patients, predominately those with antiphospholipid syndrome [157].

Although MRI is the best available imaging technique for neuropsychiatric symptoms, it is inherently limited since it detects only structural lesions. Accordingly, it cannot be the gold standard for diagnosing a neuropsychiatric SLE flare, whose pathophysiology involves primarily functional impairment. Over time, neurologic damage tends to be cumulative; however, each exacerbation may be associated with only subtle, if any, structural changes (e.g., slight edema, or atrophy, or mild change in volume of white matter lesions) that MRI is not sensitive enough to detect. A second major limitation of MRI is that it cannot reliably differentiate active from chronic lesions resulting from previous neuropsychiatric lupus episodes [158–160]. On MRI, factors suggesting active lesions include brightness on T2-weighted images, enhancement with gadolinium (both signifying increased permeability), and filamentous lesions of the gray–white matter junction [155, 158, 161]. Given the high prevalence of asymptomatic MRI lesions in patients with SLE [160, 162], distinguishing active from residual disease is critical to ascertain which patients might require corticosteroids.

The difficulty in distinguishing active lupus from previous damage is not unique to neuropsychiatric SLE. On the contrary, it is a central problem in evaluating organ system involvement in lupus. For example, interpretation of renal biopsies in SLE involves ascertaining how much of the renal pathology reflects permanent damage (scar) vs potentially reversible disease activity. (See Chapter 30.)

Refinements of MRI have generated imaging modalities that appear promising in distinguishing active from residual CNS lupus, or quantifying disease severity.

1. *Quantitiative magnetic resonance relaxometry* analyzes gray matter T2 relaxation time, which is increased in cerebral edema, a sign of active neuropsychiatric lupus. Since this technique differentiated patients with major neuropsychiatric lupus syndromes (including delirium and psychosis), from those with headache or other mild neuropsychiatric symptoms, it may prove usful in deciding which patients warrant corticosteroid treatment [163].
2. *Magnetization transfer imaging (MTI)* is more sensitive than conventional MRI in detecting and quantifying CNS injury. In patients with a history of neuropsychiatric lupus who were asymptomatic, MTI revealed residual, apparently permanent CNS damage [164]. In addition, volumetric analysis with MTI, which measures brain parenchymal homogeneity, can differentiate patients with active CNS lupus from those with residual disease or multiple sclerosis [165]. Overall brain damage, as measured by volumetric MTI, correlates directly with clinical indices of cognitive and psychiatric functioning [166].
3. *Magnetic resonance spectroscopy (MRS)* is a functional imaging technique that measures levels of brain metabolites. Decreases in *N*-acetylaspartate (NAA) seem to indicate irreversible neuronal injury [167, 168], and correlate with the severity of cognitive impairment. Nevertheless, decreased NAA occurs in both active and residual CNS lupus. Increases in choline metabolites, measured in the Cho peak, correlate with active CNS lupus, brain inflammation, and white matter disease [167], and are reversible, preceding the irreversible increase in NAA [168].
4. *Functional MRI*, which measures hemoglobin oxygen saturation levels in the brain, may prove useful in evaluating SLE patients with neuropsychiatric symptoms, but has not been adequately studied.

Use of these more sophisticated imaging modalities is limited by cost, the need for highly trained personnel for interpretation, and the lack of normative data for SLE patients without overt neuropsychiatric manifestations.

SPECT measures regional cerebral blood flow, and indirectly, metabolism. In active CNS lupus, SPECT most commonly reveals multiple small areas of hypoperfusion, but sometimes shows more focal hypoperfusion, most commonly in the parietal lobe [169, 170]. In SLE patients, the degree of hypoperfusion on SPECT did not correlate with the severity of cognitive impairment [171]. Although SPECT is extremely sensitive in

detecting perfusion abnormalities associated with neuropsychiatric SLE, it lacks specificity. In particular, it cannot distinguish active from residual CNS lupus, or infarct from edema, inflammation, or other reversible processes. SPECT must be compared to a structural imaging modality such as MRI to be interpreted, but lesions on SPECT do not correlate well with those on MRI.

PET, which measures regional oxygen and glucose uptake, has greater resolution than SPECT, but has similar limitations. It remains primarily a research tool, and is quite expensive and not widely available.

Magnetic resonance angiography is useful in evaluating patients with multiple strokes, especially due to medium to large vessel thromboembolic disease, but is not sensitive in visualizing small vessel ischemia [155]. (See Chapters 19, 26, and excellent reviews by Sibbitt [155] and Huizinga [172] for an in-depth discussion of neuroimaging in SLE.)

Overall, in clinical practice, the most crucial role of neuroimaging in evaluating patients presenting with neuropsychiatric symptoms is to rule out subdural hematoma, hemorrhagic stroke (possibly from active CNS vasculitis), or other acute emergency requiring immediate medical or surgical intervention.

Limitations to the Use of Laboratory Tests in Diagnosing Neuropsychiatric Lupus

Despite more sensitive imaging modalities, refinements in neuropsychological testing, and novel CSF autoantibody testing, lab tests play a lesser role in diagnosing neuropsychiatric SLE than in other organ system lupus flares for several reasons—both practical and theoretical. From a practical standpoint, the results of some lab tests, including autoantibody assays, are often not received until after critical treatment decisions have already been made. Consequently, although certain autoantibodies are strongly associated with neuropsychiatric lupus, and thus, may be valid diagnostically, these tests are of little use clinically. While this limitation is potentially remediable, several other factors are inherent to CNS lupus and thus, unlikely to change. First, no single lab test can define psychiatric lupus. CNS lupus encompasses a wide spectrum of syndromes, and seems to have a complex and multifactorial pathogenesis. Paradoxically, as research provides greater insight into the ways in which autoantibodies [47] and cytokines may cause brain disease, the likelihood of a single pathognomonic test decreases. Second, neuropsychiatric manifestations—perhaps because they are caused less by inflammatory processes than by autoantibodies and cytokines acting directly within the CNS—tend to follow a course independent of generalized flares affecting other organs, and independent of laboratory tests reflecting systemic involvement. Finally, and most importantly, the *risk of untreated CNS lupus sets a very high requirement for accuracy of laboratory tests. The negative predictive value of a diagnostic test for active neuropsychiatric SLE would have to be very high—close to that of the ANA itself for lupus—to obviate the need for an empiric corticosteroid trial in a patient with new, or even subacute but serious, psychiatric disease. A 2- to 3-week corticosteroid trial (at doses equivalent to 30–60 mg/day of oral prednisone) has relatively modest side effects relative to the sequelae of untreated neuropsychiatric lupus.* Consequently, risk/benefit analysis heavily favors a treat first (or test-and-treat in parallel) strategy over a test-and-wait strategy.

Summary of Diagnostic Approach

In summary, for lupus patients presenting with neuropsychiatric symptoms, the diagnostic approach focuses on ruling out conditions that may resemble CNS lupus but that require urgent specific treatment. Such conditions include (a) CNS or systemic infection, (b) cerebral infarct or hemorrhage, especially if pt has focal neurologic deficits, (c) antiphospholipid syndrome, emboli from cardiac valvular vegetations, and (d) uremia, hypertensive encephalopathy, or other medical causes of psychiatric symptoms in SLE. Accordingly, routine diagnostic workup includes: complete blood count and serum chemistries, serologic measures of systemic disease activity, coagulation tests and antiphospholipid antibody tests, and CSF tests (primarily to rule out CNS infection). In addition, CSF testing for increased immunoglobulin (usually ordered as part of a multiple sclerosis test battery) is often helpful. Special effort may be required to ensure that the anti-phospholipid workup and CSF immunoglobulin tests have been obtained. Brain MRI is helpful in most patients, except those whose neuropsychiatric symptoms have a clearly identifiable etiology. If a brain MRI cannot be obtained promptly, a cranial CT should be done in the interim to rule out intracranial bleeding.

The accuracy of this diagnostic approach may be enhanced by testing for serum anti-ribosomal P and CSF anti-neuronal antibodies [26], particularly since these tests are strongly correlated with psychiatric manifestations of SLE. However, such tests will not be incorporated into clinical practice until the lab results can be obtained expeditiously.

DIFFERENTIAL DIAGNOSIS

Psychiatric Syndromes in Other Collagen–Vascular, Autoimmune, or Multisystem Diseases

A wide variety of diseases can mimic neuropsychiatric SLE, and must be considered in its differential diagnosis. Such diseases include rheumatological disorders (e.g., Sjögren's syndrome, Behçet's disease, mixed connective tissue disease, undifferentiated connective tissue disease, temporal arteritis, polyarteritis nodosa, rheumatoid arthritis, fibromyalgia, and chronic fatigue syndrome), neurologic diseases (e.g., myasthenia gravis, multiple sclerosis), and other multisystem diseases (e.g., sarcoidosis, celiac disease, hepatitis C). Using serologic parameters, these diseases can be subdivided into three categories.

The first group of diseases is associated with a *medium to high ANA titer* (>1 : 160), and positive anti-double-stranded DNA antibodies, and includes only Sjögren's syndrome, mixed connective tissue disease, and undifferentiated connective tissue disease. (These diseases, like hepatitis C and cryoglobulinic vasculitis, are also characterized by a low serum complement.) Although these conditions may be difficult to distinguish from CNS SLE, establishing a specific diagnosis is less crucial clinically, since the treatment is the same. SSA or anti-Ro antibody identifies 80% of patients with Sjögren's syndrome. Anti-RNP, by definition, is present in 100% of patients with mixed connective tissue disease; however, RNP negative, clinically indistinguishable forms of SLE, scleroderma, and myositis are classified as undifferentiated connective tissue disease.

The second group of diseases is associated with a *low ANA titer* (≤1 : 160), and includes multiple sclerosis, and less commonly, ANA-positive rheumatoid arthritis, sarcoidosis, and hepatitis C. Multiple sclerosis is the most important disease to exclude, since 22–30% of patients have a low positive ANA titer [173, 174], and the disease is commonly associated with white matter abnormalities on brain MRI. In patients with neuropsychiatric symptoms but no systemic signs of SLE such as renal, joint, or skin involvement, the likelihood of multiple sclerosis increases. Neurosarcoid is associated with an elevated CSF angiotensin converting enzyme in 50% of patients, and sometimes, a high opening pressure on lumbar puncture, due to granulomatous obstruction to CSF flow [175].

A third group of diseases has neuropsychiatric manifestations mimicking CNS lupus, but typically has a *negative ANA* (i.e., or has the same prevalence of a false-positive ANA as in the general population). Accordingly, a strongly positive ANA and negative anti-neutrophil cytoplasmic antibodies (ANCA) virtually excludes polyarteritis nodosa, microscopic angiitis, and Wegener's granulomatosus. Likewise, chronic fatigue syndrome, fibromyalgia, and temporal arteritis should not usually be diagnosed in the presence of a positive ANA. In Behçet's disease, neurologic features are often confined to the brain stem, in contrast to the global dysfunction so characteristic of neuropsychiatric SLE. Although aseptic meningitis in Behçet's disease may present with decreased alertness, the ANA is negative. Perhaps the most problematic ANA-negative diseases to distinguish from CNS lupus are those presenting with focal neurologic signs as well as delirium, but no systemic manifestations of SLE. In such patients, possible diagnoses include CNS vasculitis (progressive or benign angiitis limited to the CNS—i.e., PACNS or BACNS, respectively), multiple sclerosis, and CNS infection (including cryptococcal, tubercular, meningococcal, and Listeria meningitis, herpes encephalitis, neurosyphilis, CNS nocardiosis, toxoplasmosis, brain abscesses, and progressive multifocal leukoencephalopathy [2, 55]. Tests crucial in excluding infection include CSF cell counts, routine bacterial, mycobacterial, and fungal stains, cryptococcal antigen, and HSV PCR, and serum HIV. A positive cerebral angiogram is required to diagnose PACNS or BACNS, but does not exclude SLE, and may not be necessary if the patient has either a positive ANA or evidence of CNS infection [176].

In summary, an algorithm with four steps can identify disorders that can mimic neuropsychiatric SLE, and that must be considered in its differential diagnosis: (1) exclude infection (2) ANA titer >1 : 160 and non-CNS features, consider Sjögren's syndrome, mixed connective tissue disease, and undifferentiated connective tissue disease. (3) ANA titer ≤1 : 160, consider Sjögren's syndrome, sarcoidosis, hepatitis C, and less commonly, multiple sclerosis (4) ANA-negative with no systemic, but severe neuropsychiatric or focal neurologic features—consider multiple sclerosis or CNS vasculitis (PACNS or BACNS). This approach to differential diagnosis is delineated in a 1999 monograph by Jennekens and Kater [177], from which Table 7 is abstracted. Vasculitis of various types comes closest to mimicking the range of neuropsychiatric features associated with SLE. As one moves from left to right across the table, the illnesses become less similar to SLE, until the multiple sclerosis column, in which depression and subcortical dementia are the only neuropsychiatric manifestations overlapping with CNS lupus.

TABLE 7 Estimated Frequency of SLE Neuropsychiatric Features over Course of Disease, Compared to Diseases Mimicking SLE[a]

	SLE (%)	Vasculitis (e.g., Wegener's granulomatosis (%)	Sjögren's syndrome (%)	Mixed or undifferentiated connective tissue disease (%)	Antiphospholipid syndrome without SLE (%)	Multiple sclerosis (%)
Total neuropsychiatric syndromes (excluding subtle cognitive deficits, minor symptoms)	30	10	<5	<5	<5	<5
Coma	<5	<5			<5	
Delirium (organic brain syndrome), dementia	12	<5	<5	<5	<5	<5
Psychosis	12			<5		
Major depression	15			<5		20
Seizure, post-ictal state	<5	<5	<5	<5	<5	
Aseptic meningitis	<5		<5	<5		
Infarct, stroke	<5	<5	<5	<5	<5	
Intracranial hemorrhage	<5	<5	<5			

[a] (<5% means the syndrome has been reported in a small percentage of patients with the illness and the frequency of the syndrome in the illness could be more than 1%. Blank means <1% of patients, or insufficient data to allow a literature-based estimate, but thought to be either <1% or not occurring in this illness.)

Psychotropic Drug-Induced Positive ANA: (in Psychiatric Patients without SLE)

Psychiatric patients who are receiving antipsychotic drugs, particularly phenothiazines such as chlorpromazine, may have positive ANAs and anti-phospholipid antibodies [178, 179]. Drug-induced lupus has also been reported with other psychotropic drugs, including carbamazepine, divalproex, other anticonvulsants, and lithium [180–184]. Nevertheless, evaluating such etiologic associations is difficult, since some cases of purported psychotropic drug-induced lupus may represent undiagnosed neuropsychiatric SLE. In drug-induced lupus, CNS manifestations are rare [184], and over 95% of patients have anti-histone antibodies. If the offending drug is discontinued, the lupus symptoms typically resolve within a few weeks, although the ANA may remain positive for over a year.

Somatization Disorder ("Psychogenic Pseudolupus" and Psychogenic Symptoms in SLE)

Systemic lupus erythematosus can be misdiagnosed in "somatizing" patients with multisystem complaints and mildly positive ANAs, which are common in young women. Somatizers crave medical attention, and typically present with a several year history of multiple, vague physical symptoms that have eluded diagnosis. Their symptoms are neither feigned nor deliberately created, but cause considerable social or occupational debilitation. Such patients have repeatedly sought medical care and have often undergone extensive diagnostic workups. The medical interview, itself, can reinforce and shape the patient's symptoms. For example, after being asked about butterfly malar rashes, the patient may adopt this description, thereby "legitimizing" her symptoms.

Interventions focused on alleviating illness tend to be ineffective in somatizers, since their symptoms are either refractory to treatment, or resolve, only to be supplanted by new ones. Such patients are seeking not cure but assurances of ongoing care.

If somatization is suspected, as in a patient who appears less ill than expected despite the plethora of symptoms, the rheumatologist should avoid mentioning SLE as a possibility until there is sufficient objective evidence to corroborate the diagnosis. Somatizers are often dismayed to learn that they do not have SLE or another rheumatologic disease. Where possible, the rheumatologist should tell the patient unequivocally, but with a positive tone, that although the nature of her illness remains unclear, the "good news" is that she does not have lupus. (The patient should also be informed of any other medical conditions with overlapping symptoms, such as fibromyalgia, that have been excluded.) Never-

theless, it is important to validate that her symptoms are real, and that they have impaired her functioning. Accordingly, the rheumatologist should emphasize that the patient will require close follow-up with her primary care physician to optimize her ability to function, despite her illness. Many somatisizers have comorbid anxiety disorders or depression, and may be amenable to psychiatric referral if it is presented as an adjunct to ongoing medical follow-up to help them cope with their illness.

At times, patients with confirmed SLE may exhibit symptoms or signs that, despite extensive diagnostic workup, do not seem attributable to a lupus flare or any other identifiable medical illness. Patients are often anxious about recurrent flares of their illness, and may become particularly attuned to, or overinterpret normal physical symptoms. However, given the vague multisystem manifestations of SLE, diagnosing somatization in a patient with known lupus should be done with caution, after a thorough evaluation for objective signs of illness.

Factitious SLE

Unlike somatizers, patients with factitious SLE fabricate symptoms, and feign or intentionally produce signs of lupus to assume the patient role. This is one variation in the universe of factitious illnesses (sometimes referred to as Munchausen's syndrome). Factitious SLE appears to be rare, but several cases have been reported [185]. Patients have simulated hematuria by pricking their finger surreptitiously to add trace amounts of blood to urine specimens, injected themselves with feces or other contaminants to cause infections, or applied rouge to their cheeks to simulate a malar rash. One patient feigned proteinuria by inserting a packet of protein into her bladder. Despite their physical signs of SLE, these patients had no serologic evidence of an autoimmune disorder. Patients with factitious disorders have often been employed in medical settings, or have health care or laboratory training. They are usually young women who are evasive and engage in subterfuge. Sometimes using aliases, they tend to present to multiple hospitals, and undergo extensive diagnostic tests and medical or surgical procedures. When the factitious nature of their illness is discovered, they typically refuse psychiatric referral, leave against medical advice, or are lost to follow-up. Factors that should engender suspicion of factitious SLE are inconsistent and markedly variable lab results without serologic evidence of SLE (i.e., negative ANA and anti-double-stranded DNA, and normal complement levels), in a patient, especially a young woman, with a health care background.

TREATMENT

Inpatient vs Outpatient Treatment

Inpatient treatment is generally required for SLE patients who are acutely suicidal, severely depressed or manic, psychotic, or delirious.

Suicidal Patients

Although few statistics are available, SLE may be one of several medical disorders associated with somewhat increased risk of suicide [186]. During a 20-year follow-up of 300 patients with SLE, 5 made suicide attempts, one of which was fatal [187]. Attempted or completed suicide has occurred in SLE patients with mood disturbances, insomnia, delirium, or psychosis [188, 189]. All SLE patients with psychiatric symptoms should be asked about hopelessness and suicidal ideation. Inquiring about suicidality will not precipitate suicide or cause a nonsuicidal patient to become suicidal. On the contrary, patients who are contemplating suicide are usually relieved to disclose it to their physicians. If the patient seems imminently suicidal, the physican must take steps to ensure her safety; for example, urgent psychiatric evaluation, and often hospitalization. Delirious patients' behavior is especially difficult to predict, and any suicidal thoughts expressed during delirium should be considered potentially serious. In nonemergency situations, the physician should evaluate the seriousness of the patient's suicidal ideation, and in most cases, arrange psychiatric consultation.

Treatment Modalities

Corticosteroids, Cytotoxic Agents, and Other Immunosuppressants for Primary CNS Manifestations of SLE

Severe neuropsychiatric SLE (e.g., psychosis, delirium), with or without systemic lupus disease activity, is generally treated with high-dose corticosteroids, and sometimes, adjuctive cyclophosphamide [190, 191]. In addition, psychotropic medications are used for symptom management. Despite widespread clinical use of high-dose corticosteroids (e.g., prednisione 1–2 mg/kg/day or methylprednisolone 1 *g* IV daily for 3 days) [2, 88], little research exists on their efficacy in CNS lupus. In patients who are on corticosteroids, determining whether new psychiatric symptoms are corticosteroid-induced or a primary CNS lupus flare is often difficult, and is outlined in Table 4. Cyclophosphamide (0.75–1 g/m^2/month) appeared effective in an uncontrolled trial in patients with severe neuropsychiatric SLE [192]. In a retrospective study, lower doses of cyclophosphamide (500 mg IV very week) were benefi-

cial in nonthrombotic severe CNS lupus. Side effects were mild, including worsening leukopenia with respiratory infection in 2 of 25 patients, but no cystitis, herpes zoster, or ovarian failure [193]. To date, no randomized, controlled trials have been done comparing methylprednisolone vs cyclophosphamide in the treatment of neuropsychiatric SLE [194]. In severe, treatment-refractory CNS lupus, other therapies include intravenous immunoglobulin, and occasionally, plasmapheresis [190]. (see Chapter 47 for an in-depth discussion).

In patients with moderate, new onset, or progressive neuropsychiatric symptoms, empiric practice is to begin prednisone 0.5 mg/kg/day for 2 to 3 weeks, or to double the current prednisone dosage as a 3-day trial [125]. For mild neuropsychiatric SLE, a small controlled study demonstrated that low-dose prednisone (0.5 mg/kg/day for 3 weeks) was beneficial in improving patients' cognition and mood [44]. Alternatively, patients with mild neuropsychiatric symptoms may be observed on psychotropic medications without immunosuppressive or cytotoxic therapy.

For those who respond to corticosteroids or cytotoxic agents, the optimal duration of treatment of neuropsychiatric SLE is unresolved. Chronic use of steroid-sparing medication, such as mycophenolate, azathioprine, or pulse cyclophosphamide for CNS lupus has not been studied in randomized trials, but may be considered in patients with recurrent or progressive neuropsychiatric symptoms.

Anticoagulants for Primary CNS Manifestations of SLE due to Thrombosis

Systemic lupus erythematosus patients with antiphospholipid antibody syndrome and thrombotic CNS complications generally require long-term anticoagulation and antiplatelet therapy [195] (see Chapter 40 for an in-depth discussion).

Psychopharmacologic Treatment

Psychopharmacologic medication is used in treating specific psychiatric symptoms that occur in SLE. (See Refs. 196–198 for excellent reviews of psychopharmacology.)

Antidepressants

Newer antidepressants, most notably the selective serotonin reuptake inhibitors (SSRIs), have been developed that have fewer side effects and low lethality in overdose compared to tricyclic antidepressants. Antidepressants are used in treating depression as well as anxiety. Except possibly in severe melancholic depression, all antidepressants appear to be equally efficacious. In addition, all currently available antidepressants have a delayed onset of action, generally 2–4 weeks (sometimes as long as 8 weeks) from the time the patient reaches a typical therapeutic dosage until the antidepressant (or antianxiety) effect occurs. Antidepressants must be taken daily; they are ineffective when taken prn.

The main differences among antidepressants are in half-life, side-effect profile, and drug interactions. Such considerations often determine the choice of antidepressant in a given patient (see Table 8).

In treating depression, *SSRIs* are generally first line drugs. Commonly used SSRIs include fluoxetine, sertraline, paroxetine, and citalopram. (Fluvoxamine is used only occasionally for depression due to its short half-life and significant drug interactions.) The most common side effect of SSRIs is sexual dysfunction (i.e., anorgasmia, decreased libido, delayed ejaculation, or erectile dysfunction), occurring in ≥33% of patients, and persisting for the duration of treatment. Other side effects are usually mild, and include insomnia (or sedation), headache, nausea, jitteriness, and transient anxiety, which can be minimized with a low initial dose and gradual upward titration. Fluoxetine and sertraline tend to be activating, whereas paroxetine is sometimes sedating, and citalopram is somewhere in-between. Fluoxetine has a very long half-life; consequently, it is particularly useful in patients who take their medications sporadically. In contrast, paroxetine (immediate-release form) has a very short half-life. Patients may experience a "discontinuation syndrome" when they miss a dose, or abruptly discontinue it (or other shorter-acting SSRIs). Discontinuation syndrome is characterized by nonspecific symptoms that are also common in SLE, for example, fatigue, headache, dizziness, and myalgias, as well as nausea, diarrhea, and shock-like sensations. Drug interactions with SSRIs are more common with fluoxetine and paroxetine, which inhibit CYP 2D6, and thus can elevate levels of warfarin, β-blockers, tricyclic antidepressants (risk of cardiotoxicity), and some antiarrhythmics, and inhibit conversion of codeine to more potent opioid metabolites. (Concomitant use of serotonergic drugs, e.g., SSRIs, amitriptyline, chlorpheniramine (TCA-like), meperidine, dextromethorphan, tramadol, and monoamine oxidase inhibitors, including selegiline, can cause serotonin syndrome, characterized by shivering, teeth chattering, fever, diaphoresis, rigidity, hyperreflexia, myoclonus, tachycardia, hypertension, and confusion.) Among the SSRIs, citalopram and sertraline have the fewest drug interactions. The potential for drug interactions is most important to consider in SLE patients on drugs with a low therapeutic index, such as warfarin.

TABLE 8 Choice of Antidepressant[a]

Depression in	Preferred antidepressant	Relatively contraindicated antidepressants
Noncompliant patient	Fluoxetine (long T $^1/_2$)	Paroxetine, (immediate-release), venlafaxine (short T $^1/_2$, even "extended-release" venlafaxine)
Pt on cyclophosphamide		Mirtazapine (risk of agranulocytosis, which may be masked by corticosteroids)
Pt on warfarin	Venlafaxine, citalopram, sertraline	Fluoxetine, paroxetine
Pt on multiple medications	Venlafaxine, citalopram (fewest drug interactions)	Fluoxetine, paroxetine, nefazodone, tricyclic antidepressants
Hx of seizures or stroke	SSRIs	?bupropion (immediate release form in high doses), tricyclic antidepressants
Hypertension	SSRIs	(Venlafaxine can be used provided bp is monitored)
Cardiac disease (esp. conduction disturbance)	SSRIs, mirtazapine	Tricyclic antidepressants
Renal disease	Citalopram, fluoxetine (minimal dosage adjustment)	Tricyclic antidepressants (venlafaxine can be used but at half the typical dosage)
Obesity	Bupropion, sertraline fluoxetine, citalopram	Tricyclic antidepressants, mirtazapine, paroxetine
Insomnia	Paroxetine, trazodone, mirtazapine	Bupropion, fluoxetine, sertraline (but SSRIs can be used concomitantly with low-dose trazodone)
Sedation	Bupropion, fluoxetine, sertraline	Paroxetine, trazodone, nefazodone, mirtazapine, tricyclic antidepressants

[a] Sayal, 2000 [201].

Venlafaxine has SSRI-like actions at low dosage, and shares similar side effects. Additional side effects include constipation, diaphoresis, and mild elevations in blood pressure. It has very few drug interactions. Since even the "extended release" venlafaxine preparation has a short half-life, it can cause an SSRI-like discontinuation syndrome.

Although classified as an antidepressant, *trazodone* is very sedating; consequently, it is more useful for treating insomnia. It is commonly used adjunctively in low doses, for example, 50–100 mg qhs, with activating SSRIs. It can cause orthostasis, and rarely, priapism.

Nefazodone is somewhat sedating, and can improve the quality of sleep. It is not associated with sexual dysfunction or weight gain. Side effects include headache, nausea, dizziness, dry mouth, and rare cases of liver failure [199, 200]. Since nefazodone has a risk of hepatotoxicity, and also has many drug interactions, it should only be used when other antidepressants have been exhausted.

Mirtazapine causes significant sedation and weight gain, but not sexual dysfunction or cardiotoxicity. Rare cases of agranulocytosis have been reported (incidence 1–3/1000), a significant concern in SLE patients who are on cyclophosphamide, and other drugs that can cause neutropenia, for example, carbamazepine, phenytoin, and ACE inhibitors. (Also, since concomitant treatment with corticosteroids might mask the development of agranulocytosis, mirtazapine probably should be used cautiously in SLE patients.)

Bupropion has stimulant-like effects, and may enhance concentration. It also facilitates smoking cessation. Bupropion has few significant drug interactions, and does not cause cardiotoxicity or weight gain. It does not cause (and sometimes alleviates SSRI-induced) sexual dysfunction. Side effects include insomnia, agitation, headache, and dizziness. Although bupropion was purported to increase the risk of seizures, this concern derived from a study of patients with eating disorders on high doses. Seizures have not been associated with the sustained-release form of bupropion. Like SSRIs, bupropion can also inhibit CYP 2D6, and cause similar drug interactions.

Tricyclic antidepressants, such as imipramine, amitriptyline, desipramine, and nortriptyline, are less commonly used. Relative to newer antidepressants, especially SSRIs, tricyclics are less well tolerated due to their side effects: sedation, orthostasis, weight gain, and anticholinergic effects—that is, tachycardia, constipation, urinary retention, blurred vision, dry mouth, and confusion, which can progress to delirium. Furthermore, their quinidine-like effects may cause cardiotoxicity (prolonged QRS and QT intervals), making them potentially lethal in overdose. Because of the risk of

delirium and cardiotoxicity, as well as their photosensitizing effects, they should be used very cautiously in SLE patients.

St. John's wort is a commonly used herbal antidepressant. Since it is a potent inducer of hepatic microsomal enzymes, it can significantly lower the levels of oral contraceptives, and possibly digoxin, warfarin, and many other drugs, and should be avoided by SLE patients.

All antidepressants may precipitate or exacerbate mania in vulnerable patients, especially those with a personal or family history of bipolar disorder. Patients on high-dose corticosteroids may also be at increased risk for mania on antidepressants (unless the patient is already on a mood stabilizer or antipsychotic).

The *most common reason for lack of antidepressant response is inadequate dosage for an insufficient period of time*. An adequate antidepressant trial is at least 8 weeks at the upper end of the typical dosage range. If the patient has a good antidepressant response, the medication should be continued for ~9 months (unless mania/hypomania ensues). Antidepressants, especially those with short half-lives, should then be tapered gradually over several weeks for a single episode of major depression, but long-term treatment is appropriate for recurrent major depression or chronic depression.

Stimulants

Psychostimulants such as dextroamphetamine and methylphenidate are very useful in treating depression in the medically ill. Their primary advantage over antidepressants is their rapid onset of action, generally within 48 h. Psychostimulants are particularly effective in alleviating apathy, fatigue, decreased energy, and poor concentration, all of which are common in SLE. When used in modest doses, psychostimulants have minimal side effects, but higher doses can cause appetite suppression and slight elevations in blood pressure and heart rate. (Very high doses may precipitate psychosis.) Psychostimulants are relatively contraindicated in patients with uncontrolled hypertension, arrhythmias, delirium, or a history of psychosis. Since psychostimulants have no effect on seizure threshold, they can be used in patients with a history of seizures. Amphetamine can prolong the action of warfarin; however, methylphenidate has not been reported to have this effect. Patients with a history of stroke tend to be more sensitive to CNS medications, including psychostimulants, and should be started on a low dose, which is then increased gradually. Tolerance and abuse are rare in medically ill patients without a prior history of substance abuse [202].

Mood Stabilizers

Mood stabilizers such as lithium, carbamazepine, and valproate, are used in the prophylaxis and treatment of mania, depression, and mood lability, including that due to corticosteroids. Because of their side effects, drug interactions, and teratogenicity, they should be prescribed and monitored by psychiatrists or other physicians familiar with their use.

Lithium has been used for mood stabilization and antidepressant augmentation. Lithium has been helpful in alleviating depression in corticosteroid-treated patients with SLE and strokes [203]. Lithium has also prophylaxed against corticotropin-induced mania or depression with psychotic features [127], and corticosteroid-induced mania and psychosis. Common side effects of lithium include nausea, tremor, polyuria (nephrogenic diabetes insipidus), polydipsia, hypothyroidism, acne, leukocytosis, weight gain, and subtle cognitive "dulling." Lithium has a very low therapeutic index. Lithium toxicity is manifested by vomiting, diarrhea, coarse tremor, ataxia, confusion, seizures, and death. Since lithium is renally excreted, and handled similarly to sodium, patients with substantial fluid and electrolyte shifts due to congestive heart failure, renal insufficiency, or diuretics (including thiazides, triamterene, and spironolactone) are at increased risk for lithium toxicity. ACE-inhibitors, angiotensin II receptor antagonists, NSAIDS, and COX 2 inhibitors also increase lithium levels. Lithium is contraindicated in SLE patients with poorly controlled seizures or renal insufficiency (but is typically easy to use in end-stage renal disease). Since lithium can cause ECG changes, it should also be used cautiously in SLE patients with cardiac disease.

Valproate, and its enterically coated form, *divalproex*, are anticonvulsants that have been effective as mood stabilizers. Some patients with SLE and various mood disturbances have had marked positive responses to valproate [204]. Side effects of valproate include nausea, sedation, weight gain ("Depa-bloat"), edema, alopecia, tremor, thrombocytopenia, increased transaminases, and less commonly, hyperammonemia and polycystic ovary syndrome. (Valproate-induced weight gain, and occasionally, ecchymoses and bleeding, may be compounded by similar side effects from corticosteroids.) Serious adverse reactions to valproate include rashes, pancreatitis, and hepatotoxicity, sometimes progressing to hepatic failure in children. Valproate is contraindicated in patients with hepatic disease. Valproate inhibits the metabolism of many drugs, including warfarin.

Like valproate, *carbamazepine* is an anticonvulsant that also has mood-stabilizing properties. Little data

exists regarding its use in neuropsychiatric SLE. Although carbamazepine generally does not cause weight gain, common side effects are sedation, dizziness, ataxia, blurry vision, diplopia, dysarthria, confusion, tremor, nausea, elevated transaminases, and hyponatremia (SIADH). Serious side effects include rash, hepatotoxicity, agranulocytosis, and aplastic anemia. Accordingly, carbamazepine is contraindicated in patients with hepatic disease, and in patients receiving cyclophosphamide or other cytotoxic agents. Since carbamazepine is a potent inducer of hepatic cytochrome P450 enzymes, it induces the metabolism of many other drugs (and itself), thereby decreasing drug levels, including warfarin.

Teratogenicity is a major concern with valproate and carbamazepine (neural tube defects), and to a lesser extent, lithium (Ebstein's anomaly). Accordingly, these medications are contraindicated in pregnancy unless the clinical benefits strongly outweigh the risks. Safer alternatives, such as antipsychotic medication or benzodiazepines, are preferable in treating agitation and mania during pregnancy.

Newer anticonvulsant agents such as *gabapentin*, *topiramate*, and *lamotrigine* have been inadequately studied as mood stabilizers, and little if any data exists regarding their use in SLE. Gabapentin (discussed later) has not been proven efficacious as a mood stabilizer. Topiramate appears to have some mood stabilizing effects, and causes weight loss, rather than weight gain. However, its side effects also include word-finding and cognitive difficulties, which may be problematic since SLE, itself, predisposes to cognitive impairment. Although lamotrigine has mood stabilizing effects, its risk of serious rashes, including Stevens-Johnson syndrome, limits its use.

Benzodiazepines, Other Anxiolytic Agents, and Sedative Hypnotics

Benzodiazepines are used in treating anxiety, agitation (including agitation due to delirium), mania, and insomnia, and also have anticonvulsant and muscle relaxant effects. Unlike antidepressants, they have no latency in onset of action. In selecting a benzodiazepine, the most important considerations are rate of onset and duration of action, and presence or absence of active metabolites.

Short-acting benzodiazepines, such as alprazolam and oxazepam, have the advantage of not accumulating, but have the disadvantages of increased risk of rebound insomnia, interdose anxiety, and withdrawal symptoms, including seizures, with abrupt discontinuation. Longer-acting benzodiazepines, such as clonazepam and diazepam, are less likely to cause rebound insomnia, interdose anxiety, or withdrawal symptoms, but with repeated dosing, levels may progressively increase for up to a week before reaching steady state. Also, long-acting benzodiazepines generally should not be used for insomnia because they cause daytime sedation.

Lorazepam, oxazepam, and temazepam undergo only glucuronidation and have no active metabolites; consequently, they are the preferred benzodiazepines in the elderly or patients with liver disease. Clonazepam also has no active metabolites.

For acute agitation, lorazepam is generally the benzodiazepine of choice because it can be administered PO, IM, or IV, has no active metabolites, and has a long duration of action after a single dose, due to its small volume of distribution in peripheral tissue. (In contrast, after repeated dosing, peripheral tissues become saturated, and the duration of action lessens since lorazepam has a short elimination half-life.) See Table 9 for adjunctive use of lorazepam in treating delirium. For chronic anxiety, long-acting benzodiazepines such as clonazepam are preferred.

The primary risk of benzodiazepines is dependence. Accordingly, except in acute situations, benzodiazepines are relatively contraindicated for treatment of anxiety or insomnia in patients with a history of alcohol or other substance abuse. Risk of dependence is highest with short-acting, high potency benzodiazepines, such as alprazolam. Additional side effects of benzodiazepines are sedation, impaired memory and coordination, and falls, which are of particular concern in SLE patients with osteoporosis due to long-term corticosteroid therapy. Benzodiazepines can exacerbate depression, and occasionally cause disinhibition. Respiratory depression can occur, particularly with rapid IV administration of benzodiazepines, or in patients with pulmonary disease. Benzodiazepine withdrawal is characterized by anxiety, insomnia, tremulousness, tachycardia, hypertension, nausea, agitation, delirium, and seizures, especially with short-acting benzodiazepines such as alprazolam. Benzodiazepines are nonlethal in overdose, except when used concomitantly with alcohol or other CNS depressants.

Buspirone is an antianxiety medication that is chemically distinct from benzodiazepines. It neither cross-reacts with benzodiazepines nor alleviates benzodiazepine withdrawal, and has no anticonvulsant effects. Although less effective than benzodiazepines, buspirone is useful for generalized anxiety, but usually not panic attacks. Its primary advantage is that it does not cause dependence. Buspirone is nonsedating and has few side effects, except for transient increase in anxiety. Like antidepressants, buspirone has the disadvantage of a several week latency in onset of action. It also must be taken daily, and is ineffective when taken prn for

TABLE 9 Treatment Protocol for Delirium (Acute Confusional State)

Initial dosage of haloperidol (mg intravenously or intramuscularly) (For oral dosing, double the IV or IM dose)				
	Severity of agitation			
	Mild	Moderate	Severe	Frequency of dosing
Young–middle-aged patients	0.5–2 mg	2–5 mg	5–10 mg	every 1–4 h (but may dose every 30 min, if necessary)
Elderly patients	0.25–0.5 mg	0.5–1 mg	1–2 mg	Every 2–4 h

If haloperidol is being administered intravenously, obtain a baseline ECG and monitor QTc interval for prolongation. Monitor serum potassium and magnesium.

If agitation persists, double the previously administered haloperidol dose.

If agitation has not resolved after 3 doses, then add lorazepam 0.5–1 mg IV or IM with each dose of haloperidol.

If the patient requires more than either 50 mg IV of haloperidol in 5 h, or 80 mg/day, consider a bolus haloperidol dose of 10 mg , then continuous intravenous haloperidol infusion at 5–10 mg/h.

Once agitation has resolved, determine the total haloperidol and lorazepam dosage the patient has required in 24 h, and administer it in divided doses (every 6-8 h).

If the patient remains stable, haloperidol and lorazepam can then be tapered over the next 3–7 days.

[a] Adapted from Wise and Terrell [208], as cited in Rundell, J.R., and Wise, M.G. [2000]. Concise Guide to Consutation Psychiatry," 3rd ed., p. 40; and [103].

occasional anxiety, in contrast to benzodiazepines. Its short half-life necessitates TID dosing.

Zolpidem and *zalephon* are nonbenzodiazepine medications used in the short-term treatment of insomnia. They appear to have a low (but not zero) abuse potential. Since both zolpidem and zalephon have fairly short half-lives, they generally do not cause daytime sedation. Both can cause sedation and respiratory depression when used concomitantly with benzodiazepines or alcohol.

Gabapentin, an anticonvulsant, appears to have some antianxiety effects. In addition, it is effective in alleviating neuropathic pain. (Whether it has mood stabilizing effects remains controversial.) Its side effects are sedation, dizziness, ataxia, and weight gain, and it has no significant drug interactions. Because of its short half-life, it requires TID dosing.

Herbs such as *kava kava*, which has antianxiety effects, and *valerian root*, which is used for insomnia, should be avoided because of the risk of greater than additive effects with other sedative-hypnotics, including benzodiazepines. Kava kava may also pose a risk of hepatotoxicity.

Antipsychotic drugs are used to alleviate agitation, mania, delirium, and psychotic symptoms, even when corticosteroid-induced. Commonly used antipsychotics include haloperidol, phenothiazines (e.g., fluphenazine, chlorpromazine), and newer agents such as clozapine, olanzapine, risperidone, quetiapine, and ziprasidone. The side effects of antipsychotic drugs, that is, extrapyramidal effects and elevated prolactin (both due to dopamine receptor blockade), sedation, anticholinergic efffects, orthostatic hypotension, and weight gain, are summarized in Table 10. Extrapyramidal side effects include acute dystonic reactions (sudden muscle spasms), akathisia (motor restlessnsess with an irresistible urge to move), and Parkinsonian symptoms (bradykinesia, rigidity, resting tremor, and masked facies). Relative to older antipsychotic drugs such as haloperidol, newer drugs, particularly clozapine, olanzapine, quetiapine, and ziprasidone, have many fewer extrapyramidal side effects, whereas risperidone is intermediate. Although chronic administration of antipsychotic drugs can cause tardive dyskinesia, the risk is minimal with short-term treatment, and is much lower with newer antipsychotic agents. Neuroleptic malignant syndrome is a rare, life-threatening reaction to antipsychotic drugs, and is characterized by rigidity, elevated CPK, and high fever, with tachycardia, fluctuating blood pressure, and confusion. Phenothiazines should probably be avoided in SLE due to the risk of photosensitivity, phenothiazine-induced immunologic abnormalities, including + ANA, and the lack of advantage over haloperidol. Clozapine requires weekly blood

TABLE 10 Side Effects of Antipsychotic Drugs

	Haloperidol	Risperidone	Ziprasidone	Olanzapine	Quetiapine	Clozapine
Extrapyramidal effects	4[a]	2	1	1	0	0
↑ Prolactin	4	3	1	1	0	0
Sedation	1	1	2	2	2	4
Anticholinergic effects	0	0	0	2	0	4
Orthostasis	0	3	1	1	1	4
Weight gain	1	2	0	4	2	4
↑Glucose + ↑triglycerides	0	0	?	2	?1	2
Other serious side effects			↑QT_c interval			*Agranulocytosis* *Seizures* (dose related) ? myocarditis ?cardiomyopathy ? thromboembolism

[a] Note: 4 ≡ severe, 3 ≡ moderate, 2 ≡ mild, 1 ≡ minimal, 0 ≡ none.

monitoring because of the risk of agranulocytosis (1% incidence), and is contraindicated in SLE patients on cyclophosphamide or other cytotoxic agents. Since clozapine also causes decreased seizure threshold (as well as hyperglycemia), and has been linked to myocarditis, cardiomyopathy [205, 206], and thromboembolism [207], it should probably be avoided in SLE. Olanzapine appears to have mood stabilizing effects in addition to its antipsychotic effects, but it can cause hyperglycemia. (Although both clozapine and olanzapine are asssociated with significant weight gain, this issue may not be relevant to short-term treatment.) With most antipsychotic agents, drug interactions are generally not a major concern, except for additive hypotensive or anticholinergic effects when used concomitantly with similar agents. Risperidone levels are increased by fluoxetine and some other SSRIs, and by B-blockers. Because of the risk of prolonged QT interval, ziprasidone should not be used in patients with preexisting prolonged QT, or those receiving class 1a and III antiarrhythmics, sotalol, mefloquine, tacrolimus, and several other drugs. Overall, haloperidol, risperidone, and quetiapine may be the preferred antipsychotics in SLE; however, haloperidol has the major advantage of IM/IV, as well as PO administration.

Electroconvulsive Therapy (ECT)

Electroconvulsive therapy (ECT) is the periodic induction of seizures to treat disorders such as severe depression, mania, and catatonia. Under general anesthesia, an electrical current is applied to the scalp to induce a controlled seizure. In SLE patients, ECT has been helpful in ameliorating catatonia [209], mania, and severe depression with or without psychotic features, including corticosteroid-induced syndromes [126]. Indications for ECT include inability to tolerate, or failure to respond to psychotropic medication, or need for rapid therapeutic response, especially in debilitated patients unable to maintain adequate PO intake. Although there are no absolute contraindications to ECT, relative contraindications include recent acute myocardial infarct, intracerebral hemorrhage, cerebral mass, or increased intracranial pressure. ECT can cause transiently elevated blood pressure and arrhythmias; however, the most common side effects are post-ictal confusion (delirium) and amnesia. Retrograde amnesia (i.e., inability to recall information learned pre-ECT) may persist, and involves patchy loss of memories from the months immediately prior to, and including the period of ECT treatment. Anterograde amnesia (i.e., inability to recall new information) is prominent during the course of ECT treatment, but almost invariably resolves within a few weeks after ECT has been completed [210–212]. Subjective complaints of memory impairment correlate directly with depression, and are often not substantiated on cognitive testing [213]. Since SLE patients may already have underlying cognitive impairment, a cognitive exam should be performed at baseline (pre-ECT), and several months post-ECT.

Psychotherapy, Psychosocial, and Behavioral Interventions

Nonadherence to Treatment: Evaluation and Interventions

To enhance treatment adherence, the rheumatologist must first ascertain the reasons for nonadherence. Nonadherence is exceedingly common, and has multiple causes relating not only to the patient and family, but

TABLE 11 Reasons for Nonadherence to Treatment[a]

- Patient-related factors
  - Socioeconomic
    - **Poverty**
    - Homelessness
    - **Inadequate insurance coverage**
    - **Lack of childcare**, **transportation**, or time off from work
  - Cognitive/educational factors
    - Illiteracy or lack of education
    - **Misunderstanding instructions**
    - **Memory impairment**
  - Psychological factors
    - **Denial of illness**
    - **Defiance** (struggle for autonomy)—especially in adolescents
    - Desire to maintain control
    - Anxiety or specific phobia (e.g., needle phobia), culminating in avoidance of medical care
    - **Depression, sense of futility**
    - **Pessimism regarding efficacy of treatment**
    - Secondary gain from sick role
    - Lack of trust in caregivers
    - **Lack of perceived self-efficacy (patient's belief in own capability of coping with illness and its treatment)**
    - **Erroneous beliefs that undermine treatment**
  - Substance abuse
- Family-related factors
  - Lack of social supports
  - Family undermining treatment
- Physician-related factors
  - **Lack of rapport with patient**
  - **Inadequate patient education**
  - **Poor communication**
  - **Lack of concordance between patient's and physician's priorities**
  - Physician's pessimism regarding patient's prognosis
  - Conflicting advice from multiple caregivers (lack of coordination of care)
- Treatment-related factors
  - **Complicated medication regimen**
  - **Disruptive treatment regimen (adverse impact on lifestyle)**
  - **Intolerable side effects**
  - Prophylactic treatment (in contrast to symptomatic treatment)
  - Chronicity of treatment

[a] Major factors are in boldface type.

also to the physician and the treatment itself (see Table 11). Indigent patients frequently cannot access care due to lack of insurance, and may be unable to afford copays for medication, or bus fare for appointments. Family and friends may fail to support the patient, or directly undermine treatment. Denial of illness often manifests as nonadherence to treatment. Other psychological factors that may impact on compliance include defiance, especially in adolescents, whose rebellion against authority is intensified by their sense of immortality. Despair about the illness, and doubts about the efficacy of treatment tend to be reinforced by experiencing lupus flares despite good treatment adherence, or having no flares despite poor adherence. Many patients feel overwhelmed and perceive themselves as incapable of coping with their illness or following treatment recommendations. In addition, patients may harbor fallacious beliefs that interfere with treatment. For example, the patient may contend that medication should be taken only when she feels ill, that taking medication regularly will cause immunity to it, or that if the prescribed dose is beneficial, an even higher dose will be better [214]. Finally, despite the desire and intent to adhere to treatment, the patient may misconstrue the instructions, especially if she has underlying cognitive impairment.

Treatment nonadherence may also be attributable to physician and treatment-related factors, such as failure to establish rapport with a patient, or educate her about the nature of SLE, rationale for treatment, and risks of nontreatment. If the patient's and rheumatologist's priorities are not in accord, patients will often disregard the treatment recommendations. For instance, to the patient distressed by fatigue, the rheumatologist's focus on hypertension or the long-term risk of renal insufficiency seems irrelevant. Frequently, patients fail to adhere to treatment because the medication regimen is too complex, too disruptive to their lifestyle, or causes side effects that are more distressing than the illness itself [214, 215].

In inquiring about the reasons for treatment nonadherence, the rheumatologist should be nonjudgmental—for example, "Sometimes patients decide not to take prednisone because they are upset about weight gain or other side effects. Has that ever happened to you?" "Sometimes patients stop taking their medication because they are feeling well and don't think they need it. Has that ever happened to you?" "What do you think would happen if you decided to take the medication every day?"

Once the reasons for nonadherence have been elucidated, the rheumatologist should address these issues directly, and enlist the patient as a partner in treatment (Table 12). In engaging the patient, the most crucial step is to ask what is most important to her regarding her care, then reorient the treatment goals in accordance with her priorities. Whenever possible, the rheumatologist should involve the patient in decision-making. It is helpful to delineate the patient's role in her care, particularly the initial steps she can take in pursuing her treatment goals, and the rheumatologist's role in assisting her. If the patient lacks confidence in her ability to carry out treatment recommendations, she may benefit from programs that bolster self-efficacy (see later).

For nearly all patients, treatment adherence varies inversely with the complexity of the medication

TABLE 12 Interventions to Enhance Treatment Adherence

Ascertain why the patient has not been adherent to treatment Be nonjudgmental in asking patient her views and practices Validate patient for being honest Rule out depression, denial
Focus on what is most important to the patient (patient's priorities and concerns)
Enlist patient as an active partner in treatment
Enhance the patient's sense of self-efficacy
Simplify medication regimen
Give explicit verbal and written instructions on medications, dosages, and rationale for each medication in language and format that patient can understand
Defuse adolescent defiance
Give positive reinforcement for any improvements in adherence to treatment

regimen. Accordingly, whenever possible, medication regimens should be simplified by reducing the number of medications prescribed and the frequency of dosing. The rheumatologist should provide clear written instructions detailing the medication regimen. Clarity can be ensured by making simple charts that list the medication name, mg strength/pill, and the number of pills to take each morning and evening. (Color coding of charts and prescription bottles can be helpful.) Dispense pills into pill boxes, or have pharmacy use blister packs. Give practical suggestions for how to remember to take medications (e.g., instructing the patient to put the pill bottle next to her toothbrush or alarm clock).

In working with a defiant adolescent, emphasize (in matter-of-fact, nonchalant way) that it is her choice whether or not to take medication or to come in for appointments, and that she, not you as the physician, will experience the consequences, both positive and negative. Portray the doctor–patient relationship as collaborative, and emphasize that without the patient's participation, treatment cannot proceed. Do not allow medications to become a control issue. Advise parents to adopt the same approach.

Finally, praise patients for even small improvements in adherence to treatment. Focus on what patient is doing right. Ask the patient what she did differently that enabled her to take the medication daily, or cut down on her smoking [214, 215].

Unfortunately, some patients are chronically nonadherent to treatment, despite having access to care, good insight into their illness, and devastating sequelae of SLE. Although seemingly incomprehensible, such noncompliance may emanate from the patient's questioning whether the benefits outweigh the losses in relinquishing current behavior and becoming more treatment adherent.

Motivational interviewing, developed by Miller and Rolnick [216] is exceedingly effective in enabling patients to change detrimental behavior. While this approach requires an in-depth explanation and training, certain aspects are relevant to addressing chronic treatment nonadherence. First, it is important to explore positive or beneficial aspects of nonadherence. Ask, "What's good about not taking your medicine?" or "What do you like most about smoking, or using cocaine?" After getting insight into positive factors reinforcing the behavior, segue into inquiring about "less than positive," or detrimental aspects. For patients who have strong factors perpetuating or reinforcing nonadherence, ask the patient, "What would have to happen for you to consider taking your medication, or quitting smoking?" The response can elucidate the patient's perception of her illness, and her motivation and readiness for change. Above all, avoid adversarial confrontations, which tend to degenerate into battles for control. Pressuring the patient to "comply" with treatment is not only ethically problematic, but also futile and counterproductive. Such approaches intensify the patient's defensiveness about current behavior, making it more impervious to change, or drive the patient out of treatment altogether.

Types of Psychotherapeutic, Psychosocial, and Behavioral Interventions in SLE

Patient and Family Education Regarding SLE

Patient and family education should include discussions about the nature, course, and prognosis of SLE, and rationale for and risks/benefits of treatment. Such discussions are essential to obtain informed consent, engage the patient and family as active partners in the care, and enhance the likelihood of treatment adherence. In discussing such issues, the rheumatologist can glean a sense of the patient's comprehension of the disease, and her primary concerns. Misperceptions about lupus can be corrected, often alleviating patient's and family members' anxiety. Topics such as pregnancy, and the impact of the illness on the family, should be explored. (See previous section on marriage and family relationships.) The rheumatologist can also gain insight into the patient's value system, and her priorities, so that treatment can be focused on achieving shared objectives. The patient may benefit from specific suggestions about what she can do to manage her illness and promote her own well-being. In addition, it is important to delineate the clinical symptoms and signs that warrant concern, and that should prompt her or her family to contact the rheumatologist.

Self-Efficacy and Self-Management Training

Self-efficacy programs develop the patient's confidence in her own ability to do whatever tasks are required to achieve a specific goal [217]. In clinical settings, such training can enhance the patient's ability to cope with chronic illness and its treatment.

Self-management courses, which are derived from self-efficacy theory, conceptualize the patient as central in managing her disease, rather than as a passive beneficiary of treatment. Patients receive training in problem-solving, decision-making, confidence-building, and symptom and stress management [218, 219]. In a longitudinal study of SLE patients attending a self-management program, their learned response to illness changed over time. Depressive symptoms decreased, while perceived self-efficacy increased, suggesting that these skills can be taught [220]. A 4-year follow-up trial in rheumatoid arthritis patients demonstrated that skills training is associated with improved health outcomes [221]. In a 6-month randomized, controlled study [222] of patients with various chronic diseases, including arthritis, those who received self-management training had improved health status and fewer hospitalization days than did those on a waiting list. Most benefits were sustained at longitudinal follow-up 2 years later, despite worsening physical disability, and were associated with enhanced self-efficacy in coping with their illness [223].

Psychotherapy

Psychotherapy can be of considerable benefit in helping patients cope with chronic illness, including SLE. Therapy can help patients explore how SLE has impacted them, and develop ways to adapt and move forward with their lives. Furthermore, for patients with depression and anxiety, numerous studies have demonstrated that psychotherapy in conjunction with psychotropic medication is usually more effective than medication alone. Indications for psychotherapy in SLE are summarized in Table 13. Given the complexity of neuropsychiatric aspects of SLE, the therapist or psychiatrist should maintain close communication with the treating rheumatologist, and collaborate in patient care.

For a patient with SLE, the goals of psychotherapy are to identify and enhance positive coping mechanisms that support and sustain her in spite of her illness. Since the patient is often unaware of such coping strategies, gaining insight into them, and bringing them to conscious awareness, can increase her sense of control. In addition, the therapist should help the patient identify and minimize negative or detrimental coping mechanisms, such as smoking, alcohol consumption, self-isolation, or regressing into the sick role. Although lupus can be devastating, psychotherapy can help the patient reframe it as an opportunity to reorient her priorities toward what is most important. Finally, psychotherapy can illuminate that no matter how many losses she has endured because of her lupus, she still retains the freedom to choose her attitude toward her illness [224].

TABLE 13 Indications for Considering Psychotherapy in SLE

Lack of response to adequate psychotropic medication trial in patient without significant cognitive impairment
Nonadherence to treatment or followup
Relationship issues
Physician–patient tensions
Marital discord
Domestic violence
Parenting or other family difficulties
Maladaptive reactions to illness
Pessimism or demoralization
Anxiety, hypochondriasis, or somatization
Social isolation
Dependency
Regression, e.g., functional impairment disproportionate to severity of illness
Denial
Anger, hostility
Difficulty coping with losses, or with other adverse effects of illness on daily life
Coping with terminal illness

Referrals for Psychiatric Care

Some SLE patients may be upset or angered by referrals for psychiatric care. Patients whose SLE was misdiagnosed as psychosomatic are particularly likely to resent the mental health field, and be sensitive to any implication that psychological factors may impact on their physical illness. In addition, unfortunately, many patients feel that having emotional difficulties, or merely being in psychiatric care is stigmatizing. When psychiatric care is recommended, the patient may be apprehensive that it signifies termination of treatment with the referring rheumatologist. Such concerns can be allayed by reassurance that the rheumatologist will continue to work with the patient, and that the psychiatric care is an adjunct to her lupus treatment. Patients may be more receptive to psychiatric referrals if they have received education about the psychiatric manifestations of lupus and its treatment, and the difficulty that many people have with coping with chronic illness. It is helpful to emphasize that the focus of the psychiatric treatment will be to help her achieve her goals and optimize her functioning in spite of her illness [225].

Patient Support Groups, Telephone Counseling, and Online Resources

Lupus support groups enable patients to meet with others who have experienced the illness firsthand. Many patients find that such groups ameliorate the sense of isolation and provide emotional support. Since the patients have experienced similar struggles, they can genuinely empathize and commiserate with each other, and voice their frustrations, fears, and anger. When another patient suggests a particular coping strategy, it is likely to be more readily accepted than advice from family members or physicians who, though usually well-intentioned, have never had lupus. Often, another patient who is functioning well despite severe lupus is an inspiration, and a role model for others to emulate.

Although support groups are usually beneficial, they can have some potentially detrimental aspects. For example, after encountering patients who have a more severe form of SLE, a patient may become increasingly anxious or depressed regarding her own illness. Some patients become unduly absorbed in other patient's difficulties, and leave feeling more burdened. Most importantly, when support groups are poorly led, patients may excessively dwell on their symptoms, complaints, and debility, thereby intensifying the sense of incapacitation, and reinforcing their identity in the "sick role." Poorly led groups can also degenerate into unconstructive "gripe sessions" in which patients complain about doctors, hospitals, employers, and family without assuming any responsibility themselves for change.

Peer counseling by telephone, where available, can be an additional source of emotional support for SLE patients [226]. It has the advantages of being accessible on demand, and to patients who may not be able to attend support groups. For patients with Internet access, other venues for information and support include online SLE websites, bulletin boards, and chat rooms.

Social Services

Some of patient's suffering may be due to insurance inadequacies, poverty, and other socioeconomic factors that neither medication nor psychotherapy can rectify. Social workers can be invaluable in addressing such concerns, and accessing resources and services for patients. Nevertheless, patients may be reluctant to disclose such matters due to feelings of shame, or a sense that the socioeconomic disparity between them and their physician precludes mutual understanding. Physicians tend to mistakenly attribute noncompliance to patients' failure to face up to their illness and be responsible. Lupus patients who have no stable residence will probably not benefit from discussions about the importance of adequate rest, and those who lack money for bus fare are unlikely to be receptive to being told they must come in regularly for appointments.

Rehabilitation

Physical and occupational rehabilitation are crucial in optimizing patient functioning after strokes or other sequelae of SLE that cause physical debility or involve prolonged recuperation. Rehabilitation generally focuses on helping patients regain the necessary skills for maximal autonomy. Most importantly, it is individualized and tailored to address each patient's needs, goals, and lifestyle.

Spiritual Counseling and Altruistic Endeavors

In coping with serious illness, some patients find that their spiritual beliefs are strengthened, and provide solace and fortitude. Faith in a power beyond oneself has sustained many patients through adversities that would otherwise seem unendurable. Conversely, if a patient blames God for causing her illness or failing to heal her, she may begin to challenge her previously held beliefs, or discard them entirely. Such spiritual issues can be very distressing to patients, and are often best addressed by clergy and pastoral counselors. Patients who have no spiritual or religious inclination will especially rely on their network of relatives and friends for support. Patients should be encouraged to pursue volunteer work or other altruistic endeavors that imbue their lives with meaning.

References

1. Kaposi, M. K. (1872). Neue beit rage zur Kenntnis des lupus erythematosus. *Arch. Dermatol. Syph.* **4**, 36–79.
2. West, S. G. (1994). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am.* **20**, 129–158.
3. Ainiala, H., Loukkola, J., Peltola, J., Korpela, M., and Hietaharju, A. (2001). The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. *Neurology* **57**, 496–500.
4. Brey, R. L., Holliday, S. L., Saklad, A. R., *et al.* (2002). Neuropsychiatric syndromes in lupus: Prevalence using standard definitions. *Neurology* **58**, 1214–1220.
5. Johnson, R. T., and Richardson, E. P. (1968). The neurological manifestations of systemic lupus erythematosus: A clinical–pathological study of 24 cases and review of the literature. *Medicine* **47**, 337–369.
6. Ellis, S. G., and Verity, M. A. (1979). Central nervous system involvement in systemic lupus erythematosus: A review of neuropathological findings in 57 cases, 1955–1977. *Semin. Arthritis Rheum.* **8**, 212–221.
7. Scolding, N. J., and Joseph, F. G. (2002). The neuropathology and pathogenesis of systemic lupus erythematosus. *Neuropathol. Appl. Neurobiol.* **28**, 173–189.

8. Amoura, Z., Piette, J. C., Bach, J. F., and Koutouzov, S. (1999). The key role of nucleosomes in lupus. *Arthritis Rheum.* **42**, 833–843.
9. Andrade, F., Cascioloa-Rosen, L., and Rosen, A. (2000). Apoptosis in systemic lupus erythematosus: Clinical implications. *Rheum. Dis. Clin. North Am.* **26**, 215–227.
10. Walport, M. J., Davies, K. A., and Botto, M. (1998). C1q and systemic lupus erythematosus. *Immunobiology* **199**, 265–285.
11. Wierzbicki, A. S. (2000). Lipids, cardiovascular disease and atherosclerosis in systemic lupus erythematosus. *Lupus* **9**, 194–201.
12. Gharavi, A. E. (2001). Anticardiolipin syndrome: Antiphospholipid syndrome. *Clin. Med.* **1**, 14–17.
13. Hanly, J. G. (2001). Neuropsychiatric lupus. *Curr. Rheumatol. Rep.* **3**, 205–212.
14. Svenungsson, E., Andersson, M., Brudin, L., *et al*. (2001). Increased levels of proinflammatory cytokines and nitric oxide metabolites in neuropsychiatric lupus erythematosus. *Ann. Rheum. Dis.* **60**, 372–379.
15. Bruyn, G. A. W. (1995). Controversies in lupus: Nervous system involvement. *Ann. Rheum. Dis.* **54**, 159–167.
16. Jennekens, F. G. I., and Kater, L. (2002). The central nervous system in systemic lupus erythematosus. Part 2. Pathogenic mechanisms of clinical syndromes: A literature investigation. *Rheumatology* **41**, 619–630.
17. Bonfa, E., Golombek, S. J., Kaufman, L. D., *et al*. (1987). Association between lupus psychosis and anti-ribosomal P protein antibodies. *N. Engl. J. Med.* **317**, 265–271.
18. Schneebaum, A. B., Singleton, J. D., West, S. G., *et al*. (1991). Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am. J. Med.* **90**, 54–62.
19. Watanabe, T., Sato, T., Uchiumi, T., and Arakawa, M. (1996). Neuropsychiatric manifestations in patients with systemic lupus erythematosus: Diagnostic and predictive value of longitudinal examination of anti-ribosomal P antibody. *Lupus* **5**, 178–183.
20. Arnett, F. C., Reveille, J. D., Moutsopoulos, H. M., Georgescu, L., and Elkon, K. B. (1996). Ribosomal P autoantibodies in systemic lupus erythematosus: Frequencies in different ethnic groups and clinical and immunogenetic associations. *Arthritis Rheum.* **39**, 1833–1839.
21. Georgescu, L., Mevorach, D., Arnett, F. C., Reveille, J. D., and Elkon, K. B. (1997). Anti-P antibodies and neuropsychiatric lupus erythematosus. *Ann. N. Y. Acad. Sci.* **823**, 263–269.
22. Isshi, K., and Hirohata, S. (1998). Differential roles of antiribosomal P antibody and antineuronal antibody in the pathogenesis of central nervous system involvement in systemic lupus erythematosus. *Arthritis Rheum.* **41**, 1819–1827.
23. Teh, L. S., and Isenberg, D. A. (1994). Anti-ribosomal P protein antibodies in systemic lupus erythematosus: A reappraisal. *Arthritis Rheum.* **37**, 307–315.
24. Gerli, R., Caponi, L., Tincani, A., *et al*. (2002). Clinical and serological associations of ribosomal P autoantibodies in systemic lupus erythematosus: Prospective evaluation in a large cohort of Italian patients. *Rheumatology* **41**, 1357–1366.
25. Shiozawa, S., Kuroki, Y., Kim, M., *et al*. (1992). Interferon-alpha in lupus psychosis. *Arthritis Rheum.* **35**, 417–422.
26. West, S. G., Emlen, W., Wener, M. H., and Kotzin, B. L. (1995). Neuropsychiatric lupus erythematosus: A prospective study on the value of diagnostic tests. *Am. J. Med.* **99**, 153–163.
27. Yeh, T. S., Wang, G. W., *et al*. (1994). The study of anticardiolipin antibodies and interleukin-6 in cerebrospinal fluid and blood of Chinese patients with systemic lupus erythematosus and central nervous system involvement. *Autoimmunity* **18**, 69–175.
28. Levine, J. S., Branch, D. W., and Rauch, J. (2002). The antiphospholipid syndrome. *N. Engl. J. Med.* **346**, 752–763.
29. Sabet, A., Sibbitt, W. L., Stidley, C. A., Danska, J., and Brooks, W. M. (1998). Neurometabolite markers of cerebral injury in the antiphospholipid antibody syndrome of systemic lupus erythematosus. *Stroke* **29**, 2254–2260.
30. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann. Intern. Med.* **112**, 682–698.
31. Coull, B. M., Levine, S. R., and Brey, R. L. (1992). The role of antiphospholipid antibodies in stroke. *Neurol. Clin.* **10**, 125–143.
32. Hanly, J. G., Hong, C., Smith, S., and Fisk, J. D. (1999). A prospective analysis of cognitive function and anticardiolipin antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 728–734.
33. Menon, S., *et al*. (1999). A longitudinal study of anticardiolipin antibody levels and cognitive functioning in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 735–741.
34. Chapman, J., Cohen-Armon, M., Shoenfeld, Y., and Korcyzn, A. D. (1999). Antiphospholipid antibodies permeabilize and depolarize brain synaptosomes. *Lupus* **8**, 127–133.
35. Lacki, J. K., Leszczynski, P., Keleman, J., Muller, W., and Mackiewicz, S. H. (1997). Cytokine concentration in serum of lupus erythematosus patients: The effect of the acute phase response. *J. Med.* **28**, 99–107.
36. Belmont, H. M., Buyon, J., Giorno, R., and Abramson, S. (1994). Up-regulation of endothelial cell adhesion molecules characterizes disease activity in systemic lupus erythematosus. The Schwartzman phenomenon revisited. *Arthritis Rheum.* **37**, 376–383.
37. Trysberg, E., Carlsten, H., and Tarlowski, A. (2000). Intrathecal cytokines in systemic lupus erythematosus with central nervous system involvement. *Lupus* **9**, 498–503.
38. Hirohata, S., and Miyamoto, T. (1990). Elevated levels of interleukin-6 in cerebrospinal fluid from patients with systemic lupus erythematosus and central nervous system involvement. *Arthritis Rheum.* **33**, 644–649.
39. Alcocer-Varela, J., Aleman-Hoey, D., and Alarcon-Segovia, D. (1992). Interleukin-1 and interleukin-6 activities are increased in the cerebral spinal fluid of patient

with CNS lupus erythematosus and correlate with local late T-cell activation markers. *Lupus* **33**, 644–649.
40. Kronfol, Z., and Remick, D. G. (2000). Cytokines and the brain: Implications for clinical psychiatry. *Am. J. Psychiatry* **157**, 683–694.
41. Wichers, M., and Maes, M. (2002). The psychoneuroimmuno-pathophysiology of cytokine-induced depression in humans. *Int. J. Neuropsychopharmacol.* **5**, 375–388.
42. Schaefer, M., Engelbrecht, M. A., Oliver, G., *et al*. (2002). Interferon-alpha and psychiatric syndromes: A review. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **26**, 731–746.
43. Dellalibera-Joviliano, R., Dos Reis, M. L., Cunha, F. de Q., and Donadi, E. A. (2003). Kinins and cytokines in plasma and cerebrospinal fluid of patients with neuropsychiatric lupus. *J. Rheumatol.* **30**, 485–492.
44. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1994). Corticosteroids and neuropsychological functioning in patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 1311–1320.
45. Hanly, J. G., Cassell, K., and Fisk, J. D. (1997). Cognitive function in systemic lupus erythematosus: Results of a 5-year prospective study. *Arthritis Rheum.* **40**, 1542–1543.
46. DeGiorgio, L. A., Konstantinov, K. N., Lee, S. C., *et al*. (2001). A subset of lupus anti-DNA antibodies cross-reacts with the NR2 glutamate receptor in systemic lupus erythematosus. *Nat. Med.* **7**, 1189–1193.
47. Zanardi, V. A., Magna, L. A., and Costallat, L. T. (2001). Cerebral atrophy related to corticotherapy in systemic lupus erythematosus. *Clin. Rheumatol.* **20**, 245–250.
47a. Bluestein, H. G., Williams, G. W., and Steinberg, A. D. (1981). Cerebrospinal fluid antibodies to neuronal cells: Association with neuropsychiatric manifestations of systemic lupus erythematosus. *Am. J. Med.* **70**, 240–246.
47b. Bell, C. L., Partington, C., Robbins, M., *et al*. (1991). MRI of CNS lesions in patients with lupus erythematosus: Correlation with clinical remission and antineurofilament and anticardiolipin antibody titers. *Arthritis Rheum.* **34**, 432–441.
48. Karassa, F. B., Ioannidis, J. P. A., Touloumi, G., Boki, K. A., and Moutsopoulos, H. M. (2000). Risk factors for central nervous system involvement in systemic lupus erythematosus. *Q. J. Med.* **93**, 169–174.
49. Liou, H. H., Wang, C. R., Chen, C. J., *et al*. (1996). Elevated levels of anticardiolipin antibodies and epilepsy in lupus patients. *Lupus* **5**, 307–312.
50. Herranz, M. T., Rivier, G., Khamashta, M. A., Blaser, K. U., and Hughes, G. R. (1994). Association between antiphospholipid antibodies and epilepsy in patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 568–571.
51. Verrot, D., San-Marco, M., Dravat, C., *et al*. (1997). Prevalence and significance of antinuclear and anticardiolipin antibodies in patients with epilepsy. *Am. J. Med.* **103**, 33–37.
52. Karassa, F. B., Ioannidis, J. P., Kyriaka, A., *et al*. (2000). Predictors of clinical outcome and radiologic progression in patients with neuropsychiatric manifestations of systemic lupus erythematosus. *Am. J. Med.* **109**, 628–634.
53. Nath, S. K., Kelly, J. A., Reid, J., *et al*. (2002). SLEB3 in systemic lupus erythematosus (SLE) is strongly related to SLE families ascertained through neuropsychiatric manifestations. *Hum. Genet.* **111**, 54–58.
54. Mok, C. C., and Lau, C. S. (2000). Profile of sex hormones in male patients with systemic lupus erythematosus. *Lupus* **9**, 252–257.
55. Warnatz, K., Peter, H. H., Schumacher, M., *et al*. (2003). Infectious CNS disease as a differential diagnosis in systemic rheumatic diseases: Three case reports and a review of the literature. *Ann. Rheum. Dis.* **62**, 50–57.
56. Rogers, M. P. (1983). Psychiatric aspects. In "The Clinical Management of Systemic Lupus Erythematosus" (P. H. Schur, Ed.), pp. 189–210. Grune & Stratton, New York.
57. Perry, S., and Miller, F. (1992). Psychiatric aspects of systemic lupus erythematosus. In "Systemic Lupus Erythematosus (R. Lahita, Ed.), p. 853. 2nd ed, Churchill Livingstone, New York.
58. Goldstein, M. A. (2000). "Travels with the Wolf: A Story of Chronic Illness," p. 104. Ohio State Univ. Press, Columbus.
59. Engle, E. W., Callahan, L. F., Pincus, T., and Hochberg, M. C. I. (1990). Learned helplessness in systemic lupus erythematosus: Analysis using the Rheumatology Attitudes Index. *Arthritis Rheum.* **33**, 281–286.
60. Liang, M. H., Rogers, M., Larson, M., *et al*. (1984). The psychosocial impact of systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* **27**, 13–19.
61. Wallace, D. J. (1987). The role of stress and trauma in rheumatoid arthritis and systemic lupus erythematosus. *Semine Arthritis Rheum.* **16**, 153–157.
62. Hinrichsen, H., Barth, J., Ferstl, R., and Kirch, W. (1989). Changes of immunoregulatory cells induced by acoustic stress in patients with systemic lupus erythematosus, sarcoidosis, and in healthy controls. *Eur. J. Clin. Invest.* **19**, 372–377.
63. Hinrichsen, H., Barth, J., Ruckemann, M., *et al*. (1992). Influence of prolonged neuropsychological testing on immunoregulatory cells and hormonal parameters in patients with systemic lupus erythematosus. *Rheumatol. Int.* **12**, 47–51.
64. Jacobs, R., Pawlak, C. R., Mikeska, *et al*. (2001). Systemic lupus erythematosus and rheumatoid arthritis patients differ from healthy controls in their cytokine pattern after stress exposure. *Rheumatology* **40**, 868–875.
65. Pawlak, C., Heiken, H., Witte, T., *et al*. (1999). A prospective study of daily stress and disease activity in patients with systemic lupus erythematosus. *Neuroimmunomodulation* **64**, 241.
66. Herrmann, M., Scholmerick, J., and Straub, R. H. (2000). Stress and rheumatic disease. *Rheum. Dis. Clin. North Am.* **26**, 737–763.
67. Otto, R., and Mackay, J. R., (1967). Psycho-social and emotional disturbances to systemic lupus erythematosus. *Med. J. Aust.* **2**, 488–493.
68. Blumenfield, M. (1978). Psychological aspects of systemic lupus erythematosus. *Primary Care* **5**, 159–171.
69. Ropes, M. W. (1976). "Systemic Lupus Erythematosus," p. 19. Harvard Univ. Press, Cambridge, Massachusetts.

70. Hall, R. C. W., Stickney, S. K., and Gardner, E. R. (1981). Psychiatric symptoms in patients with systemic lupus erythematosus. *Psychosomatics* **22**, 15–24.
71. Wekking, E. M., Nossent, J. C., van Dam, A. P., and Swaak, A. J. J. G. (1991). Cognitive and emotional disturbances in systemic lupus erythematosus. *Psychother. Psychosom.* **55**, 126–131.
72. Wekking, E. M. (1993). Psychiatric symptoms in systemic lupus erythematosus: An update. *Psychosom. Med.* **55**, 219–228.
73. Wallace, D. J., and Metzger, A. L. (1994). Can an earthquake cause flares of rheumatoid arthritis or lupus nephritis? *Arthritis Rheum.* **37**, 1826–1828.
74. Ward, M. M., Marx, A. S., and Barry, N. N. (2002). Psychological distress and changes in the activity of systemic lupus erythematosus. *Rheumatology* **41**, 184–188.
75. Adams, S. G., Jr., Dammers, P. M., Saia, T. L., *et al*. (1994). Stress, depression, and anxiety predict average symptom severity and daily symptom fluctuation in systemic lupus erythematosus. *J. Behav. Med.* **17**, 459–477.
76. Kahana, R. J., and Bibring, G. L. (1964). Personality types in medical management. In "Psychiatry and Medical Practice in a General Hospital" (N. Zinberg, Ed.), p. 108. International Univ. Press, New York.
77. Cohen, W. S. (2002). Late night exasperation of a nonrheumatologist.
78. Tan, E. M., Cohen, A. S., Fries, J. F., *et al*. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271–1277.
79. ACR Ad Hoc Committee on Neuropsychiatric Lupus Nomenclature (1999). The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum.* **42**, 599–608.
80. Ainiala, H., Hietaharju, A., Loukkola, J., *et al*. (2001). Validity of the American College of Rheumatology criteria for neuropsychiatric lupus syndromes: A population-based evaluation. *Arthritis Care Res.* **45**, 419–423.
81. Ruggieri, A. P. (2001). Neuropsychiatric lupus: Nomenclature, classification, criteria, and other confusional states. *Arthritis Care Res.* **45**, 406–409.
82. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1997). Psychological aspects of systemic lupus erythematosus: Cognitive function, mood, and self-report. *J. Rheumatol.* **24**, 998–1003.
83. Carbotte, R. M., Denburg, S. D., and Denburg, J. A. (1995). Cognitive dysfunction in SLE is independent of active disease. *J. Rheumatol.* **22**, 863–867.
84. Gladman, D. D., Urowitz, M. B., Slonim, D., *et al*. (2000). Evaluation of predictive factors for neurocognitive dysfunction in patients with inactive systemic lupus erythematosus. *J. Rheumatol.* **27**, 2367–2371.
85. Long, A. A., Denburg, D. S., Carbotte, R. M., *et al*. (1990). Serum lymphocytotoxic antibodies and neurocognitive function in systemic lupus erythematosus. *Ann. Rheum. Dis.* **49**, 249–253.
86. Kelly, M. C., Denburg, J. A. (1987). Cerebrospinal fluid immunoglobuling and neuronal antibodies in neuropsychiatric systemic lupus erythematosus and related conditions. *J. Rheumatol*. **14**, 740–744.
87. Hay, E. M., Black, D., Huddy, A., *et al*. (1994). A prospective study of psychiatric disorder and cognitive function in systemic lupus erythematosus. *Ann. Rheum. Dis.* **53**, 298–303.
88. Sibbitt, W. L., Jung, R. E., and Brooks, W. M. (1999). Neuropsychiatric systemic lupus erythematosus. *Comp. Ther.* **25**, 198–208.
89. Beck, A. T., Rush, A. J., Shaw, B., and Emery, G. (1979). "Cognitive Therapy of Depression." Guilford Press, New York.
90. Giang, D. W. (1991). Systemic lupus erythematosus and depression. *Neuropsych. Neuropsychol. Behav. Neurol.* **4**, 78–82.
91. Kozora, E., and Thompson, L. L. (1996). Analysis of cognitive and psychological deficits in systemic lupus erythematosus patients without overt central nervous system disease. *Arthritis Rheum.* **39**, 2035–2045.
92. Utset, T. O., Golden, M., Siberry, G., *et al*. (1994). Depressive symptoms in patients with systemic lupus erythematosus: Association with central nervous system lupus and Sjogren's syndrome. *J. Rheumatol.* **21**, 2039–2045.
93. Lim, L., Ron, M. A., and Ormerod, I. E. C. (1988). Psychiatric and neurological manifestations in systemic lupus erythematosus. *Q. J. Med.* **66**, 27–38.
94. Kremer, J. M., Rynes, R. I., Bartholomew, L. E., *et al*. (1981). Non-organic non-psychotic psychopathology (NONPP) in patients with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **11**, 182–189.
95. Hay, E. M., Black, D., Huddy, A., *et al*. (1992). Psychiatric disorder and cognitive impairment in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 411–416.
96. Shortall, E., Isenberg, D., and Newman, S. P. (1995). Factors associated with mood and mood disorders in SLE. *Lupus* **4**, 272–279.
97. Nollet, D., Herreman, G., Piette, J. C., *et al*. (1985). Psychic disorders in systemic lupus erythematosus. Prospective study of 35 cases. *Presse Med.* **14**, 401–404.
98. Purandare, K. N., Wagle, A. C., and Parker, S. R. (1999). Psychiatric morbidity in patients with systemic lupus erythematosus. *Q. J. Med.* **92**, 283–286.
99. Cavanaugh, S., Clark, D. C., and Gibbons, R. D. (1983). Diagnosing depression in the hospitalized medically ill. *Psychosomatics* **24**, 809–815.
100. Pyne, D., and Isenberg, D. A. (2002). Autoimmune thyroid disease in systemic lupus erythematosus. *Ann. Rheum. Dis.* **61**, 70–72.
101. Omdal, R., Waterloo, K., Koldingsnes, W., Husby, G., and Mellgren, S. I. (2001). Somatic and psychological features of headache in systemic lupus erythematosis. *J. Rheumatol.* **28**, 772–779.
102. Robb-Nicholson, L. C., Daltroy, L., Eaton, H., *et al*. (1989). Effects of aerobic conditioning in lupus fatigue: A pilot study. *Br. J. Rheumatol.* **28**, 500–505.
103. Trepacz, P., Breitbart, W., Franklin, J., Levenson, J., *et al*. (1999). American Psychiatric Association practice guide-

lines for the treatment of delirium. *Am. J. Psychiatry* **156**, 1–20.
104. Mesulam, M.-M. (1986). Frontal cortex and behavior. *Ann. Neurol.* **19**, 320–324.
105. Mesulam, M.-M. (2000). "Principles of Behavioral and Cognitive Neurology," 2nd ed., p. 384. Oxford Univ. Press, Oxford.
106. Sibley, J. T., Olszynski, W. P., Decoteau, W. E., and Sundaram, M. B. (1992). The incidence and prognosis of central nervous system disease in systemic lupus erythematosus. *J. Rheumatol.* **19**, 47–52.
107. Hugo, F. J., and Halland, A. M. (1996). DSM-III-R classification of psychiatric symptoms in systemic lupus erythematosus. *Psychosomatics* **37**, 262–269.
108. Mok, C. C., Lau, C. S., and Wong, R. W. (2001). Neuropsychiatric manifestations and their clinical associations in southern Chinese patients with systemic lupus erythematosus. *J. Rheumatol.* **28**, 766–771.
109. Cade, R., Spooner, G., Schlein, E., *et al.* (1973). Comparison of azathioprine, prednisone, and heparin alone or combined in treated lupus nephritis. *Nephron* **10**, 37–56.
110. Boston Collaborative Drug Surveillance Program (1972). Acute adverse reactions to prednisone in relation to dosage. *Clin. Pharmacol. Ther.* **13**, 694–698.
111. Lewis, D. A., and Smith, R. E. (1983). Steroid-induced psychiatric syndromes: A report of 14 cases and a review of the literature. *J. Affect. Disord.* **5**, 319–332.
112. Brown, E. S., Suppes, T., Kahn, D. A., and Carmody, T. J. (2002). Mood changes during prednisone bursts in outpatients with asthma. *J. Clin. Psychopharmacol.* **22**, 55–61.
113. Ling, M. H. M., Perry, P. J., and Tsuang, M. T. (1981). Side effects of corticosteroid therapy: Psychiatric aspects. *Arch. Gen. Psychiatry* **38**, 471–477.
114. Glazer, G. H. (1952). Lesions of the central nervous system in disseminated systemic lupus erythematosus. *Arch. Neurol.* **67**, 7–45.
115. Wada, K., Yamada, N., Sato, T., *et al.* (2001). Corticosteroid-induced psychotic and mood disorders: Diagnosis defined by DSM IV and clinical picture. *Psychosomatics* **42**, 461–466.
116. Perry, P. J., Tsuang, M. T., and Hwang, M. H. (1984). Prednisolone psychosis: Clinical observations. *Drug Intell Clin. Pharmacol.* **18**, 603–608.
117. Hall, R. C. W., Popkin, M. K., Stickney, S. K., *et al.* (1979). Presentation of the steroid psychoses. *J. Nerv. Mental Disord.* **167**, 229–236.
118. Sharfstein, S. S., Sack, D. S., and Fauci, A. S. (1982). Relationship between alternate-day corticosteroid therapy and behavioral abnormalities. *JAMA* **248**, 2987–2989.
119. Brown, E. S., and Suppes, T. (1998). Mood symptoms during corticosteroid therapy: A review. *Harvard Rev. Psychiatry* **5**, 239–246.
120. Wolkowitz, O. W. (1990). Cognitive effects of corticosteroids. *Am. J. Psychiatry* **147**, 1297–1303.
121. Varney, N. R. (1984). Reversible steroid dementias in patients without steroid psychosis. *Am. J. Psychiatry* **141**, 369–372.
122. Baker, M. (1973). Psychopathology in systemic lupus erythematosus. 1. Psychiatric observations. *Semin. Arthritis Rheum.* **3**, 95–110.
123. Perry, S., and Miller, F. (1992). Psychiatric aspects of systemic lupus erythematosus. In "Systemic Lupus Erythematosus" (R. Lahita, Ed.), 2nd ed., pp. 851–852. Churchill Livingstone, New York.
124. Kohen, M., Asheron, R. A., Gharavi, A. E., and Lahita, R. G. (1993). Lupus psychosis: Differentiation from the steroid-induced state. *Clin. Exp. Rheumatol.* **11**, 323–326.
125. McCune, W. J. (1988). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am.* **14**, 149–167.
126. Sutor, B., Wells, L. A., and Rummans, T. A. (1996). Steroid-induced depressive psychosis responsive to electroconvulsive therapy. *Convuls. Ther.* **12**, 104–107.
127. Falk, W. E., Mahnke, M. W., and Peskanzer, D. C. (1979). Lithium prophylaxis of corticotrophin-induced psychosis. *JAMA* **241**, 1011–1012.
128. Axelrod, L. (1995). Corticosteroid therapy. In "Principles and Practice of Endocrinology and Metabolism" (K. L. Becker, Ed.), pp. 695–706. Lippincott, Philadelphia.
129. Wolkowitz, O. M., and Rapaport, M. (1989). Long-lasting behavioral changes following prednisone withdrawal. *JAMA* **261**, 1731–1732.
130. Some drugs that cause psychiatric symptoms. (1998). *Med. Lett.* **40**, 21–24.
131. Levenson, J. L., and Dwight, M. (2000). Cardiology. In "Psychiatric Care of the Medical Patient" (A. Stoudemire, B. S. Fogel, and D. B. Greenberg, Eds.), 2nd ed., pp. 725–726. Oxford Univ. Press, New York.
132. Schneider, R. K., and Levenson, J. L. (2000). Infectious disease syndromes. In "Psychiatric Care of the Medical Patient" (A. Stoudemire, B. S. Fogel, and D. B. Greenberg, Eds.), 2nd ed., p. 867. Oxford Univ. Press, New York.
133. Moran, M. G. (2000). Connective tissue diseases. In "Psychiatric Care of the Medical Patient" (A. Stoudemire, B. S. Fogel, and D. B. Greenberg, Eds.), 2nd ed., pp. 897–898. Oxford Univ. Press, New York.
134. House, R. M. (2000). Transplantation surgery. In "Psychiatric Care of the Medical Patient" (A. Stoudemire, B. S. Fogel, and D. B. Greenberg, Eds.), 2nd ed., p. 1058. Oxford Univ. Press, New York.
135. Steinlin, M. I., Blaser, S. I., Gilday, D. L., *et al.* (1995). Neurologic manifestations of systemic lupus erythematosus. *Pediatr. Neurol.* **13**, 191–197.
136. Quintero-Del-Rio, A., and Miller, V. (2000). Neurologic symptoms in children with systemic lupus erythematosus. *J. Child Neurol.* **15**, 803–807.
137. Yancey, C. L., Doughty, R. A., and Athreya, B. H. (1981). Central nervous system involvement in childhood systemic lupus erythematosus. *Arthritis Rheum.* **24**, 1389–1395.
138. Lee, T., von Scheven, E., and Sandborg, C. (2001). Systemic lupus erythematosus and antiphospholipid syndrome in children and adolescents. *Curr. Opin. Rheumatol.* **13**, 415–421.
139. Sibbitt, W. L., Brandt, J. R., Johnson, C. R., *et al.* (2002). The incidence and prevalence of neuropsychiatric syn-

dromes in pediatric onset systemic lupus erythematosus. *J. Rheumatol.* **29**, 1536–1542.
140. Itoh, Y., Hamada, H., Imai, T., *et al*. (1997). Antinuclear antibodies in children with chronic nonspecific complaints. *Autoimmunity* **25**, 243–250.
141. Nashel, D., and Ulmer, C. (1982). Systemic lupus erythematosus. Important considerations in the adolescent. *J. Adolesc. Health Care* **2**, 273–278.
142. Silber, T. J., Chatoor, I., and White, P. H. (1984). Psychiatric manifestations of systemic lupus erythematosus in children and adolescents: A review. *Clin. Pediatr.* **23**, 331–335.
143. Tucker, L. B. (1998). Caring for the adolescent with systemic lupus erythematosus. *Adolesc. Med.* **9**, 59–67.
144. Turkel, S. B., Miller, J. H., and Reiff, A. (2001). Case series: Neuropsychiatric symptoms with pediatric systemic lupus erythematosus. *J. Acad. Child Adolesc. Psychiatry* **40**, 482–485.
145. Hofmann, A. D. (1975). The impact of illness in adolescence and coping behavior. *Acta Paediatr. Scand. Suppl* **256**, 29–33.
146. Ruiz-Irastorza, G., Khamashta, M. A., Castellino, G., and Hughes, G. R. V. (2001). Systemic lupus erythematosus. *Lancet* **357**, 1027–1032.
147. Ruiz-Irastorza, G., Lima, F., Alves, J., *et al*. (1996). Increased rate of lupus flare during pregnancy and the puerperium: A prospective study of 78 pregnancies. *Br. J. Rheumatol.* **35**, 133–138.
148. Mok, C. C., and Wong, R. W. (2001). Pregnancy in systemic lupus erythematosus. *Postgrad. Med. J.* **77**, 157–165.
149. Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). Mini-mental state: A practical method for grading the cognitive status of patients for the clinician. *J. Psychiatr. Res.* **12**, 189–198.
150. Breitbach, S. A., Alexander, R. W., Daltroy, L. H., *et al*. (1998). Determinants of cognitive performance in systemic lupus erythematosus. *J. Clin. Exp. Neuropsychol.* **20**, 157–166.
151. Waterloo, K., Omdal, R., Husby, G., and Mellgren, S. I. (2002). Neuropsychological function in systemic lupus erythematosus: A five-year longitudinal study. *Rheumatology* **41**, 411–415.
152. Tzioufas, A. G., Tzortzakis, N. G., Panou-Pomonis, E., *et al*. (2000). The clinical relevance of antibodies to ribosomal-P common epitope in two targeted systemic lupus erythematosus populations: A large cohort of consecutive patients and patients with active central nervous system disease. *Ann. Rheum. Dis.* **59**, 99–104.
153. Portela, L. V., Brenol, J. C., Walz, R., *et al*. (2002). Serum S100B levels in patients with lupus erythematosus: Preliminary observation. *Clin. Diagnostic Lab. Immunol.* **9**, 164–166.
154. Waterloo, K., Omdal, R., Jacobsen, E. A., Klow, N. E., Husby, G., Torbergsen, T., and Mellgren, S. L. (1999). Cerebral computed tomography and electroencephalography compared with neuropsychological findings in systemic lupus erythematosus. *J. Neurol.* **246**, 706–711.
155. Sibbitt, W. L., Sibbitt, R. R., and Brooks, W. M. (1999). Neuroimaging in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* **42**, 2026–2038.
156. Sanna, G., Piga, M., Terryberry, J. W., *et al*. (2000). Central nervous system involvement in systemic lupus erythematosus: Cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. *Lupus* **9**, 573–583.
157. Provenzale, J. M., Barboriak, D. P., Allen, N. B., and Ortel, T. L. (1996). Patients with antiphospholipid autoantibodies: CT and MR findings of the brain. *Am. J. Roentgenol.* **167**, 1573–1578.
158. McCune, W. J., MacGuire, A., and Aisen, A. (1988). Identification of brain lesions in neuropsychiatric lupus erythematosus by magnetic resonance scanning. *Arthritis Rheum.* **31**, 159–166.
159. Jarek, M. J., West, S. G., Baker, M. R., *et al*. (1994). Magnetic resonance imaging in systemic lupus erythematosus patients without a history of neuropsychiatric lupus erythematosus. *Arthritis Rheum.* **37**, 1609–1613.
160. Sabbadini, M. G., Manfredi, A. A., Bozzolo, E., Ferrario, L., Rugarli, C., *et al*. (1999). Central nervous system involvement in systemic lupus erythematosus patients without overt neuropsychiatric manifestations. *Lupus* **8**, 1–2.
161. Sibbitt, W. L., Brooks, W. M., Haseler, L. J., *et al*. (1994). Spin–spin relaxation of brain tissues in systemic lupus erythematosus. A method for increasing the sensitivity of magnetic resonance imaging for neuropsychiatric lupus. *Arthritis Rheum.* **38**, 810–818.
162. Nomura, K., Yamano, S., Ikeda, Y., *et al*. (1999). Asymptomatic cerebrovascular lesions detected by magnetic resonance imaging in patients with systemic lupus erythematosus lacking a history of neuropsychiatric events. *Intern. Med.* **38**, 785–795.
163. Petropoulos, H., Sibbitt, V. L., and Brooks, V. I. M. (1999). Automated T(2) quantitation in neuropsychiatric lupus erythematosus: A marker of active disease. *Magn. Reson. Imaging* **9**, 39–43.
164. Bosma, G. P. T. H., Rood, M. J., Zwinderman, A. H., *et al*. (2000). Evidence of central nervous system damage in patients with neuropsychiatric systemic lupus erythematosus, demonstrated by magnetization transfer imaging. *Arthritis Rheum.* **43**, 48–54.
165. Bosma, G. P. T. H., Rood, M. J., Huizinga, T. J. W., *et al*. (2000). Detection of cerebral involvement in patients with active neuropsychiatric systemic lupus erythematosus by the use of volumetric magnetization transfer imaging. *Arthritis Rheum.* **43**, 2428–2436.
166. Bosma, G. P., Middelkoop, H. A., Rood, M. J., Bollen, E. L., Huizinga, T. W., and van Buchem, M. A. (2002). Association of global brain damage and clinical functioning in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* **46**, 2665–2672.
167. Sibbitt, W. L., Jr., Haseler, L. J., Griffey, R. R., Friedman, S. D., and Brooks, W. M. (1997). Neurometabolism of active neuropsychiatric lupus determined with proton MR spectroscopy. *Am. J. Neuroradiol.* **18**, 1271–1277.
168. Axford, J. S., Howe, F. A., Heron, C., and Griffiths, J. R. (2001). Sensitivity of quantitative (1)H magnetic

resonance spectroscopy of the brain in detecting early neuronal damage in systemic lupus erythematosus. *Ann. Rheum. Dis.* **60**, 106–111.
169. Huang, W. S., Chiu, P. Y., Tsai, C. H., Kao, A., and Lee, C. C. (2002). Objective evidence of abnormal regional cerebral blood flow in patients with systemic lupus erythematosus on Tc-99m ECD brain SPECT. *Rheumatol. Int.* **22**, 178–181.
170. Chen, J. J., Yen, R. F., Kao, A., Lin, C. C., and Lee, C. C. (2002). Abnormal regional cerebral blood flow found by technetium-99m ethyl cysteinate dimer brain single photon emission computed tomography in systemic lupus erythematosus patients with normal brain MRI findings. *Clin. Rheumatol.* **21**, 516–519.
171. Waterloo, K., Omdal, R., Sjoholm, H., *et al*. (2001). Neuropsychological dysfunction in systemic lupus erythematosus is not associated with changes in cerebral blood flow. *J. Neurol.* **248**, 595–602.
172. Huizinga, T. W. J., Steens, S. C. A., and van Buchem, M. A. (2001). Imaging modalities in central nervous system systemic lupus erythematosus. *Curr. Opin. Rheumatol.* **13**, 383–388.
173. Barned, S., Goodman, A. D., and Mattson, D. H. (1995). Frequency of anti-nuclear antibodies in multiple sclerosis. *Neurology* **45**, 384–385.
174. Collard, R. C., Koehler, R. P., and Mattson, D. H. (1997). Frequency and significance of antinuclear antibodies in multiple sclerosis. *Neurology* **49**, 857–861.
175. Gullapalli, D., and Phillips, L. H. (2002). Neurologic manifestations of sarcoidosis. *Neurol. Clin.* **20**, 59–83.
176. Nadeau, S. E. (2002). Neurologic manifestations of connective tissue disease. *Neurol. Clin.* **20**, 151–178.
177. Jennekens, F. G. I., and Kater, L. (1999). "Neurology of Inflammatory Connective Tissue Disease," p. 4. Saunders, Philadelphia.
178. Canuso, R. T., de Oliveira, R. M., and Nixon, R. A. (1990). Neuroleptic-associated autoantibodies: A prevalence study. *Biological Psychiatry* **27**, 863–870.
179. Yannitsi, S. G., Manoussakis, M. N., Mavridis, A. K., *et al*. (1990). Factors related to the presence of autoantibodies in patients with chronic mental disorders. *Biological Psychiatry* **27**, 747–756.
180. Drory, V. E., and Korczyn, A. D. (1993). Hypersensitivity vasculitis and systemic lupus erythematosus induced by anticonvulsants. *Clin. Neuropharmacol.* **16**, 19–29.
181. Toepfer, M., Sitter, T., Lochmuller, H., Pongratz, D., and Muller-Felber, W. (1998). Drug-induced systemic lupus erythematosus after 8 years of treatment with carbamazepine. *Eur. J. Clin. Pharmacol.* **54**, 193–194.
182. Jain, K. K. (1991). Systemic lupus erythematosus (SLE)-like syndromes associated with carbamazepine therapy. *Drug Safety* **6**, 350–360.
183. Bleck, T. P., and Smith, M. C. (1990). Possible induction of systemic lupus erythematosus by valproate. *Epilepsia* **31**, 343–345.
184. Stratton, M. A. (1985). Drug-induced systemic lupus erythematosus. *Clin. Pharmacol*. **4**, 657–663.
185. Tlacuilo-Parra, J. A., Guevara-Gutierrez, E., and Garcia-De La Torre, I. (2000). Factitious disorders mimicking systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **18**, 89–93.
186. Harris, E. C., and Barraclough, B. M. (1994). Suicide as an outcome for medical disorders. *Medicine* **73**, 281–286.
187. Karassa, F. B., Magliano, M., and Isenberg, D. A. (2003). Suicide attempts in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **62**, 58–60.
188. Matsukawa, Y., Sawada, S., Hayama, T., *et al*. (1994). Suicide in patients with SLE: A clinical analysis of seven suicidal patients. *Lupus* **3**, 31–35.
189. Futrell, N., Schultz, L. R., and Millikan, C. (1992). Central nervous system disease in patients with systemic lupus erythematosus. *Neurology* **42**, 1649–1657.
190. Navarette, M. G., and Brey, R. L. (2000). Neuropsychiatric systemic lupus erythematosus. *Curr. Treat. Options Neurol.* **2**, 473–485.
191. Baca, V., Lavalle, C., Garcia, R., *et al*. (1999). Favorable response to intravenous methylprednisolone and cyclophosphamide in children with severe neuropsychiatric lupus. *J. Rheumatol.* **26**, 432–439.
192. Boumpas, D. T., *et al*. (1991). Pulse cyclophosphamide for severe neuropsychiatric lupus. *Q. J. Med.* **81**, 975–984.
193. Ramos, P. C., Mendez, M. J., Ames, P. R., Khamashta, M. A., and Hughes, G. R. (1996). Pulse cyclophosphamide in the treatment of neuropsychiatric systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **14**, 295–299.
194. Trevisani, V. F., Castro, A. A., Neves Neto, J. F., and Atallah, A. N. (2002). Cyclophosphamide versus methylprednisolone for treating neuropsychiatric involvement in systemic lupus erythematosus (Cochrane Review). *In the Cochrane Library, Issue 3*.
195. Khamashta, M. A., Cuadrado, M. J., Mujic, F., *et al*. (1995). The management of thrombosis in the antiphospholipid-antibody syndrome. *N. Engl. J. Med.* **332**, 993–997.
196. Arana, G. W., and Rosenbaum, J. (2000). "Handbook of Psychiatric Drug Therapy," 4th ed. Lippincott Williams & Wilkins, Philadelphia.
197. Labatte, L., Arana, G. W., and Rosenbaum, J. (2002). "Psychiatric Drug Therapy for PDA." Lippincott Williams & Wilkins, Philadelphia.
198. Stahl, S. M. (2002). "Essential Psychopharmacology of Antipsychotics and Mood Stabilizers." Cambridge Univ. Press, London.
199. Aranda-Michel, J., Koehler, A., Bejarano, P. A., *et al*. (1999). Nefazodone-induced liver failure: Report of three cases. *Ann. Intern. Med.* **130**, 285–288.
200. Van Battum, P. L., van de Vrie, W., Metselaar, H. J., *et al*. (2000). Acute liver failure ascribed to nefazodone: Importance of "postmarketing surveillance" for recently introduced drugs. *Ned. Tijdschr. Geneedskd.* **144**, 1964–1967.
201. Sayal, K. S., Duncan-McConnell, D. A., McConnell, H. W., and Taylor, M. D. (2000). Psychotropic drug interactions with warfarin. *Acta Psychiatr. Scand.* **102**, 250–255.
202. Masand, P. S., and Tesar, G. E. (1996). Use of stimulants in the medically ill. *Psychiatr. Clin. North Am.* **19**, 515–547.

203. Terao, T., Mizuki, T., Ohji, T., *et al*. (1994). Antidepressant effect of lithium in patients with systemic lupus erythematosus and cerebral infarction treated with corticosteroid. *Br. J. Psychiatry* **164**, 109–111.
204. Kahn, D., Stevenson, E., and Douglas, C. J. (1988). Effect of sodium valproate in three patients with organic brain syndrome. *Am. J. Psychiatry* **145**, 1010–1011.
205. Hagg, S., Spigset, O., Bate, A., and Soderstrom, T. G. (2001). Myocarditis related to clozapine treatment. *J. Clin. Psychopharmacol.* **21**, 382–388.
206. Coulter, D. M., Bate, A., Meyboom, R. H., Lindquist, M., and Edwards, I. R. (2001). Antipsychotic drugs and heart muscle disorder in international pharmacovigilance: Data mining study. *Br. Med. J.* **322**, 1207–1209.
207. Hagg, S., Spigset, O., and Soderstrom, T. G. (2000). Association of venous thromboembolism and clozapine. *Lancet* **355**, 1155–1156.
208. Wise, M. G., and Terrell, C. D., as cited in Rundell, J. R., and Wise, M. G. (2000). "Concise Guide to Consultation Psychiatry," 3rd ed., p. 40. American Psychiatric Press, Washington, D.C.
209. Malur, C., Pasol, E., and Francis, A. (2001). ECT for prolonged catatonia. *J. ECT* **17**, 55–59.
210. Squire, L. R. (1986). Memory functions as affected by electroconvulsive therapy. *Ann. N. Y. Acad. Sci.* **462**, 307–314.
211. Calev, A., Gaudino, E. A., Squires, N. K., Zervas, I. M., and Fink, M. (1995). ECT and non-memory cognition: A review. *Br. J. Clin. Psychol.* **34**, 505–515.
212. Weiner, R. D. (1995). Electroconvulsive therapy. In "Treatments of Psychiatric Disorders" (G. O. Gabbard, Ed.), 2nd ed., pp. 1237–1262. American Psychiatric Press, Washington, D.C.
213. Coleman, E. A., Sackeim, H. A., Prudic, J., Devanand, D. P., McElhiney, M. C., and Moody, B. J. (1996). Subjective memory complaints prior to and following electroconvulsive therapy. *Biological Psychiatry* **39**, 346–356.
214. Meichenbaum, D., and Turk, D. C. (1987). "Facilitating Treatment Adherence: A Practitioner's Guidebook," pp. 30, 41–68, 94, and 236. Plenum, New York.
215. Drotar, D. (2000). "Promoting Adherence to Medical Treatment in Chronic Childhood Illness: Concepts, Methods, and Interventions," pp. 78–93 and 119. Lawrence Erlbaum Associates, Mahwah, New Jersey.
216. Miller, W. R., and Rollnick, S. (2002). "Motivational Interviewing, 2nd Edition: Preparing People for Change." Guilford Press, New York.
217. Bandura, A. (1997). "Self-Efficacy: The Exercise of Control." Freeman, San Francisco.
218. Lorig, K. R., Holman, H. R., Sobel, D. S., *et al*. (1993). "Living a Healthy Life with Chronic Conditions." Bull Publishing, Palo Alto, California.
219. Lorig, K. R., Sobel, D. S., Ritter, P. L., Laurent, D., and Hobbs, M. (2001). Effect of a self-management program on patients with chronic disease. *Effect. Clin. Pract.* **4**, 256–262.
220. Braden, C. J. (1991). Patterns of change over time in learned response to chronic illness among participants in a systemic lupus erythematosus self-help course. *Arthritis Care Res.* **4**, 158–167.
221. Lorig, K. R., Mazonson, P. D., and Holman, H. R. (1993). Evidence suggesting that health education for self-management in patients with chronic arthritis has sustained health benefits while reducing health care costs. *Arthritis Rheum.* **36**, 439–446.
222. Lorig, K. R., Sobel, D. S., Stewart, A. L., *et al*. (1999). Evidence suggesting that a chronic disease self-management program can improve health status while reducing hospitalization: A randomized trial. *Med. Care* **37**, 5–14.
223. Lorig, K. R., Ritter, P., Stewart, A. L., *et al*. (2001). Chronic disease self-management program: 2-year health status and health care utilization outcomes. *Med. Care* **39**, 1217–1223.
224. Frankl, V. E. (1984). "Man's Search for Meaning: An Introduction to Logotherapy," 3rd ed. Simon & Schuster, New York.
225. Bursztajn, H., and Barsky, A. J. (1985). Facilitating patient acceptance of a psychiatric referral. *Arch. Intern. Med.* **145**, 73–75.
226. Horton, R., Peterson, M. G., Powell, S., Engelhard, E., and Paget, S. A. (1997). Users evaluate LupusLine, a telephone peer counseling service. *Arthritis Care Res.* **10**, 257–263.

28

COGNITIVE DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

Susan D. Denburg
Lynne Larocque
Judah A. Denburg

OVERVIEW

Considerable progress has been made in understanding cognitive function in various rheumatic diseases, and principally in systemic lupus erythematosus (SLE) [1]. The systematic study of cognitive function in patients diagnosed with SLE began in the 1980s [2–6]; these early studies stimulated considerable interest and investigation internationally which continues to the present time [7–20]. Several groups of investigators have adapted methods for, and implemented the assessment of, cognitive function in cohorts of lupus patients, finding almost invariably that lupus is attended by significant impairment in a number of cognitive domains, even in the absence of overt neuropsychiatric manifestations. Although estimates of prevalence of cognitive impairment have varied, presumably a reflection of differences in patient selection, test batteries, criteria for impairment, and control groups, there is a fair degree of consensus that cognitive function may provide a "window" on the nervous system in SLE, where processes related to lupus itself may interfere with normal higher cortical function and lead to abnormalities in cognition and behavior. Neuropsychiatric SLE has been the subject of several interdisciplinary meetings including a special symposium of the New York Academy of Sciences [21], an ad hoc workshop on nomenclature sponsored by the American College of Rheumatology (ACR) [22], and a workshop convened by the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) at the National Institutes of Health in May, 1999. In all these fora, the diagnosis and interpretation of cognitive impairment has been the focus of considerable attention, in the context of discussions about the diagnosis and classification of neuropsychiatric SLE.

This chapter briefly reviews neuropsychological methodology, and examines the data from numerous studies regarding the prevalence of cognitive deficit in SLE patients. The effects of corticosteroid treatment, psychological distress, or non CNS (central nervous system) complications that might account for the high prevalence of cognitive deficit in SLE are discussed. We review existing theories of the pathogenesis of nervous system involvement in SLE and attempt to relate potential pathogenetic mechanisms such as brain cross-reactive antibodies and cytokines to specific kinds of cognitive/behavioral alterations. Finally, we consider the clinical implications of documenting cognitive deficit. Because this chapter focuses on changes in mental functioning and their underlying basis, there is necessarily some overlap with other chapters in this volume dealing with neuropsychiatric complications in SLE.

DIAGNOSIS AND CLASSIFICATION OF NERVOUS SYSTEM INVOLVEMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

Cognitive function is of interest in SLE primarily because of the high prevalence of nervous system involvement in that disease [23–29]. The documented sensitivity of neuropsychological procedures to subclinical disturbance of CNS integrity in a variety of clinical populations [30] suggests that neuropsychological studies of SLE patients have the potential to contribute substantially both to the evaluation of the more subtle symptoms and complaints of these patients, and to a redefinition of the criteria for diagnosing CNS involvement. Such involvement, frequently termed neuropsychiatric (NP), is typically diagnosed on the basis of clinical neurologic or psychiatric events and syndromes such as seizures, neuropathies, hemiparesis, organic brain syndrome, psychosis, and depression. While these symptoms and signs often represent fairly clear-cut clinical syndromes, patients with SLE experience a wide variety of "softer" neurologic and psychiatric problems including paresthesias, headaches, anxiety, mood swings, and reported difficulties in concentration, memory, and word-finding. Estimates of the prevalence of NP involvement vary widely, ranging from 14 to 75%, reflecting variable diagnostic methodologies and criteria [23–30].

A significant difficulty in establishing diagnostic criteria for NP-SLE is the absence of an acceptable gold standard against which these criteria can be validated; that is, while neurologic, psychiatric and cognitive events occur in the context of SLE, there is little evidence that they are *caused* by SLE. Nevertheless, in an attempt to standardize diagnosis for research purposes, we initially proposed a system for classification of NP symptomatology in SLE [4, 31] based on modification and extension of previous clinical classifications [32–36]. Neuropsychiatric manifestations not clearly attributable to causes other than the disease process itself were divided into "major" or "minor" signs or symptoms, with "focal" or "diffuse" presentations. Subsequently, a consensus process for standardizing the classification of NP-SLE [37] showed a level of agreement among raters regarding items important in the diagnosis of NP-SLE, which increased substantially following clarification of terminology and discussion of clinical scenarios. This process has been refined as part of an ACR-sponsored workshop organized for the purpose of developing a standardized nomenclature system for the neuropsychiatric syndromes of NP-SLE [22]. Through these consensus processes, cognitive dysfunction has been given further definition and clinical importance, both as an independent NP-SLE syndrome and in association with other manifestations of NP-SLE [38], where it may co-occur with the event itself or persist as residual brain compromise. Because of the ubiquity of cognitive dysfunction in SLE, assessment of cognitive function can serve as a standardized approach to assessing the functional integrity of the brain in the context of very diverse clinical NP presentations and even in their absence. Neuropsychological or neurocognitive assessment can also represent a distinct approach to diagnosis and classification of CNS involvement and may eventually facilitate the identification of distinct pathogenetic mechanisms within the broad category of NP-SLE.

The description of functional problems of the nervous system, specifically in the areas of cognition and emotion, has developed as a major research thrust in SLE. To facilitate an understanding of these studies, a *primer* on neuropsychological assessment will be provided.

NEUROPSYCHOLOGICAL ASSESSMENT

Background and General Considerations

Clinical neuropsychology is an approach to studying the functional integrity of the central nervous system. An individual's performance is evaluated on a variety of tests which have been found to be sensitive to the effects of well identified brain lesions, and for which there are prior normative data from healthy individuals, without neurologic, psychiatric, or systemic medical problems [39, 40].

Neuropsychological assessment is not a monolithic entity. Diverse approaches reflect different theoretical frameworks. Some consist of a fixed battery of measures and very specific criteria for identifying impaired CNS function [41, 42]. Others adopt a more eclectic and flexible approach [43], may incorporate procedures from the experimental neuropsychological literature, and tend to include analysis of a patient's test-taking strategies (termed qualitative assessment), because of their diagnostic and rehabilitative implications. The majority of studies of cognitive function in SLE have used an eclectic approach to test selection although, for the most part, there has been limited attention paid to patient test-taking strategies. The *common theme* in all three approaches is that a wide range of functions is assessed, including attention, memory, language, concept formation, visuospatial abilities, executive skills, and motor skills. In addition, there are specific, standardized rules for administering and scoring the tests, and results are analyzed with reference to expected level of performance expressed as normative data, or as an estimate of the individual's premorbid or best level of competence. All approaches in wide use have been validated

in both research and clinical settings with respect to their utility in identifying the presence of disturbed brain function.

Sensitivity of Neuropsychological Assessment

In the general neuropsychological literature, correlations between neuropsychological assessment and findings from brain imaging technology, both structural (e.g., computed tomography (CT), magnetic resonance imaging (MRI)), and functional (e.g., positron emission tomography (PET), quantified electroencephalogram (QEEG), single photon emission computed tomography (SPECT), functional MRI (fMRI)), have been demonstrated [44–47]. Neuropsychological data can be highly sensitive in identifying subtle sequelae (i.e., not readily evident clinically) of CNS events such as stroke or head injury, and in monitoring response to drug treatment [48]. Depending on the tests chosen and the use of appropriate control subjects, these approaches have long been known to be sensitive to the presence of CNS dysfunction in a variety of medical conditions in which no structural lesions are evident, such as solvent or lead neurotoxicity [49], metabolic disorders [50], and anoxia [51]. In these conditions, significant impairments in functional integrity of the brain are indicated by performance deficits which cannot be accounted for by such confounding factors as the presence of significant psychological disturbance. Cognitive measures have also proved to be sensitive to the subtle sequelae of relatively minor head trauma, with neuropsychological deficits correlating with the results from MRI and SPECT, sometimes in the face of negative findings on CT scan [44, 47].

In the case of SLE, both structural and functional brain imaging techniques have been shown to be sensitive to changes associated with clinically defined NP syndromes [52–62]. Some techniques have also been sensitive to changes that may reflect subclinical brain involvement [52, 60, 63–65]. Those studies that have compared neuroimaging approaches with neuropsychological assessment have yielded variable results. For example, we reported a study of three SLE patients with well-defined neurologic syndromes, using PET and neuropsychological assessment at two points in time [66] and found good concordance between data derived from these two methodologies, underscoring the utility of neuropsychological data for delineating the behavioral problems associated with CNS lesions imaged by structural or functional scanning in SLE. One study of cognitive function using a brief screening tool identified a pattern of cognitive function reflective of subcortical pathology [67], consistent with previous imaging studies in SLE [68, 69]. Results of proton magnetic resonance spectroscopic imaging have been found to correlate with brain function assessed via neurocognitive testing [70]. In contrast, abnormalities on routine investigative methods such as CT and conventional EEG were generally not correlated with neuropsychological findings in a sample of NP and non-NP-SLE patients [71], nor were MRI abnormalities associated with neurocognitive findings in patients without overt CNS disease [72]. It is important to note that brain imaging techniques, whether in SLE or in other diseases, cannot directly assess the extent of behavioral/cognitive consequence that may accompany a brain lesion; these may be clinically significant even in the face of minimal findings on neuroimaging.

Goals of Neuropsychological Assessment

Neuropsychological assessment provides a detailed description of a person's cognitive and emotional strengths and weaknesses. As such, it can provide unique and complementary information on which to base decisions regarding diagnosis and treatment. Figure 1 depicts the multiple reasons for undertaking neuropsychological assessment, each of which addresses different but often interrelated clinical needs. Although *diagnosis* of brain involvement is often the focus of a referral question, behavioral evaluation remains very important even in cases in which the lesion is well characterized. This can include possible changes in emotions, since these too are frequently altered by brain dysfunction. Debate exists as to whether these alterations represent a reaction to the loss of skills and competence, or constitute a direct expression of an altered neural

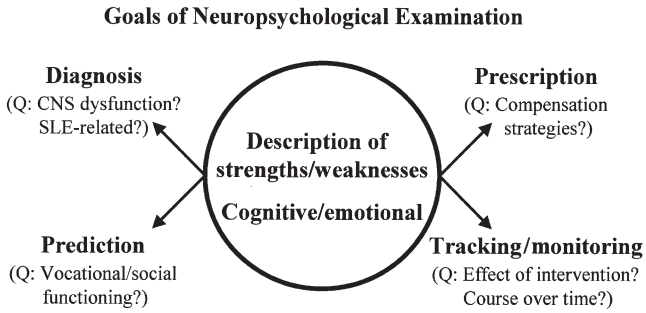


FIGURE 1 Neuropsychological assessment provides a detailed description of a person's strengths and weaknesses. This description facilitates diagnosis, predicts daily functioning, allows the physician to prescribe compensatory or rehabilitative strategies to counteract deficits, and tracks cognitive function over time. Such an evaluation not only monitors the course of disease but can also assess the effects of treatment. (From the *Journal of Musculoskeletal Medicine* **16**, 356, 1999, with permission.)

substrate, as is the case in poststroke depression [73, 74], or disturbances in mood regulation after frontal lesions [75, 76]. An understanding of the resulting alterations in the patient's functional capacity allows the clinician to make *predictions* about daily functioning, although it should be noted that the ecological validity of neuropsychological assessment (i.e., the relationship between deficits elicited during formal assessment and "real life" abilities) is imperfect and requires more study [77–82]. It also allows the clinician to *prescribe* compensatory or rehabilitative strategies to counteract deficits. All of this is necessary to help the patient and family or caregivers anticipate and cope with the functional changes [83–85]. Neuropsychological assessment, by virtue of being standardized, repeatable, and quantifiable, is also a valuable tool for *tracking* the course of disease-related brain function over time as well as the effects of any treatment intervention.

Finally, it is important to note that patients with SLE frequently complain of fluctuating or deteriorating cognitive efficiency. It is recognized that there is often limited concordance between subjective report and objective assessments of cognitive function (e.g., [86]); and further, that perception and report of poor cognitive function can be influenced by depressed mood or anxiety [87, 88]. Nevertheless, the opportunity to confirm or disconfirm a patient's complaints using objective measures that have a certain degree of "face validity" (i.e., they manifestly tap functions about which the patients frequently complain, such as attention, concentration, memory, word-finding, spatial orientation) can have considerable relevance in the clinical management of SLE patients.

NEUROPSYCHOLOGICAL STUDIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Methodology

Early studies of cognitive function in SLE used selected groups, typically referred for evaluation because of suspected nervous system problems; as such, they were probably not representative of the SLE population as a whole. Accordingly, in our early studies we set out to examine consecutive SLE patients, irrespective of NP presentation, using a comprehensive neuropsychological test battery assessing a wide range of functions subserved by diverse areas of the brain [39, 40]. Given the clinical heterogeneity of NP presentations in SLE patients, clinically significant deficits in individual patients may not correspond to the areas of statistically significant differences identified on group comparisons of SLE patients and controls. For this reason, we undertook in our initial research studies to *adapt and standardize the clinical approach* to identifying neuropsychological deficit in individual patients. Over time, this approach has proved to be more sensitive and fruitful in studying cognitive problems both in SLE and in other diseases with CNS involvement than have group comparisons of patients and controls [4, 5, 89, 90]. The principles underlying this approach to deficit specification are now widely adhered to, although the details may vary across different study centers.

The establishment of consistent and objective criteria for identifying impairment in individual patients required the standardization of the decision rules used in the clinical setting. The details of this approach have been reported previously [4, 5]. Briefly, it consisted of administering a fairly detailed test battery to SLE patients, medical controls (rheumatoid arthritis (RA)), and community controls, converting raw scores to z-scores for ready combination and comparison, grouping scores according to a face valid analysis of the processes involved in the various tests (factor analysis was precluded at this early stage due to limited patient numbers), estimating a premorbid (best) level of functioning for each individual, and comparing performance on each of the 17 cognitive summary variables against this "best estimate." A discrepancy greater than 2 standard deviations (SD) defined a given score as impaired; this modified criterion for identifying impairment was justified by the finding of highly significant correlations in normal individuals between premorbid estimates and many of the summary variables [5]. The test profile as a whole was designated as significantly impaired (quantitatively) if 3 or more of these 17 summary scores were impaired. This *criterion for overall impairment*, reflecting a moderate extent of intertest variability, was *established empirically*; that is, it yielded the best discrimination between our 35 normal controls and the first 21 SLE patients with diagnosed active NP symptomatology at the time of testing. With this quantitative criterion, 6% (2/35) of the protocols from our normal controls reflected significant impairment, which compared very well with the 9% prevalence of impairment in a much larger *control* group of young men in a multicenter study of polydrug abusers evaluated with the Halstead-Reitan battery and its associated decision criteria for impairment [91].

In accordance with the "process approach" to assessment, we also developed a criterion for designating test protocols as *qualitatively* impaired. This involved the examiner rating each patient's test-taking behavior with respect to the degree of cognitive control, using such indicators as confabulation, perseveration, and rule-breaking. Protocols with a rating of 5 or 6 on a scale of 0 to 6 were designated as showing significant qualitative impairment. Our criteria were sufficiently specific that high interrater reliability was achieved for

these judgements [5]. This qualitative approach was not adopted in other centers, and so was subsequently eliminated from analyses to facilitate data comparison with other centers [1]. Nevertheless, it yielded interesting and converging data, which will be reviewed briefly later, and contributed to the utility of the assessment as a tool in clinical management.

Prevalence of Cognitive Impairment in SLE

Studies of cognitive function in SLE have yielded overall prevalence estimates ranging from 17 to 59% for *quantitatively* defined cognitive dysfunction (see Table 1) [4, 6–8, 11, 12, 19, 20, 67, 92–98]. These studies have involved fairly diverse study samples in terms of the proportion of patients with active or inactive NP involvement (i.e., current or previous NP event), which may reflect, in part, different referral patterns to the clinics from which patients have been drawn. They have also used very different sets of neuropsychological tests, measuring different aspects of cognitive functioning. Most importantly, they have adopted different criteria for defining impairment; for example, whether current performance was compared with a premorbid level of function and/or with normative data and whether the sensitivity and specificity of the selected cutoff was addressed in relation to an appropriate control group varied considerably across studies. Nevertheless, despite the widely divergent prevalence estimates, there appears to be a consensus that even in the absence of overt NP symptoms, a sizable proportion of SLE patients can be shown objectively to have significant cognitive problems.

The importance of careful attention to the sensitivity and specificity of a test battery, and the value of converging data regarding cognitive impairment, can be illustrated by reference to findings from our own laboratory. In our initial study of cognitive function in SLE [4], 55% of a sample of 62 clinic patients was categorized as cognitively impaired on quantitative criteria alone, with 67% of the active/inactive NP groups, 38% of the never NP group, and 6% of the community controls so designated. When either quantitative or qualitative criteria were used for evaluating the test protocols, over 80% of patients with active NP symptomatology (active NP-SLE) showed significant impairment, compared to 14% of the normal controls. This corresponded to a sensitivity of .81 and a specificity of .86 for the procedures (described previously) used as a diagnostic test for identifying CNS dysfunction. These values were as good as, if not better than, those obtained with other procedures that were being routinely used at that time for diagnosing ongoing NP involvement [99–101]. Following this first published report of cognitive function in SLE, we made a minor change to the

TABLE 1 Estimates of the Prevalence of Cognitive Impairment in SLE

		Prevalence of cognitive impairment (%)			
References	**Act/inact: never**	**Active/ inactive**	**Never**	**Overall**	**Healthy controls**
Carbotte *et al.* (1986) [4]	36:26	67	38	55	6
Koffler (1987) [6]	16:29	88	14	40	0
Kutner *et al.* (1988) [11]	8:14	75	50	59	na[a]
Papero *et al.* (1990) [19]	2:19	?[b]	?	43	na
Wekking *et al.* (1991) [92]	9:11	55	54	55	na
Denburg *et al.* (1992) [20]	74:95	54	35	43	6
Hanly *et al.* (1992) [7]	15:55	27	20	21	4
Hay *et al.* (1992) [8]	13:53	62	17	26	na
Kozora *et al.* (1996) [12]	0:51	na	29	29	11
Sailer *et al.* (1997) [98]	20:15	40	13	29	?
Breitbach *et al.* (1998) [93]	25:32	?	?	30	17
Carlomagno *et al.* (1999) [94]	27:24	37	21	29	na
Sabbadini *et al.* (1999) [96]	56:101	43	45	44	na
Gladman *et al.* (2000) [95]	35:23	49	35	43	19
Leritz *et al.* (2000) [67]	0:93	na	17[c]	17[c]	na
Monastero *et al.* (2001) [97]	23:52	52	27	35	na

[a] na, group not included.
[b] ?, information not available.
[c] Based on MMSE cutoff of ≤24.

test battery which resulted in an increased rate of impairment in the healthy controls, using the original criterion of three or more impaired summary scores. A new criterion of four or more impaired scores, adopted to maintain a 6% rate of impairment in the community controls, was then applied to the data from 169 SLE patients, including 95 classified as never NP [20]. Figure 2 depicts the percentage of patients and controls impaired according to this more conservative cutoff. It also depicts the percentage of patients and controls categorized as showing impaired performance on the Cognitive Laterality Battery (CLB), an experimental battery with a large normative database and consisting of eight subtests measuring specific verbal and visuospatial functions [102]. A criterion of two or more scores greater than 1.64 SD below normative values (without consideration of premorbid levels) resulted in a 7% impairment rate in healthy controls on the CLB; this same criterion yielded 33% impairment in the never NP group and 59% in the active/inactive NP group [103]. Figure 2 highlights the strong *convergence of data* regarding the prevalence of cognitive impairment in SLE obtained using different test batteries, administered to somewhat different patient samples, using different criteria for defining impairment, while maintaining an acceptably low rate of impairment in healthy controls.

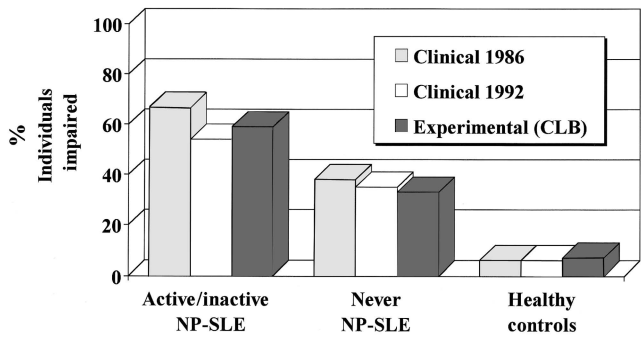


FIGURE 2 A comparison of impairment rates in SLE patient subgroups and healthy controls using different test batteries and different impairment criteria. Clinical 1986: Clinical neuropsychological battery; active/inactive NP-SLE ($N = 36$), never NP-SLE ($N = 26$), healthy controls ($N = 35$). Impairment is defined as 3 or more scores >2 SD below estimated premorbid score [4]. Clinical 1992: Clinical neuropsychological battery; active/inactive NP-SLE ($N = 74$), never NP-SLE ($N = 95$), healthy controls ($N = 35$). Impairment is defined as 4 or more scores >2 SD below estimated premorbid score [20]. Experimental (CLB): Cognitive laterality battery [102], 1994; active/inactive NP-SLE ($N = 28$), never NP-SLE ($N = 12$), healthy controls ($N = 29$). Impairment is defined as 2 or more scores >1.64 SD below normative data (without consideration of premorbid levels) [103]. (From Denburg *et al. Ann. N.Y. Acad. Sci.* **823**; 44, 1997, with permission.)

Another source of converging evidence for impaired cognition in SLE can be found in our early data obtained using a *qualitative* rating, designed to capture aspects of test-taking behavior that were considered pathonogmonic of brain lesions (e.g., rule-breaking, perseveration) [40]. The data from the qualitative assessment alone yielded a gradient of impairment which paralleled the quantitative estimates of impairment in the active/inactive and never NP-SLE groups and healthy controls. (As with the quantitative estimates, the qualitative results for the active and inactive NP groups could not be differentiated and were combined.) Thus, 55% of the active/inactive NP, 17% of the never NP, and 11% of the controls received a "severe" (impaired) rating; conversely, only 5% of the active/inactive NP group, in contrast to 34% of the never NP group and fully 54% of controls, were rated as showing "none-to-mild" qualitative problems. Interestingly, the majority (51%) of the never NP group was rated as showing "moderate" degrees of qualitative impairment. While this was not rated as impaired performance, it represented a fair degree of compromise in "cognitive control." Qualitative analysis was discontinued to facilitate cross-study comparison and because of its time-consuming nature. Nevertheless, such an approach, which has the advantage of providing converging data within patients, may warrant reconsideration as a means of more fully describing the cognitive dysfunction documented in SLE.

Types of Cognitive Deficit in SLE

Studies of cognitive functioning in SLE have yielded data regarding the prevalence and/or type of cognitive deficit in this patient population. Examination of these different studies, which have adopted very different approaches to deficit specification, suggests that the cognitive deficits shown by SLE patients are fairly wide-ranging [5], in keeping with the heterogeneity evident in their clinical NP presentation and consistent with the likelihood of more than one pathogenetic mechanism underlying CNS involvement [23–26]. This diversity in type of cognitive problem demonstrated in SLE patients as a whole, is also evident within the NP and non NP subgroups. Preliminary data from early unpublished studies of cognitive function in selected groups of SLE patients referred for suspected nervous system involvement [2, 3], suggested group deficits in motor speed, new learning, and concentration, in keeping with the subjective reports of many SLE patients. Problems that have subsequently been identified in more representative patient samples include attention and concentration, various aspects of verbal and nonverbal memory including working memory, verbal fluency/

productivity, visuospatial skills, psychomotor speed, and cognitive flexibility [5, 7, 8, 10, 12–20, 92, 97, 98, 104–107]. Table 2 represents a schematic summary of the types of cognitive deficit identified in the majority of studies where these were noted. Deficits were grouped according to areas of function following the factor analytic model proposed by Ryan *et al.* [108] and adopted in our own studies [109]. When we examined the types of cognitive problems that occurred significantly more frequently in SLE patients than in healthy controls, we were struck by the commonalities that existed in deficit areas across patient groups, *irrespective of major NP involvement*. Thus, even never NP patients were demonstrating significant levels of deficit, ranging from 25 to 39%, in such areas as visuospatial skills, verbal and nonverbal memory, and verbal fluency [110]. It is interesting to note that a study using a highly abbreviated cognitive screening tool identified a *pattern* of cognitive function consistent with subcortical dysfunction, even in SLE patients whose overall performance did not fall in the deficient range [67].

Course of Cognitive Function in SLE

One of the striking findings in early studies of cognitive function in SLE, besides the significant impairment in almost 40% of never NP patients, was the high prevalence (74%) of cognitive impairment (quantitatively defined) in *inactive* NP-SLE patients, whose NP symptomatology had been quiescent for at least 4 weeks (and more typically for several years) at the time of testing [4]. The degree of impairment was as severe for these patients as for those with active NP symptoms, with an average of greater than four cognitive summary scores impaired in both groups. While cognitive functioning does appear to improve clinically over time in many individuals who have had an NP event, it appeared that significant *residual* cognitive compromise was being documented in these patients.

Prospective longitudinal data on the natural history of cognitive deficits in SLE are, at present, limited. Two groups of investigators have reported fluctuating cognitive impairment within patients over 1- [9], 2- [111], and 5- [112] year follow-up periods. This has been interpreted to suggest that cognitive dysfunction is not a significant factor in SLE, either as a manifestation of current nervous system involvement, or as a harbinger of future involvement. The conclusion that cognitive impairment can be explained on the basis of coexisting psychiatric disorder [111] is not in keeping with the findings of most cognitive studies, either in SLE or in other medical disorders with proposed neuroimmune pathogenesis, such as acquired immune deficiency syndrome (AIDS) [113] or multiple sclerosis (MS) [114, 115]. Methodological problems further complicate these conclusions: for example, relatively brief follow-up periods, insufficient attention to anticipated test–retest gains, the possible role of steroids in cognitive improvement,

TABLE 2 Areas of Cognitive Impairment in SLE:[a] An Overview

References	General intelligence[b]	Verbal learning and memory[b]	Visuospatial skills (including memory)[b]	Psychomotor speed and manual dexterity[b]	Attention and mental flexibility[b]
Denburg *et al.* (1987) [5]	+[c]	+	+	+	+
Denburg *et al.* (1992) [20]	+	+	+	+	–[d]
Ginsburg *et al.* (1992) [10]	–	–	+	–	+
Hanly *et al.* (1992) [7]	–	+	+	+	+
Hay *et al.* (1992) [8]	–	–	+	–	+
Kozora *et al.* (1996) [12]	+	+	–	–	+
Glanz *et al.* (1997) [105]	–	+	+	+	–
Holliday *et al.* (1997) [17]	+	–	–	+	+
Sailer *et al.* (1997) [98]	–	+	+	+	+
Waterloo *et al.* (1997) [16]	+	+	+	+	+
Skeel and Johnstone (2000) [106]	–	–	–	–	+
Ainiala *et al.* (2001) [107]	–	+	+	+	+
Monastero *et al.* (2001) [97]	na[e]	+	+	–	–

[a] Criteria for impaired cognitive area differed across studies.
[b] Cognitive groupings and their labels were adopted from Ryan *et al.* [108].
[c] +, cognitive area impaired.
[d] –, cognitive area not impaired.
[e] na, cognitive area not assessed.

and relatively large patient attrition. Nevertheless, it is important to consider that NP-SLE and its associated cognitive problems may represent a range of reversible and nonreversible brain-related abnormalities, as has been observed in MS [116]. Just as there are well-documented fluctuations in other systemic manifestations, serological measures, and overall disease activity index scores in SLE, cognitive function need not follow an inexorable downhill course in order to serve as an index of current CNS status, and possibly, in some instances, as a risk factor for CNS deterioration. Moreover, it is possible that even seemingly reversible or fluctuating cognitive dysfunction can eventuate in irreversible cognitive compromise in the long term.

We continue to follow patients in a longitudinal study examining both the course of cognitive function over time and the contribution of various parameters, such as specific autoantibody positivity, to cognitive decline, and to the emergence of major NP events. Data on the first 47 originally never NP patients are depicted in Table 3; 35 of these patients retained their never NP status (Group 1: "never-to-never" NP), while 12 had an intervening NP event during the approximately 4-year follow-up interval (Group 2: "never-to-active/inactive" NP). The Group 1 NP patients showed a small overall improvement at Time 2 ($\Delta = +.24$) that fell within 1 SD of the mean control group change ($+.17 \pm .21$); the Group 2 NP patients showed an overall decline ($\Delta = -.20$) that was almost 2 SD below the control group change. We also examined significant change within individuals, defined using the criterion of greater than 1.64 SD beyond the mean change of a group of 11 healthy controls. The patterns of changed performance of the two groups and of individuals within the groups showed only a trend toward significance, reflecting the variability *within* groups, irrespective of NP event. Most interesting, however, is the finding that the Group 2 patients, whose members were originally categorized as never NP-SLE, showed significantly poorer cognitive performance at baseline, *prior* to any overt NP event ($t = 2.46$, $p = .02$). This suggests an hypothesis that cognitive dysfunction, however subtle, might be a harbinger of more serious NP events in SLE.

Interpretation of Cognitive Impairment

In the neuropsychological evaluation of medical patients, impaired test performance, rather than being a reflection of the disease process itself, could potentially result from confounding factors such as the adverse side effects of medication, emotional distress, or simply the "nonspecific" effects of medical illness and/or constitutional symptoms at the time of testing. The following sections address the issue of whether any of these factors likely contributes significantly to the presence of cognitive impairment. The bulk of the evidence so far indicates that their contribution is not substantial, suggesting that the cognitive impairment documented in SLE may, instead, constitute an autoimmune/inflammatory, SLE-related CNS dysfunction.

Corticosteroids

Corticosteroids are regularly prescribed for many of the complications of SLE. However, various sources have suggested that corticosteroid therapy could plausibly contribute to the presence of cognitive impairment in SLE patients; these include the documented association between corticosteroids and psychosis [117, 118], the significant improvement in cognitive functioning that has been reported on discontinuation of steroid therapy in individuals with diseases other than SLE

TABLE 3 Longitudinal Cognitive Data in Originally Never NP-SLE

	Performance change				
	Individual data[a]			Group data[b]	
NP group	↑[c]	=[d]	↓[e]	Time 1	Time 2 – Time 1
Group 1 (never-to-never; $N = 35$)	29%	51%	20%	.01 ± .76	.24 ± .64
Group 2 (never-to-active/inactive; $N = 12$)	8%	42%	50%	–.46 ± .49	–.20 ± .81

[a] Significant change is defined as >1.64 from mean change of controls ($N = 11$).
[b] Considered in relation to the average control group change (Time 2 – Time 1) of +.17 ± .21; data are expressed as z-scores.
[c] ↑, improved performance.
[d] =, no change in performance.
[e] ↓, decline in performance.

[119], the cognitive deficits that have been described following acute administration of corticosteroids in experimental animals and humans (reviewed in [120]), and the site-preferential effects of corticosteroids on hippocampal neurons [121–123].

In our original data, we found no significant association between being on or off steroids at the time of testing and being designated as cognitively impaired (25/35 vs 16/28; $\chi^2 = 1.18$, n.s.) [4]. Moreover, none of the correlations between steroid dosage and the 17 cognitive summary variables was significantly negative, as would be expected if steroids adversely affected performance [4]. In fact, the one significant correlation indicated better retrieval of information from remote memory, with increasing steroid dosage. Only one study to date has documented an association between cognitive impairment and steroid use in SLE patients that was independent of generalized disease activity [7]; corticosteroid use did not correlate with changes in cognitive function over a 1-year follow-up period in this same cohort of patients [9]. A significant association has been reported between pulse methylprednisone and chronic cognitive dysfunction [124] and between steroid dose or use and isolated tests of auditory attention [16]. Nevertheless, the vast majority of studies have found no significant association between cognitive impairment and either steroid therapy per se [2, 4, 8, 10, 16, 19, 71, 94–97, 112, 125] or steroid dosage [2, 4, 7, 12, 17, 71, 94, 96]. A similar conclusion has been reached with respect to the possible effects of corticosteroids on the memory impairment documented in patients with MS [126].

On the basis of retrospective data that suggested improved cognitive performance in association with steroid use [127], we undertook a series of single patient drug/placebo trials, using measures of cognitive function, mood, and disease-related symptomatology as end points. Using relatively low-dose steroids (0.5 mg/kg) in patients without active NP-SLE but with cognitive complaints, we documented significant gains in these different domains in five of the eight patients who completed the 6-month trial, no change in two patients, and significant decline in one patient with a fixed neurologic lesion [128]. Of particular note is the fact that the specific memory problems that have been associated with high-dose corticosteroid use in healthy volunteers (i.e., errors of commission) [129] were not noted to be elevated in these patients. *Variable response to steroid medication may reflect a variable neural substrate, with positive drug effects occurring in the context of underlying inflammation.* In support of this, we have found that immunosuppression with cyclophosphamide in an animal model of NP-SLE attenuates leukocyte infiltration in the brain and reduces levels of autoantibodies, while improving behavioral abnormalities [130, 131].

Physical Illness

The contribution of chronic disease and/or constitutional symptoms to cognitive impairment has been addressed in virtually all studies of cognitive function in SLE, initially through the use of such disease activity indices as the Lupus Activity Criteria Count (LACC) [132] and more recently the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [133]. It should be noted that we found a high level of concordance between the data obtained using these two indices of disease activity in a random sample of 20 SLE patients [134]. The majority of studies to date have demonstrated no association between cognitive dysfunction and disease activity, irrespective of the index of disease activity chosen [7, 8, 10, 12, 14, 16, 17, 71, 94, 97, 98], particularly when NP symptoms are eliminated from the calculation of the activity index, and when patients with active NP involvement are eliminated from the study sample so as to focus on the presence of active systemic disease apart from the brain [134]. In addition to the absence of a significant association between an overall designation of cognitive impairment and disease activity, we reported neither an increased risk of impaired performance in any particular area of cognitive function in the presence of active disease nor a significant association between cognitive impairment and the involvement of any specific organ system, including renal involvement [134]. Such observations make it unlikely that nonspecific inflammatory mechanisms (e.g., cytokines released during renal or joint involvement) are sufficient causes of cognitive impairment in SLE; these may, however, play a role in other behavioral abnormalities such as depression (see later), and thus, interact with brain-specific immune mechanisms in the development of cognitive dysfunction in SLE. It remains to be seen whether potentially more sensitive indices of disease status, such as subjective measures of pain or fatigue [135], may correlate with cognitive dysfunction in SLE. It is important to consider, however, that these parameters may not only represent potential interfering effects of systemic disease on cognition but may also reflect aberrant CNS processes and hence might correlate with subclinical nervous system compromise, as reflected in cognitive dysfunction [136].

Psychological Distress

Emotional or psychological distress, whether as a component of a clinical psychiatric syndrome or in its absence, occurs commonly in chronic rheumatologic disorders [136–138], and has been documented in a significant proportion of SLE patients, independent of

clinical psychiatric or neurologic syndromes [4, 12, 92, 139–141]. Because clinically significant psychological distress is often accompanied by significant performance deficits [38, 40, 142–144], the contribution of psychological distress to the cognitive impairment documented in SLE has been addressed in a number of studies. It is important to point out that psychological distress, which may include depressed and/or anxious mood and which is typically assessed using self-report inventories, must be differentiated from clinical psychiatric syndromes. The relationship of each to cognitive function should not be equated; failure to draw this distinction can lead to an oversimplification of the findings in SLE. While cognitive problems frequently accompany psychiatric disorders, the attempt to explain cognitive deficits in SLE on the basis of coexisting psychiatric disorders [145] is not consistent with findings from most of the studies in the area (see later).

In our original studies, we assessed emotional status with the Minnesota Multiphasic Personality Inventory (MMPI) because of its demonstrated sensitivity to the presence of generalized psychological disturbance, despite it being well established that it is not useful for assigning psychiatric diagnoses [146]. Our initial criterion for significant distress was the presence of two or more significant elevations (above a T-score of 70) on the clinical scales, excluding those scales which are characterological in their interpretation and relatively insensitive to the presence of acute distress [4]. We subsequently [5] eliminated two additional scales which contain many items reflecting somatic concerns, because of the strong likelihood that physically ill patients might show elevations simply by reporting their physical status accurately, leading to an overestimation of the prevalence of emotional disturbance (cf [147]). Using a criterion of two or more elevations on the remaining six clinical MMPI scales, we found significant distress in 34% (24/70) of SLE patients relative to 9% of nonmedical controls. The association between the presence of significant distress and cognitive impairment was not statistically significant; the frequency of significant impairment was as great in nondistressed (27/46) as in distressed (17/24) patients. Moreover, when only those patients with distress and/or impairment were considered, 67% (34/51) of these women were distressed *or* impaired but *not both*. Of the three nonmedical controls whose responses suggested significant distress, only one also showed cognitive impairment [4]. These findings strongly suggested that emotional distress was unlikely, by itself, to be a predictor of cognitive impairment and that cognitive distress was not a necessary response to the experience of being cognitively impaired.

With a few exceptions [97, 148], the majority of cognitive studies in SLE that have examined emotional status have found no significant association between cognitive dysfunction and psychological distress [5, 17, 94, 95, 98, 112, 125, 149], consistent with findings in other medical disorders with major neurologic involvement, such as AIDS [113] and MS [90, 114]. Interestingly, although one study documented equivalent rates of cognitive impairment in the SLE and RA patient samples [12], the SLE patients were rated as significantly more distressed than were the RA patients (42 vs 9%), making it particularly difficult to attribute the findings of cognitive impairment to distress. Comparing SLE patients to chronic whiplash patients reporting subjective cognitive complaints, Waterloo *et al*. documented better cognitive performance in the whiplash patients despite higher scores on measures of mood and depression, again suggesting that chronic disease and depression cannot account for cognitive dysfunction [16].

Rather than considering the effects of depressed mood or clinical depression on cognitive function in SLE, and given the strong likelihood of finding cognitive deficits in patients diagnosed with clinical depression [142–144], it might be possible to use neuropsychological methodology to address the question of whether depression that occurs in the context of SLE is, at least in part, reflecting primary brain involvement in SLE, rather than or in addition to the effects of chronic illness [138] or a coincidental depression, given the demographics of the SLE population (i.e., young women in their childbearing years) [150].

Support for the ideas that depression reflects primary CNS involvement in SLE comes from studies relating its occurrence to specific autoantibodies with potential pathogenetic significance, such as anti-ribosomal P antibodies [151] or to neurologic events [152]. Consistent with this view, it should be noted that in our original studies, the patients with major psychiatric symptomatology, whether active or inactive at the time of testing, showed the same degree of cognitive impairment as those with major neurologic symptomatology. When these groups were compared directly on both the original test scores and the summary scores, there was only one significant difference; given the large number of comparisons, this likely represented a chance finding. These studies are contrasted with those that attribute depression and other psychiatric manifestations in SLE largely to social stressors and to the effects of chronic illness [8, 111, 139, 153, 154]. This latter view has been supported by findings that depression appears to occur no more commonly in SLE than in RA [140, 154] or in hospitalized patients with diverse medical conditions [155].

It is possible that examination of the quality of depression in SLE, rather than its prevalence, might yield clues with respect to underlying mechanism. For

this reason, we undertook a pilot study of 11 SLE patients with clinical depression, 7 nondepressed SLE patients, and 8 depressed psychiatric outpatients. No differences have been found between the two depressed groups with regard to symptom endorsements on several self-report symptom inventories, suggesting that the clinical presentation of depression in the two groups may be indistinguishable. In contrast, the depressed SLE patients performed significantly more poorly than the depressed and/or nondepressed control groups on measures of sustained mental effort, verbal and nonverbal learning, and visuospatial planning [156]. These data highlight the potential value of neuropsychological approaches in studying the nature and etiology of depression, which constitutes a fairly common NP manifestation in SLE. Based on these pilot data, we are currently examining cognitive function in SLE patients with and without depression and in nonmedical depressed controls to assess the differences in areas of deficit, antibody and cytokine profiles, and treatment responsiveness.

PATHOGENESIS OF COGNITIVE AND BEHAVIORAL ABNORMALITIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

The type of cognitive deficit demonstrated by SLE patients has been noted to be wide-ranging and variable according to the numerous studies cited earlier. Attempts to relate cognitive deficit to underlying pathogenetic mechanisms have been made, with varying success. Before these are discussed (see Neuropsychological Correlates of Specific Anti-Brain Antibodies), a review of potential mechanisms will be presented. It should be noted that procedures which would allow us to define more homogeneous groups of SLE patients (e.g., those with deficits in particular cognitive areas), should prove more fruitful for exploring immunopathogenetic mechanisms underlying disturbed brain function; these may include the effects of both brain-reactive autoantibodies and/or dysregulated proinflammatory cytokines on the neural substrate.

Common Lymphocyte/Brain Antigens

The biological underpinnings of a relationship between autoimmunity, as manifested clinically in SLE, and nervous system disease rest historically on observations of antigens common to the lymphoid and nervous systems [157–161]. The notion has been advanced that lymphocyte antigens, shared with brain tissue, become targets of the autoimmune response in SLE, thus leading to a "cross-reaction" of the immune response with elements of the nervous system; such autoantibody-mediated neuronal dysfunction or cytotoxicity could lead to subclinical or clinical neuropsychiatric disease, including cognitive dysfunction [31, 100, 162–167]. Whether or not antibodies directed against common lymphocyte/brain antigens can account for more than a limited number of clinical NP-SLE manifestations/syndromes remains controversial [26, 56, 168–170]. Indeed, because of advancing knowledge on the existence of multiple—and thus probably, noncausal—autoantigen-autoantibody reactions, and even of several, alternative (nonantibody) mechanisms which may be operative in SLE (see later), the preeminence of cross-reactive lymphocyte-brain autoantibodies in the pathogenesis of NP-SLE, and its attendant cognitive and behavioral problems, has receded more recently.

Nonetheless, there is a rationale for the presence of lymphocyte/brain antigens; some of these are shared with microbial antigens [171], while others, such as thymic-brain antigens [157], may be involved in disturbed neuroimmunoregulation [172], since helper T cells bear on their surface the antigenic marker CD4, a specific receptor for human immunodeficiency virus (HIV) [173, 174]; this CD4 is also present on cells from the brain which HIV can enter [175, 176], so that antibodies to CD4 may provide an explanation for some aspects of neuropsychiatric disease, including NP-SLE. In fact, the model of HIV-induced autoimmunity may explain the development of many autoantibody-directed reactions in SLE [177–179]. Likewise, the suppressor T lymphocyte marker molecule, CD8, has been identified on myelin-producing oligodendrocytes [180], providing a possible explanation for involvement of T suppressor cells in the demyelinating autoimmune disorder, MS [181]. Given that T suppressor cell lymphopenia and lymphocytotoxic antibodies are common in SLE [163, 164, 172, 182], it can be proposed that some cases of NP-SLE, including neurocognitive disorder, may be due to T cell/brain cross-reactive autoimmune responses, resulting in neuronal or glial cell loss, demyelination, and/or inflammation in the brain.

Bacterial and Other Nonprotein Antigens

Numerous other candidate bacterial antigens which are also present in brain, while not in and of themselves lymphocyte/brain antigens, have been proposed as providing explanations for cross-reactive autoimmune responses which may be directed against the nervous system in SLE [171]. An interesting class of nonprotein antigens which may be present on formed elements of the blood (including lymphocytes), as well as in brain, includes phospholipids. Anti-phospholipid antibodies represent the targeting by the immune response of a

very important antigenic family in SLE, presumably leading to vasculopathy, thrombosis, and/or infarction with focal neuropsychiatric deficits (extensively reviewed as "the anti-phospholipid antibody or lupus anticoagulant syndrome") [183–186]. Some of these antibodies may be directed at endothelial antigens and thus putatively eventuate in vasculopathy [187], leading to cognitive dysfunction (see later).

Specific Antigens on Neuroglia and Lymphocytes

Several potential lymphocyte/brain antigens have been studied by immunoprecipitation techniques [165, 188–192]. Many SLE patients have significant elevations of autoantibodies which bind to neuroblastoma cells [192, 193] or which are lymphocytotoxic *in vitro* [165, 166, 182, 190]; the nature of the surface antigens against which IgG class antibodies are directed in sera and in cerebrospinal fluid of SLE patients has been explored [31, 191–194]. Neuronal antigens identified include 50-kDa and 97-kDa proteins on the surface of neuroblastoma cells [191, 192, 195]. There has been an interesting report of a specific brain autoantigen (LBA-1) identified through cloning and expression of a unique sequence present in mouse (MRL) brain, and distributed in the hippocampus, hypothalamus, and cingulate gyrus [196]. Whether this antigen is specifically and causally involved in the development of behavioral abnormalities in this mouse model remains to be determined; autoimmune targeting of the LBA-1 antigen as a sufficient cause of NP-SLE needs to be proved. This subject has been reviewed extensively [197, 198].

In addition, certain surface proteins on lymphocytes, either from the peripheral blood or on CD4+ lymphocytic cell lines, have been shown to be targeted by a large majority of lupus sera with autolymphocytotoxic activity [165]. Both a 52- to 55-kDa moiety and a 32- to 34-kDa moiety can be shown, by immunoprecipitation and radiolabeling, to be present on the surface of helper T cells and be bound by autoantibodies present in these sera [191]. Moreover, these reactivities correlate positively with the presence of NP-SLE and/or cognitive impairment, suggesting (but not proving) a causal role in certain individuals with SLE [199]. The possibility that these antigens are specifically expressed in certain areas of the central nervous system has been raised on the basis of the patterns of serology and cognitive dysfunction observed [167, 199] (and see later).

Several other antigens have been proposed as potentially involved in the pathogenesis of NP-SLE, based on correlative studies of serology with imaging in SLE patients [200]: these include the gliotypic antigen, GFAP (glial fibrillary acidic protein), and the neutrophil serine protease, PR-3 (c-ANCA). Confirmation of the roles of these antigens in NP-SLE awaits larger, prospective studies.

Brain Cross-Reactive Antigens in Other Diseases

More cogency for the argument of antigenic localization and autoantibody-mediated disease comes from elegant studies done by Posner and Furneaux [201] on the pathogenesis of paraneoplastic syndromes. Both 62- and 34-kDa proteins, termed Yo, are precipitated by an autoantibody present in serum and CSF of patients with subacute cerebellar degeneration syndrome associated primarily with gynecological carcinomas [201]. The specificity of this antibody for Purkinje cells in the cerebellum, and antigen extractability from both Purkinje cells and the tumors in these patients, support its role in the syndrome's pathogenesis. Another antigenic target, Hu, is a 35- to 40-kDa band immunoprecipitated by antibodies present mainly in patients with oat cell carcinoma and a syndrome of limbic encephalitis characterized by total loss of memory as well as sensory neuropathy [201]. Such a syndrome has been described in a patient with SLE but without attribution to any specific neuronal or lymphocyte autoantibody or to anti-Hu [202]. Whether or not Yo or Hu represent any of the target antigens in NP-SLE remains to be determined. However, the concept that a distinct neuropsychiatric syndrome and cognitive dysfunction can eventuate from specific autoantibody responses, localizable to some but not all areas of the brain, is attractive and can potentially explain some of the cognitive deficits found in SLE patients, whether or not they go on to develop overt neuropsychiatric disease.

Other potential models for localized brain dysfunction in relation to autoimmunity include: tropical spastic paraparesis associated with HTLV-1 infections [203]; chorea after rheumatic fever [204]; and, various types of neuropathy associated with monoclonal gammopathies directed against myelin [205].

Phospholipid Antigens

The subject of the anti-phospholipid antibody syndrome, its clinical as well as pathogenetic consequences including neuropsychiatric diseases, has been reviewed extensively elsewhere in this volume. The question of whether or not anti-phospholipid antibodies, via a thrombotic or thromboembolic mechanism, can account for cognitive deficits is being addressed in several ongoing and proposed studies of cognitive function in SLE patients with anti-phospholipid antibodies, either lupus anti-coagulant or anti-cardiolipin antibodies. Of

interest from the point of view of pathogenesis is the finding that several of these patients have evidence for an acquired protein S deficiency in association with surrogate markers for ongoing thrombosis [206]. Low to therapeutic doses of sodium warfarin are able to reverse this coagulopathy; whether or not cognitive function also improves in these patients remains to be elucidated in clinical studies.

Other Mechanisms: Apoptosis

The notion that disordered apoptosis occurs in SLE derives from the presence of an animal model, the MRL-lpr mouse, in which a mutated Fas receptor leads to lymphoproliferation and manifestations of autoimmunity (see later). Moreover, great interest has developed in apoptosis as a mechanism in human SLE in studies and reviews by Rosen and co-workers [207–211]. It has been demonstrated by these investigators that autoantibodies in SLE, some of them for ubiquitous nuclear antigens, can target these proteins on the cell surface as blebs on apoptotic cells. This has afforded a new way of looking at typical autoantibody–autoantigen reactions in SLE as potentially pathogenic: double-stranded DNA (dsDNA); extractable nuclear antigens (ENA) such as Ro and La; ribonuclear proteins; and, ribosomal proteins—the latter which have been implicated in depression and psychosis syndromes in NP-SLE (P antigens) [151, 212, 213]—could each potentially be a target of autoantibody response on the surface of an apoptotic cell in the neural substrate, thus leading to cognitive and/or behavioral abnormalities. Although this hypothesis is attractive, and could explain some of the findings in the MRL-lpr model to be discussed later, there is very little information at present causally relating disordered apoptosis and autoantibodies directed against dsDNA or ENA in the development of NP-SLE or of cognitive dysfunction in SLE patients. There is a syndrome of Fas/APO-1 (CD95) mutation leading to clinical manifestations somewhat like SLE [214] but, to date, there has been no study of the relationship of this syndrome to cognitive dysfunction. The area of apoptosis and its relationship to cognitive and behavioral abnormalities in SLE would be worthy of further investigation.

Another, similar mechanism relates to disordered clearance of apoptotic cells by macrophages, as proposed by Herrmann *et al*. [215]. In this situation, there is purported aberrant presentation of autoantigens because of access of antigen-presenting cells to nuclear antigens, rather than the bleb-on-surface hypothesis proposed previously. The underlying causes of this disordered presentation are not yet clear, and its relationship to cognitive dysfunction in SLE is unknown.

Finally, while there have been a plethora of descriptions of abnormal cytokines in SLE, and relationships of various of these to NP-SLE (as described elsewhere in this volume), a report suggesting the association of an imbalance in interleukin-10 (IL-10) promoter haplotypes to the development of NP-SLE is of interest [216]. While IL-10 in general is an immunosuppressive cytokine for several prominent T helper cell type 2 (TH2) responses, as exemplified by its role in allergic inflammation, there is some reason to propose that IL-10 may *favor* the development of autoimmunity; diseases such as SLE could thus be construed to result from a predominent TH1 response. More studies and information on the roles and interactions of immunoregulatory cytokines in the development of NP-SLE and its attendant cognitive dysfunction are warranted.

Behavioral Abnormalities in Lupus-Prone Mice

In spontaneous SLE syndromes in mice, including the NZB/W, MRL/lpr, and BXSB strains, there is not only a high prevalence of behavioral abnormalities but also of antibodies directed against brain extracts, homogenates, or tissue [217], along with an upregulation of proinflammatory cytokines such as interleukin (IL)-1 and interleukin (IL)-6 [218]. While examination of the specificities of these antibodies or behavioral effects of these cytokines is developing, the presence of brain-reactive antibodies, brain leukocyte infiltrates, and/or upregulated cytokines can be shown to be related to learning deficits in aging and/or autoimmunity [103, 131, 217–221]. Avoidance learning, for example, has been shown to be defective in murine lupus, and this is associated with the presence of brain-reactive antibodies [217, 222]. Anxiety-like behavior in NZB/W mice has likewise been related to upregulated cytokines, possibly interferons [223], and not simply a result of neuronal ectopies [224, 225]. Some of the latter behavioral abnormalities can, in fact, be ameliorated by chronic, soluble interferon-γ receptor treatment [226]. To what extent brain autoantibodies and cytokines interact to cause (or ameliorate) learning and behavioral abnormalities in lupus-prone mice is the subject of active investigation [218].

Studies using the MRL/lpr murine model have demonstrated the coemergence of autoantibodies against nuclear antigens and specific behavioral disturbances including perseveration, motor incoordination, disruption of nocturnal/diurnal rhythms, and hesitancy or "emotionality" [218, 227–231]. These abnormalities appear before the onset of overt manifestations of lupus such as lymphadenopathy, arthritis, renal disease, or

anemia [227–229]. The animals have high systemic levels of brain-reactive antibodies and proinflammatory cytokines; however, the higher antibody titres in the MRL/lpr compared to the congenic MRL/$^{++}$ strain are not sufficient to account for the wide range of behavioral and learning abnormalities. Findings suggest that the development of depressive-like symptomatology and other illness behaviors in MRL-lpr mice can be reproduced in MRL^{++} and control mice by transgenic upregulation of IL-6 expression [231, 232]. Furthermore, reductions in neuronal cell mass and dendritic arborization of neurites in MRL-lpr mice are associated with infiltrations of T and B cells in the brain [131, 233], and with increased apoptosis of both T cells and neuroglia [234] in these animals. Treatment with the immunosuppressive drug, cyclophosphamide, or with an IL-6 receptor antagonist can reverse the dendritic changes and improve some of the abnormal behavior (AABS) in the MRL-lpr mouse model [130]. Thus, multiple mechanisms may be involved in the development of AABS.

Table 4 lists possible mediators of cognitive dysfunction in SLE, based on a synthesis of data from human and animal work.

NEUROPSYCHOLOGICAL CORRELATES OF SPECIFIC ANTI-BRAIN ANTIBODIES

Previous studies of the relationship between anti-brain antibodies and CNS involvement in SLE have defined such involvement on the basis of major neuropsychiatric syndromes. Relatively little attention was paid to subclinical nervous system involvement such as might be indicated by neuropsychological impairment.

In order to reduce the cognitive heterogeneity evident in the SLE population, cognitive status, as a marker of nervous system involvement, has been examined in relation to specific autoantibodies that react with nervous system antigens. Significant associations have been documented between an overall designation of cognitive impairment and positivity for neuronal (NA) [31], lymphocytotoxic (LCA) [165], and anti-phospholipid (aPL) [109] antibodies, as well as between sequential changes in cognitive function and neuronal antibodies [235]; negative findings in this area have also been reported, using different criteria for cognitive impairment [19, 236]. Of special interest in terms of subgrouping patients, have been the findings of associations between antibodies and specific cognitive function; these include LCA positivity and visuospatial deficits [167, 199], and aPL positivity and a pattern of cognitive dysfunction characterized by difficulties with verbal memory, productivity, and speeded output (Table 5) [109, 237–239], even in the never NP patient group [109] (although one study found aPL positive patients to have worse performance in virtually all areas of function with increased duration of disease [239].) While

TABLE 4 Pathogenetic Mechanisms Possibly Involved in the Development of Cognitive Dysfunction in SLE

Mediator(s)	Mechanism(s)
Brain-specific autoantibodies	Interference with neurotransmission Neuronal cytotoxicity and cell loss Loss of neuronal plasticity
Anti-phospholipid antibodies	Thrombosis/ischemia/infarction Vasculopathy (endothelial dysfunction)
Cytokines	
Proinflammatory (e.g., IL-6)	Neuronal cytotoxicity and cell loss Loss of neuronal plasticity
Immunoregulatory (e.g., IL-2)	Microgliosis

TABLE 5 Relationship between Anti-phospholipid Antibody Positivity and Areas of Cognitive Impairment[a]

References	General intelligence[b]	Verbal learning and memory[b]	Visuospatial skills (including memory)[b]	Psychomotor speed and manual dexterity[b]	Attention and mental flexibility[b]
Denburg *et al.* (1997) [109]	–[c]	+[d]	–	+	+
Hanly *et al.* (1999) [237]	–	–	–	+	+
Menon *et al.* (1999) [238]	–	–	–	+	+
Whitelaw *et al.* (1999) [239]	+	+	+	na[e]	+

[a] Criteria for impaired cognitive area differed across studies.
[b] Cognitive groupings and their labels were adopted from Ryan *et al.* [108].
[c] –, cognitive area not impaired.
[d] +, cognitive area impaired.
[e] na, cognitive area not assessed.

cross-sectional associational studies raise hypotheses regarding the mechanism(s) of cognitive dysfunction in SLE, (e.g., that ongoing aPL-related microthrombotic events or vasculopathy can lead to CNS compromise manifested as cognitive dysfunction), longitudinal data are essential for their confirmation. In this regard, antibody data from our ongoing longitudinal study (see earlier) are promising. Specifically, rates of NA and LCA positivity at baseline were equivalent in the "never-to-never" and "never-to-active/inactive" NP groups; however, positivity for aPLs, measured both as lupus anticoagulant (LA) and anti-cardiolipin antibody (aCL), differed significantly between the two groups.

In addition to the specific cognitive deficits found in association with specific antibodies, we have documented significant verbal memory problems in all three antibody positive groups. This is consistent with the high prevalence of verbal memory problems documented in our original sample of SLE patients [5] and in studies from other centers (see Table 2). It also raises the possibility of another pathogenetic mechanism, possibly involving autoantibodies specific to brain areas that mediate memory, in particular medial temporal areas. The suggestion that specific immunological involvement underlies the neuropsychological deficits apparent in both paraneoplastic limbic encephalopathy and SLE has been raised [240]; the nature of that mechanism has yet to be delineated. The role of other immunological factors also warrants investigation. For example, the proinflammatory cytokine, IL-6 has been shown to contribute uniquely to measures of learning beyond the effects of depression, prednisone therapy, and hormonal measures [125].

THERAPEUTIC IMPLICATIONS OF COGNITIVE DYSFUNCTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

Medical Intervention for Acute CNS Involvement

The general practice with respect to CNS manifestations in SLE that appear to be primary (i.e., cannot be explained on the basis of toxic, metabolic, infectious, or other causes), continues to be to treat the condition with increasing doses of corticosteroids. However, it should be possible to rationalize an approach on the basis of tests aimed at delineating the pathogenetic mechanisms involved in each case. Thus, a pattern of clinical and/or cognitive findings suggesting vasculopathy and/or focal abnormalities, in the presence of anti-phospholipid antibodies, might warrant anticoagulation and/or antiplatelet drugs. Encephalopathy or "diffuse" syndromes, primarily involving psychiatric or behavioral disturbances and significant cognitive abnormalities, in the presence of antibodies against neurons or lymphocytes, or elevated cytokine levels, might be treated with corticosteroids and/or immunosuppressives. A retrospective analysis of the utility of pulse doses of cyclophosphamide in refractory cases of NP-SLE is instructive [241], as are reports of intrathecal immunosuppressives [242, 243]; however, effects on cognitive function of these interventions are unknown. Nevertheless, depending on the particular clinical NP picture, and in the absence of compelling data regarding an autoimmune/inflammatory mechanism, consideration must always be given to the potential efficacy of anticonvulsants, neuroleptics, or antidepressants.

Controlled trials of corticosteroid therapy in NP-SLE are nonexistent. The heterogeneity of NP manifestations, the frequent lack of association between such manifestations and other evidence of disease activity, and the variability of markers of NP activity, together make evaluation of the efficacy of steroid therapy in NP-SLE difficult. Further, because of the likelihood of diverse pathogenetic mechanisms underlying NP-SLE [23–26], patients may well show marked differences in their response to corticosteroid treatment. One prospective, single patient (N of 1) trial to examine the efficacy of relatively low-dose corticosteroids on CNS functioning, as reflected specifically in cognitive and mood status, has been reported and discussed previously [128]. The issue is best addressed through prospective, multicenter clinical trials of corticosteroids, immunosuppressives, and/or anticoagulants in patients with SLE, including cognitive end points to assess effects on brain function and functional indices to assess impact on everyday functioning [244, 245].

Long-Term Functional Issues

Changes in mental status can be a strong indicator of developing CNS involvement that may require medical intervention. Clinicians should also be aware of the handicap posed by residual/subclinical cognitive deficits, once an acute episode has been treated and resolved. Effective care in any chronic illness such as SLE must take into account the difficulties in daily life that are a consequence of functional impairment. SLE patients regularly complain of diminished cognitive efficiency, which may or may not correlate well with objectively documented cognitive deficit [86, 87, 136]. The lack of a reliable correlation may be due to a number of factors, including concomitant depressive symptomatology [87], but should not, a priori, invalidate the subjective functional complaints. The therapeutic implications of identifying chronic or even transient CNS

dysfunction are as important for SLE patients as for any other group with CNS compromise, such as MS [246] or head injury [247]. Most patients and family members respond positively to having specific deficits defined and their impact on daily life explored. Diminished retention of information, poor attention, distractibility, social withdrawal, and lability of mood can be easier to live with and compensate for, once identified and recognized as manifestations of subtle alterations in CNS function, rather than being perceived as carelessness or characterological faults. Memory and organizational dysfunction, when unrecognized, potentially compromise the patient's ability to adhere to prescribed treatment strategies. Together with a general reduction in mental efficiency, these impairments can have a significant impact on occupational functioning [244, 245]; activities that were previously well within the patient's competence may become highly demanding and very fatiguing. Although measures of impact are beginning to be included in studies of cognitive function in SLE (e.g., [112]), considerably more work needs to be done applying well-validated measures of daily function in large scale cognitive studies, before the question of impact of cognitive deficit can be answered. For some patients who experience significant dysfunction, adjustments in the home or work environment and schedule may substantially facilitate their ability to function productively and remain fully involved. However, for other individuals, more extensive changes may be needed, along with appreciable modifications in expectations and activities, in order to accommodate substantially reduced mental capacities. Cognitive rehabilitation techniques, as used with patients who have sustained head injury or other neurologic disorders, may be appropriate for a subset of SLE patients whose impairment persists or worsens over time [248]. Even in those patients who have never had major neurologic or psychiatric symptomatology, cognitive impairment (as a reflection of brain compromise) may predict increased susceptibility to adverse CNS side effects of prescribed medication, such as hypnotics and anxiolytics. Moreover, alcohol and sleep disruption may have a much greater impact on the performance of these patients than would be the case if there were no compromise of CNS integrity [249, 250].

FUTURE DIRECTIONS FOR COGNITIVE STUDIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Research over the last almost two decades has focused on identifying the extent and nature of cognitive dysfunction in SLE, using diverse batteries of clinical neuropsychological tests that have proven utility in identifying compromised brain function. However, the ability of these tests to identify specific functional deficits is limited, owing in particular to their multifactorial nature. Individual tests tend to tap a variety of skills at different levels of complexity, making it difficult to specify the particular function that is impaired. A few isolated studies have used experimental measures to tap subtle problems in attentional capacity and information processing in SLE [14, 15]. They postulated that these problems might underlie the cognitive deficits documented by the clinical tests and might account for the subjective experience of cognitive dysfunction experienced by many SLE patients; the latter is not always corroborated by conventional neuropsychological tools. The use of experimental tests, as well as the analysis of patterns of responses on clinical neuropsychological tests, should be pursued as this approach has proved to be productive in the study of a variety of patient populations, such as MS, HIV, chronic fatigue syndrome (CFS), and depression, when standard neuropsychological measures have not necessarily identified significant deficits or been able to localize the underlying lesion [251–258]. The modification of current methods and the adoption of new experimental approaches represent important directions for future investigation. The selection of tests should be hypothesis-driven, based on existing cognitive data in SLE and on the underlying models of cognitive deficit that would relate to proposed pathogenetic mechanisms [259].

Longitudinal studies are essential to assess the predictive utility of various risk factors that have been associated cross-sectionally with NP involvement (e.g., autoantibodies, cognitive dysfunction) and to identify key factors associated with cognitive improvement or decline. In addition, multicenter randomized controlled intervention trials should be undertaken where the existing data support them: for example, the use of anticoagulant therapy in aPL positive patients [183–186] with the inclusion of cognitive data as an end point [109, 237–239].

The heterogeneous presentation of NP-SLE complicates studies of its pathogenesis. Neuropsychological measures that correlate specifically with a postulated pathogenetic mechanism (e.g., autoantibodies) can serve to limit the focus of study to subsets of patients. The same can be accomplished by differentiating the larger patient group into discrete clinical presentations (e.g., stroke, depression) that may then be studied in relation to cognitive and biological correlates, the latter augmented through the use of structural and functional brain imaging. Neuropsychological measures may themselves be subjected to statistical approaches for

subgrouping patients for further study of clinical correlates and underlying mechanisms [260, 261].

Finally, another important direction in cognitive research in SLE is the development of brief, well-validated test batteries (e.g., [114, 262]). This would have clinical utility for routine patient monitoring, as well as research utility, as part of large, multicenter investigations of the assessment, etiology, and treatment of NP-SLE. The development of a consensual approach to brief neuropsychological assessment was one of the foci of an ad hoc workshop on nomenclature and classification sponsored by the ACR [22]. A 1-hour battery was proposed, using existing clinical tests to assess the areas of complex attention, memory, visual–spatial processing, language, psychomotor speed, motor speed and dexterity, and executive function. A subjective measure of cognitive function and an assessment of impact on daily living were also recommended. There remains the need to validate such a short battery against a full neuropsychological assessment and to collect norms on healthy controls in order to ensure its diagnostic efficacy and capacity to identify clinically significant change over time. Until this is done, the use of a brief cognitive battery in the clinical setting remains problematic in the absence of appropriate professional consultation. Nevertheless, the consistent use of such a battery across various study centers would greatly facilitate future research efforts and, ultimately, clinical approaches to NP-SLE. The various ways that cognitive data might contribute to future studies of NP-SLE are summarized in Table 6.

TABLE 6 Future Directions: Cognition as a "Tool" to Study NP-SLE[a]

Longitudinal studies: risk factors for NP involvement, including cognitive dysfunction
Randomized controlled trials of steroid/anticoagulant/immunosuppressive therapy in subsets of patients defined by NP-SLE presentation and/or potential pathogenetic mechanisms (e.g., aPL)
Depression as a primary manifestation of NP-SLE
Statistical approaches to subgrouping patients according to cognitive profiles
Experimental cognitive paradigm to specify underlying cognitive deficits
Validation of brief cognitive batteries for clinical use and research applications
Collaborative studies using cognitive and imaging approaches

[a] All of the research directions outlined above would be facilitated and enhanced through multicentered collaborations.

SUMMARY

Broad-based neuropsychological assessment strategies have proved to be sensitive to the presence of cognitive impairment in patients with SLE, and have uncovered significant impairment even in patients without overt neurologic or psychiatric symptomatology. Although responses to self-report questionnaires indicate emotional distress in a fair number of SLE patients, a significant association between the presence of cognitive impairment and distress is not evident. Moreover, the documented cognitive impairment cannot be attributed to possible adverse side effects of corticosteroids, and is not strongly associated with the presence of active disease. Thus, cognitive impairment, when documented in SLE patients, most likely reflects central nervous system dysfunction; this may be residual, in the case of patients with inactive CNS symptomatology, or perhaps an early marker or predictor of CNS involvement, in the case of patients who have never had major CNS symptomatology. It is important to note that there is limited concordance between the deficits that are identified by directly comparing control and SLE groups and the deficits that are identified in individual test protocols, applying decision criteria commonly used in clinical neuropsychological assessment. This suggests a relatively limited clinical utility for data from group studies, no matter what the patient population, unless there is a well established homogeneity in neurologic presentation.

Thus, the cognitive deficits described in SLE patients, most probably due to no other process but SLE itself, represent an extremely important index of central nervous system dysfunction, probably related to, or as a consequence of, immunological mechanisms such as brain-specific autoantibodies, "neurotoxic" or "neuroactive" cytokines, and/or inflammation. Attempts to reliably investigate the pathogenesis of NP-SLE in its clinical or subclinical manifestations and ascribe it to an autoimmune and/or inflammatory process would be considerably enhanced by the use of standardized neuropsychological assessments of brain function complemented by brain imaging studies, particularly if this enabled the identification of patient subgroups, across various centers. Such multicenter studies also have the potential to validate brief cognitive batteries that would be relevant to the assessment and monitoring of cognitive function in clinical practice.

References

1. Carbotte, R. M., Denburg, S. D., and Denburg, J. A. (1995). Cognitive deficit associated with rheumatic diseases: Neuropsychological perspectives. *Arthritis Rheum.* **38**, 1363–1374.

2. Sonies, B. C., Klippel, J. H., Gerber, R. B., and Gerber, L. H. (1982). Cognitive performance in systemic lupus erythematosus (abstract). *Arthritis Rheum.* **25**, S80.
3. Bresnihan, B., and O'Connell, A. (1982). Systemic lupus erythematosus, cerebral disease and intellectual function (abstract). *Arthritis Rheum.* **25**, S80.
4. Carbotte, R. M., Denburg, S. D., and Denburg, J. A. (1986). Prevalence of cognitive impairment in systemic lupus erythematosus. *J. Nerv. Ment. Dis.* **174**, 357–364.
5. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1987). Cognitive impairment in systemic lupus erythematosus: A neuropsychological study of individual and group deficits. *J. Clin. Exp. Neuropsychol.* **9**, 323–339.
6. Koffler, S. (1987). The role of neuropsychological testing in systemic lupus erythematosus. In "Systemic Lupus Erythematosus" (R. G. Lahita, Ed.), pp. 847–853. Wiley, New York.
7. Hanly, J. G., Fisk, J. D., Sherwood, G., Jones, E., Jones, J. V., and Eastwood, B. (1992). Cognitive impairment in patients with systemic lupus erythematosus. *J. Rheumatol.* **19**, 562–567.
8. Hay, E. M., Black, D., Huddy, A., Creed, F., Tomenson, B., Bernstein, R. M., and Holt, P. J. L. (1992). Psychiatric disorder and cognitive impairment in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 411–416.
9. Hanly, J. G., Fisk, J. D., Sherwood, G., and Eastwood, B. (1994). Clinical course of cognitive dysfunction in systemic lupus erythematosus. *J. Rheumatol.* **21**, 1825–1831.
10. Ginsburg, K. S., Wright, E. A., Larson, M. G., Fossel, A. H., Albert, M., Schur, P. H., and Liang, M. H. (1992). A controlled study of the prevalence of cognitive dysfunction in randomly selected patients with systemic lupus erythematosus. *Arthritis Rheum.* **35**, 776–782.
11. Kutner, K. C., Busch, H. M., Mahmood, T., Racis, S. P., and Krey, P. R. (1988). Neuropsychological functioning in systemic lupus erythematosus. *Neuropsychology* **2**, 119–126.
12. Kozora, E., Thompson, L. L., West, S. G., and Kotzin, B. L. (1996). Analysis of cognitive and psychological deficits in systemic lupus erythematosus patients without overt central nervous system disease. *Arthritis Rheum.* **39**, 2035–2045.
13. Ferstl, R., Niemann, T., Biehl, G., Hinrichsen, H., and Kirch, W. (1992). Neuropsychological impairment in auto-immune disease. *Eur. J. Clin. Invest.* **22**, 16–20.
14. Beers, S. R., Morrow, L., Ryan, C. M., Wasko, M. C., Rarie, J., and Manzi, S. (1996). The use of information processing measures to detect cognitive deficits in systemic lupus erythematosus. *J. Int. Neuropsychol. Soc.* **2**, 15–16.
15. Kerr, E. N., Edworthy, S. M., Samuels, M. T., and Violato, C. (1994). Attentional capacity in patients with systemic lupus erythematosus (SLE) (abstract). *Arthritis Rheum.* **37**, S178.
16. Waterloo, K., Omdal, R., Mellgren, S. I., and Husby, G. (1997). Neuropsychological functions in systemic lupus erythematosus: A comparison with chronic whiplash patients. *Eur. J. Neurol.* **4**, 171–177.
17. Holliday, S., Brey, R., Escalante, G., and Ledbetter, M. (1997). Neuropsychological functioning in SLE: Computer-assisted testing in a predominantly Hispanic sample (abstract). *J. Int. Neuropsychol. Soc.* **3**, 17.
18. Rummelt, J. K., Sobota, W. L., Brickman, C. M., and Doyle, T. H. (1991). Memory and motor scores discriminate systemic lupus (SLE) patients from matched controls (abstract). *J. Clin. Exp. Neuropsychol.* **13**, 59.
19. Papero, P. H., Bluestein, H. G., White, P., and Lipnick, R. N. (1990). Neuropsychologic deficits and antineuronal antibodies in pediatric systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **8**, 417–424.
20. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1992). Cognitive deficit in non-neuropsychiatric SLE (fact or fiction?) (abstract). *Arthritis Rheum.* **35**, S208.
21. Moore, P. M., and Lahita, R. G., Eds. (1997). Neuropsychiatric manifestations of systemic lupus erythematosus. *Ann. N. Y. Acad. Sci*. **823**.
22. ACR ad hoc committee on neuropsychiatric lupus nomenclature (1999). The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum.* **42**, 599–608.
23. Denburg, J. A., Carbotte, R., and Denburg, S. (1993). Central nervous system lupus. *Rheumatol. Rev.* **2**, 123–132.
24. Bluestein, H. G. (1992). The central nervous system in systemic lupus erythematosus. In "Systemic Lupus Erythematosus" (R. G. Lahita, Ed.), 2nd ed., pp. 639–655. Churchill Livingstone, New York.
25. West, S. G. (1994). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am.* **20**, 129–158.
26. West, S. G. (1996). Lupus and the central nervous system. *Curr. Opin. Rheumatol.* **8**, 408–414.
27. Adelman, D. C., Saltiel, E., and Klinenberg, J. R. (1986). The neuropsychiatric manifestations of systemic lupus erythematosus: An overview. *Semin. Arthritis Rheum.* **15**, 185–199.
28. Burnstein, S. L., Janoff, L., and McCormick, K. (1987). Neuropsychiatric involvement in systemic lupus erythematosus: Case report and review of the literature. *J. Am. Osteopath. Assoc.* **87**, 626–631.
29. McCune, W. J., and Golbus, J. (1988). Neuropsychiatric lupus. *Rheum. Dis. Clin. North Am.* **14**, 149–167.
30. Tarter, R. E., Edwards, K. L., and Van Thiel, D. H. (1988). Perspective and rationale for neuropsychological assessment of medical disease. In "Medical Neuropsychology: The Impact of Disease on Behavior" (R. E. Tarter, D. H. Van Thiel, and K. L. Edwards, Eds.), pp. 1–10. Plenum, New York.
31. Denburg, J. A., Carbotte, R. M., and Denburg, S. D. (1987). Neuronal antibodies and cognitive function in systemic lupus erythematosus. *Neurology* **37**, 464–467.
32. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85–95.
33. Feinglass, E. J., Arnett, F. C., Dorsch, C. A., Zizic, T. M., and Stevens, M. B. (1976). Neuropsychiatric manifestations of systemic lupus erythematosus: Diagnosis, clinical spectrum, and relationship to other features of the disease. *Medicine* **55**, 323–339.

34. Bresnihan, B. (1982). CNS lupus. *Clin. Rheum. Dis.* **8**, 183–195.
35. Kassan, S. S., and Lockshin, M. D. (1979). Central nervous system lupus erythematosus: The need for classification. *Arthritis Rheum.* **22**, 1382–1385.
36. Yancey, C. L., Doughty, R. A., and Athreya, B. H. (1981). Central nervous system involvement in childhood systemic lupus erythematosus. *Arthritis Rheum.* **24**, 1389–1395.
37. Singer, J., Denburg, J. A., and the Ad Hoc Neuropsychiatric Lupus Workshop Group (1990). Diagnostic criteria for neuropsychiatric systemic lupus erythematosus: The results of a consensus meeting. *J. Rheumatol.* **17**, 1397–1402.
38. Grant, I., and Adams, K. M., Eds. (1986). "Neuropsychological Assessment of Neuropsychiatric Disorders." Oxford Univ. Press, New York.
39. Walsh, K. W. (1978). "Neuropsychology: A Clinical Approach." Churchill Livingstone, New York.
40. Lezak, M. D. (1995). "Neuropsychological Assessment." 3rd ed. Oxford Univ. Press, New York.
41. Reitan, R. M. (1986). Theoretical and methodological bases of the Halstead-Reitan neuropsychological test battery. In "Neuropsychological Assessment of Neuropsychiatric Disorders" (I. Grant and K. M. Adams, Eds.), pp. 3–30. Oxford Univ. Press, New York.
42. Golden, C. J., Moses, J. A., Fishburne, F. J., Engum, E., Lewis, G. P., Wisniewski, A. M., Conley, F. K., Berg, R. A., and Graber, B. (1981). Cross-validation of the Luria-Nebraska Neuropsychological Battery for the presence, lateralization, and localization of brain damage. *J. Consult. Clin. Psychol.* **49**, 491–507.
43. Milberg, W. P., Hebben, N., and Kaplan, E. (1986). The Boston process approach to neuropsychological assessment. In "Neuropsychological Assessment of Neuropsychiatric Disorders" (I. Grant and K. M. Adams, Eds.), pp. 65–86. Oxford Univ. Press, New York.
44. Levin, H. S., Williams, D. H., Eisenberg, H. M., High, W. M., and Guinto, F. C. (1992). Serial MRI and neurobehavioural findings after mild to moderate closed head injury. *J. Neurol. Neurosurg. Psychiatry* **55**, 255–262.
45. de Leon, M. J., George, A. E., and Ferris, S. H. (1986). Computed tomography and positron emission tomography correlates of cognitive decline in aging and senile dementia. In "Handbook for Clinical Memory Assessment of Older Adults" (L. W. Poon, Ed.), pp. 367–382. American Psychological Association, Washington, D.C.
46. Albert, M. S., Naeser, M. A., Duffy, F. H., and McAnulty, G. (1986). CT and EEG validators for Alzheimer's Disease. In "Handbook for Clinical Memory Assessment of Older Adults" (L. W. Poon, Ed.), pp. 383–392. American Psychological Association, Washington, D.C.
47. Kesler, S. R., Adams, H. F., and Bigler, E. D. (2000). SPECT, MR and quantitative MR imaging: Correlates with neuropsychological and psychological outcome in traumatic brain injury. *Brain Inj.* **14**, 851–857.
48. McEvoy, J. P., McCue, M., Spring, B., Mohs, R. C., Lavori, P. W., and Farr, R. M. (1987). Effects of amantadine and trihexyphenidyl on memory in elderly normal volunteers. *Am. J. Psychiatry* **144**, 573–577.
49. Hartman, D. E. (1988). "Neuropsychological Toxicology." Pergammon, New York.
50. Hart, R. P., and Kreutzer, J. S. (1988). Renal system. In "Medical Neuropsychology: The Impact of Disease on Behavior" (R. E. Tarter, D. H. Van Thiel, and K. L. Edwards, Eds.), pp. 99–120. Plenum, New York.
51. Prigatano, G. P., and Levin, D. C. (1988). Pulmonary system. In "Medical Neuropsychology: The Impact of Disease on Behavior" (R. E. Tarter, D. H. Van Thiel, and K. L. Edwards, Eds.), pp. 11–26. Plenum, New York.
52. Ritchlin, C. T., Chabot, R. J., Alper, K., Buyon, J., Belmont, H. M., Roubey, R., and Abramson, S. B. (1992). Quantitative electroencephalography. A new approach to the diagnosis of cerebral dysfunction in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 1330–1342.
53. McCune, W. J., MacGuire, A., Aisen, A., and Gebarski, S. (1988). Identification of brain lesions in neuropsychiatric systemic lupus erythematosus by magnetic resonance scanning. *Arthritis Rheum.* **31**, 159–166.
54. Sibbitt, W. L., Sibbitt, R. R., Griffey, R. H., Eckel, C., and Bankhurst, A. D. (1989). Magnetic resonance imaging in the evaluation of acute neuropsychiatric disease in systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**, 1014–1022.
55. Sibbitt, W. L., Brooks, W. M., Haseler, L. J., Griffey, R. H., Frank, L. M., Hart, B. L., and Sibbitt, R. R. (1995). Spin–spin relaxation of brain tissues in systemic lupus erythematosus. *Arthritis Rheum.* **38**, 810–818.
56. Bell, C. L., Partington, C., Robbins, M., Graziano, F., Turski, P., and Kornguth, S. (1991). Magnetic resonance imaging of central nervous system lesions in patients with lupus erythematosus: Correlation with clinical remission and antineurofilament and anticardiolipin antibody titers. *Arthritis Rheum.* **34**, 432–441.
57. Hiraiwa, M., Nonaka, C., Abe, T., and Iio, M. (1983). Positron emission tomography in systemic lupus erythematosus: Relation of cerebral vasculitis to PET findings. *Am. J. Neuroradiol.* **4**, 541–543.
58. Stoppe, G., Wildhagen, K., Seidel, J. W., Meyer, G. J., Schober, O., Heintz, P., Kunkel, H., Deicher, H., and Hundeshagen, H. (1990). Positron emission tomography in neuropsychiatric lupus erythematosus. *Neurology* **40**, 304–308.
59. Volkow, N. D., Warner, N., McIntyre, R., and Valentine, A. (1988). Cerebral involvement in systemic lupus erythematosus. *Am. J. Physiol. Imaging* **3**, 91–98.
60. Nossent, J. C., Hovestadt, A., Schonfeld, D. H., and Swaak, A. J. (1991). Single-photon-emission computed tomography of the brain in the evaluation of cerebral lupus. *Arthritis Rheum.* **34**, 1397–1403.
61. Kovacs, J. A. J., Urowitz, M. B., Gladman, D. D., and Zeman, R. (1995). The use of single photon emission computerized tomography in neuropsychiatric SLE: A pilot study. *J. Rheumatol.* **22**, 1247–1253.
62. Otte, A., Weiner, S. M., Hoegerle, S., Wolf, R., Juengling, F. D., Peter, H. H., and Nitzsche, E. U. (1998). Neuropsychiatric systemic lupus erythematusos before and

after immunosuppressive treatment: A FDG PET study. *Lupus* **7**, 57–59.
63. Awada, H. H., Mamo, H. L., Luft, A. G., Ponsin, J. C., and Kahn, M. F. (1987). Cerebral blood flow in systemic lupus with and without central nervous system involvement. *J. Neurol. Neurosurg. Psychiatry* **50**, 1597–1601.
64. Kushner, M. J., Chawluk, M. D., Fazekas, F., Burke, A., Jagg, J., Rosen, M., and Reivich, M. (1987). Cerebral blood flow in systemic lupus erythematosus with or without cerebral complications. *Neurology* **37**, 1596–1598.
65. Pinching, A. J., Travers, R. L., Hughes, G. R. V., Jones, T., and Moss, S. (1978). Oxygen-15 brain scanning for detection of cerebral involvement in systemic lupus erythematosus. *Lancet* **1**, 898–900.
66. Carbotte, R. M., Denburg, S. D., Denburg, J. A., Nahmias, C., and Garnett, E. S. (1992). Fluctuating cognitive abnormalities and cerebral glucose metabolism in neuropsychiatric systemic lupus erythematosus. *J. Neurol. Neurosurg. Psychiatry* **55**, 1054–1059.
67. Leritz, E., Brandt, J., Minor, M., Reis-Jensen, F., and Petri, M. (2000). "Subcortical" cognitive impairment in patients with systemic lupus erythematosus. *J. Int. Neuropsychol. Soc.* **6**, 821–825.
68. Chinn, R. J. S., Wilkinson, I. D., Hall-Craggs, M. A., Paley, M. N. J., Shortall, E., Carter, S., Kendall, B. E., Isenberg, D. A., Newman, S. P., and Harrison, M. J. G. (1997). Magnetic resonance imaging of the brain and cerebral proton spectroscopy in patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 36–46.
69. Emmi, L., Bramati, M., De Cristofaro, M. T. R., Mascalchi, M., Dal Pozzo, G., Marconi, G. P., Massai, G., and Passaleva, A. (1993). MRI and SPECT investigations of the CNS in SLE patients. *Clin. Exp. Rheumatol.* **11**, 13–20.
70. Sibbitt, W. L., Jr., Sibbitt, R. R., and Brooks, W. M. (1999). Neuroimaging in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* **42**, 2026–2038.
71. Waterloo, K., Omdal, R., Jacobsen, E. A., Klow, N. E., Husby, G., Torbergsen, T., and Mellgren, S. I. (1999). Cerebral computed tomography and electroencephalography compared with neuropsychological findings in systemic lupus erythematosus. *J. Neurol.* **246**, 706–711.
72. Kozora, E., West, S. G., Kotzin, B. L., Julian, L., Porter, S., and Bigler, E. (1998). Magnetic resonance imaging abnormalities and cognitive deficits in systemic lupus erythematosus patients without overt central nervous system disease. *Arthritis Rheum.* **41**, 41–47.
73. Starkstein, S. E., and Robinson, R. G. (1989). Affective disorders and cerebral vascular disease. *Br. J. Psychiatry* **154**, 170–182.
74. Gianotti, G., and Marra, C. (2002). Determinants and consequences of post-stroke depression. *Curr. Opin. Neurol.* **15**, 85–89.
75. Grafman, J., Vance, S. C., Weingartner, H., Salazar, A. M., and Amin, D. (1986). The effects of lateralized frontal lesions on mood regulation. *Brain* **109**, 1127–1148.
76. Braun, C. M., Larocque, C., Daigneault, S., and Montour-Proulx, I. (1999). Mania, pseudomania, depression, and pseudodepression resulting from focal unilateral cortical lesions. *Neuropsychiatr. Neuropsychol. Behav. Neurol.* **12**, 35–51.
77. Heinrichs, R. W. (1990). Current and emergent applications of neuropsychological assessment: Problems of validity and utility. *Professional Psychology: Research and Practice* **21**, 171–176.
78. Ready, R. E., Stierman, L., and Paulsen, J. S. (2001). Ecological validity of neuropsychological and personality measures of executive functions. *Clin. Neuropsychol.* **15**, 314–323.
79. Silver, C. H. (2000). Ecological validity of neuropsychological assessment in childhood traumatic brain injury. *J. Head Trauma Rehabil.* **15**, 973–988.
80. Goldberg, E., and Podell, K. (2000). Adaptive decision making, ecological validity, and the frontal lobes. *J. Clin. Exp. Neuropsychol.* **22**, 56–68.
81. Burgess, P. W., Alderman, N., Evans, J., Emslie, H., and Wilson, B. A. (1998). The ecological validity of tests of executive function. *J. Int. Neuropsychol. Soc.* **4**, 547–558.
82. Goodman, C. R., and Zarit, S. H. (1995). Ecological measures of cognitive functioning: A validation study. *Int. Psychogeriatr.* **7**, 39–50.
83. Tupper, D. E., and Cicerone, K. D., Eds. (1990). "The Neuropsychology of Everyday Life: Assessment and Basic Competencies." Kluwer Academic Publishers, Boston.
84. Lezak, M. D. (1988). Brain damage is a family affair. *J. Clin. Exp. Neuropsychol.* **10**, 111–123.
85. Cicerone, K. D., Dahlberg, C., Kalmar, K., Langenbahn, D. M., Malec, J. F., Bergquist, T. F., Felicetti, T., Giacino, J. T., Harley, J. P., Harrington, D. E., Herzog, J., Kneipp, S., Laatsch, L., and Morse, P. A. (2000). Evidence-based cognitive rehabilitation: Recommendations for clinical practice. *Arch. Phys. Med. Rehabil.* **81**, 1596–1615.
86. Zelinski, E. M., Gilewski, M. J., and Anthony-Bergstone, C. R. (1990). Memory functioning questionnaire: Concurrent validity with memory performance and self-reported memory failures. *Psychol. Aging* **5**, 388–399.
87. Gass, C. S., and Apple, C. (1997). Cognitive complaints in closed-head injury: Relationship to memory test performance and emotional disturbance. *J. Clin. Exp. Neuropsychol.* **19**, 290–299.
88. Cull, A., Hay, C., Love, S. B., Mackie, M., Smets, E., and Stewart, M. (1996). What do cancer patients mean when they complain of concentration and memory problems? *Br. J. Cancer* **74**, 1674–1679.
89. Heaton, R. K., Grant, I., Butters, N., White, D. A., Kirson, D., Atkinson, J. H., McCutchan, J. A., Taylor, M. J., Kelly, M. D., Ellis, R. J., Wolfson, T., Velin, R., Marcotte, T. D., Hesselink, J. R., Jernigan, T. L., Chandler, J., Wallace, M., Abramson, I., and HNRC Group (1995). The HNRC 500—Neuropsychology of HIV infection at different disease stages. *J. Int. Neuropsychol. Soc.* **1**, 231–251.
90. Minden, S. L., Moes, E. J., Orav, J., Kaplan, E., and Reich, P. (1990). Memory impairment in multiple sclerosis. *J. Clin. Exp. Neuropsychol.* **12**, 566–586.
91. Grant, I., Adams, K. M., Carlin, A. S., Rennick, P. M., Judd, L. L., and Schooff, K. (1978). The collaborative

neuropsychological study of polydrug users. *Arch. Gen. Psychiatry* **35**, 1063–1074.
92. Wekking, E. M., Nossent, J. C., van Dam, A. P., and Swaak, A. J. J. G. (1991). Cognitive and emotional disturbances in systemic lupus erythematosus. *Psychother. Psychosom.* **55**, 126–131.
93. Breitbach, S. A., Alexander, R. W., Daltroy, L. H., Liang, M. H., Boll, T. J., Karlson, E. W., Partiridge, A. J., Roberts, W. N., Stern, S. H., Wacholtz, M. C., and Straaton, K. V. (1998). Determinants of cognitive performance in systemic lupus erythematosus. *J. Clin. Exp. Neuropsychol.* **20**, 157–166.
94. Carlomagno, S., Migliaresi, S., Ambrosone, L., Sannino, M., Sanges, G., and Di Iorio, G. (2000). Cognitive impairment in systemic lupus erythematosus: A follow-up study. *J. Neurol.* **247**, 273–279.
95. Gladman, D. D., Urowitz, M. B., Slonim, D., Glanz, B., Carlen, P., Noldy, N., Gough, J., Pauzner, R., Heslegrave, R., Darby, P., and MacKinnon, A. (2000). Evaluation of predictive factors for neurocognitive dysfunction in patients with inactive systemic lupus erythematosus. *J. Rheumatol.* **27**, 2367–2371.
96. Sabbadini, M. G., Manfredi, A. A., Bozzolo, E., Ferrario, L., Rugarli, C., Scorza, R., Origgi, L., Vanoli, M., Gambini, O., Vanzulli, L., Croce, D., Campana, A., Messa, C., Fazio, F., Tincani, A., Anzola, G., Cattaneo, R., Padovani, A., Gasparotti, R., Gerli, R., Quartesan, R., Piccirilli, M., Farsi, A., Emmi, E., Passaleva, A., *et al*. (1999). Central nervous system involvement in systemic lupus erythematosus patients without overt neuropsychiatric manifestations. *Lupus* **8**, 11–19.
97. Monastero, R., Bettini, P., Del Zotto, E., Cottini, E., Tincani, A., Balestrieri, G., Cattaneo, R., Camarda, R., Vignolo, L. A., and Padovani, A. (2001). Prevalence and pattern of cognitive impairment in systemic lupus erythematosus patients with and without overt neuropsychiatric manifestations. *J. Neurol. Sci.* **184**, 33–39.
98. Sailer, M., Burchert, W., Ehrenheim, C., Smid, H. G., Haas, J., Wildhagen, K., Wurster, U., and Deicher, H. (1997). Positron emission tomography and magnetic resonance imaging for cerebral involvement in patients with systemic lupus erythematosus. *J. Neurol.* **244**, 186–193.
99. Gibson, T., and Myers, A. R. (1976). Nervous system involvement in systemic lupus erythematosus. *Ann. Rheum. Dis.* **35**, 398–406.
100. Denburg, J. A., and Temesvari, P. (1983). The pathogenesis of neuropsychiatric lupus. *Can. Med. Assoc. J.* **128**, 257–260.
101. O'Connor, P. (1988). Diagnosis of central nervous system lupus. *Can. J. Neurol. Sci.* **15**, 257–260.
102. Gordon, H. W. (1986). The cognitive laterality battery: Tests of specialized cognitive function. *Int. J. Neurosci.* **29**, 223–244.
103. Denburg, S. D. (1994). Cognitive dysfunction in patients with SLE: The research and clinical challenge. Study group presentation for the American College of Rheumatology. *Arthritis Rheum.* **37**.
104. Fisk, J. D., Eastwood, B., Sherwood, G., and Hanly, J. G. (1993). Patterns of cognitive impairment in patients with systemic lupus erythematosus. *Br. J. Rheumatol.* **32**, 458–462.
105. Glanz, B. I., Slonim, D., Urowitz, M. B., Gladman, D. D., Gough, J., and MacKinnon, A. (1997). Pattern of neuropsychologic dysfunction in inactive systemic lupus erythematosus. *Neuropsychiatr. Neuropsychol. Behav. Neurol.* **10**, 232–238.
106. Skeel, R. L., and Johnstone, B. (2000). Neuropsychological deficit profiles in systemic lupus erythematosus. *Appl. Neuropsychol.* **7**, 96–101.
107. Ainiala, H., Loukkola, J., Peltola, J., Korpela, M., and Hietaharju, A. (2001). The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. *Neurology* **57**, 496–500.
108. Ryan, C. M., Morrow, L. A., Bromet, E. J., and Parkinson, D. K. (1987). The assessment of neuropsychological dysfunction in the workplace: Normative data from the Pittsburgh Occupational Exposures Test Battery. *J. Clin. Exp. Neuropsychol.* **9**, 665–679.
109. Denburg, S. D., Carbotte, R. M., Ginsberg, J. S., and Denburg, J. A. (1997). The relationship of antiphospholipid antibodies to cognitive function in patients with systemic lupus erythematosus. *J. Int. Neuropsychol. Soc.* **3**, 377–386.
110. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1997). Cognition and mood in SLE: Evaluation and pathogenesis. *Ann. N. Y. Acad. Sci.* **823**, 44–59.
111. Hay, E. M., Huddy, A., Black, D., Mbaya, P., Tomenson, B., Bernstein, R. M., Holt, P. J. L., and Creed, F. (1994). A prospective study of psychiatric disorder and cognitive function in systemic lupus erythematosus. *Ann. Rheum. Dis.* **53**, 298–303.
112. Hanly, J. G., Cassell, K., and Fisk, J. D. (1997). Cognitive function in systemic lupus erythematosus: Results of a 5-year prospective study. *Arthritis Rheum.* **40**, 1542–1543.
113. Grant, I., Olshen, R. A., Atkinson, J. H., Heaton, R. K., Nelson, J., McCutchan, J. A., and Weinrich, J. D. (1993). Depressed mood does not explain neuropsychological deficits in HIV-infected persons. *Neuropsychology* **7**, 53–61.
114. Rao, S. M., Leo, G. J., Bernardin, L., and Unverzagt, F. (1991). Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. *Neurology* **41**, 685–691.
115. Mapou, R. L., and Spector, J., Eds. (1995). "Clinical Neuropsychological Assessment: A Cognitive Approach." Plenum, New York.
116. Bernard, I., Le Gall, D., Truelle, J. L., Joseph, P. A., and Emile, J. (1995). Cognitive disturbances in multiple sclerosis: A ten year follow-up study (abstract). *J. Int. Neuropsychol. Soc.* **1**, 201.
117. Ling, M. H. M., Perry, P. H., and Tsuang, M. T. (1981). Psychiatric side effects of corticosteroid therapy. *Arch. Gen. Psychiatry* **38**, 471–477.
118. Perry, P. J., Tsuang, M. T., and Hwang, M. H. (1984). Prednisolone psychosis: Clinical observation. *Drug Intell. Clin. Pharm.* **18**, 603–609.

119. Varney, N. R., Alexander, B., and MacIndoe, J. H. (1984). Reversible steroid dementia in patients without steroid psychosis. *Am. J. Psychiatry* **141**, 369–372.
120. Lupien, S. J., and McEwen, B. S. (1997). The acute effects of corticosteroids on cognition: Integration of animal and human model studies. *Brain Res. Brain Res. Rev.* **24**, 1–27.
121. Sapolsky, R. M., Krey, L. C., and McEwen, B. S. (1984). Stress down-regulates corticosterone receptors in a site-specific manner in the brain. *Endocrinology* **114**, 287–292.
122. Woolley, C. S., Gould, E., and McEwen, B. S. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res.* **531**, 225–231.
123. Wolkowitz, O. M., Reus, V. I., Canick, J., Levin, B., and Lupien, S. (1997). Glucocorticoid medication, memory and steroid psychosis in medical illness. *Ann. N. Y. Acad. Sci.* **823**, 96.
124. Zonana-Nacach, A., Barr, S. G., Magder, L. S., and Petri, M. (2000). Damage in systemic lupus erythematosus and its association with corticosteroids. *Arthritis Rheum.* **43**, 1801–1808.
125. Kozora, E., Laudenslager, M., Lemieux, A., and West, S. G. (2001). Inflammatory and hormonal measures predict neuropsychological functioning in systemic lupus erythematosus and rheumatoid arthritis patients. *J. Int. Neuropsychol. Soc.* **7**, 745–754.
126. Grafman, J., Rao, S. M., and Litvan, I. (1990). Cognitive dysfunction in multiple sclerosis: Disorders of memory. In "Neurobehavioral Aspects of Multiple Sclerosis" (S. M. Rao, Ed.), pp. 102–117. Oxford Univ. Press, New York.
127. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1987). The effects of corticosteroids on neurocognitive functioning: A trend toward improvement (abstract). *Can. J. Psychol.* **28**, 132.
128. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1994). Corticosteroids and neuropsychological functioning in patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 1311–1320.
129. Wolkowitz, O. M., Reus, V. I., Weingartner, H., Thompson, K., Breier, A., Doran, A., Rubinow, D., and Pickar, D. (1990). Cognitive effects of corticosteroids. *Am. J. Psychiatry* **147**, 1297–1303.
130. Sakic, B., Szechtman, H., Denburg, S. D., and Denburg, J. A. (1995). Immunosuppressive treatment prevents behavioral deficit in autoimmune MRL-lpr mice. *Physiol. Behav.* **58**, 797–802.
131. Farrell, M., Sakic, B., Szechtman, H., and Denburg, J. A. (1997). Effect of cyclophosphamide on leukocytic infiltration in the brain of MRL/lpr mice. *Lupus* **6**, 268–274.
132. Urowitz, M. B., Gladman, D. D., Tozman, E. C. S., and Goldsmith, C. H. (1984). The lupus activity criteria count (LACC). *J. Rheumatol.* **11**, 783–787.
133. Bombardier, C., Gladman, D. D., Urowitz, M. B., Caron, D., Chang, C. H., and the Committee on prognosis studies in SLE (1992). Derivation of the SLEDAI: A disease activity index for lupus patients. *Arthritis Rheum.* **35**, 630–640.
134. Carbotte, R. M., Denburg, S. D., and Denburg, J. A. (1995). Cognitive dysfunction in systemic lupus erythematosus is independent of active disease. *J. Rheumatol.* **22**, 863–867.
135. Krupp, L. B., LaRocca, N. C., Muir-Nash, J., and Steinberg, A. D. (1989). The fatigue severity scale. Applications to patients with multiple sclerosis and systemic lupus erythematosus. *Arch. Neurol.* **46**, 1121–1123.
136. Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1997). Psychological aspects of SLE: Cognitive function, mood and self-report. *J. Rheumatol.* **24**, 998–1002.
137. Katz, P. P., and Yelin, E. H. (1993). Prevalence and correlates of depressive symptoms among persons with rheumatoid arthritis. *J. Rheumatol.* **20**, 790–796.
138. DeVellis, B. M. (1993). Depression in rheumatological diseases. *Baillieres Clin. Rheumatol.* **7**, 241–257.
139. Shortall, E., Isenberg, D., and Newman, S. P. (1995). Factors associated with mood and mood disorders in SLE. *Lupus* **4**, 272–279.
140. Liang, M. H., Rogers, M., Larson, M., Eaton, H. M., Murawski, B. J., Taylor, J. E., Swafford, J., and Schur, P. (1984). The psychosocial impact of systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* **27**, 13–19.
141. Kremer, J. M., Rynes, R. I., Bartholomew, L. E., Rodichok, L. D., Pelton, E. W., Block, E. A., Tassinari, R. B., and Silver, R. J. (1981). Non-organic non-psychotic psychopathology (NONPP) in patients with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **11**, 182–189.
142. Caine, E. D. (1986). The neuropsychology of depression: The pseudodementia syndrome. In "Neuropsychological Assessment of Neuropsychiatric Disorders" (I. Grant and K. M. Adams, Eds.), pp. 221–243. Oxford Univ. Press, New York.
143. Cassens, G., Wolfe, L., and Zola, M. (1990). The neuropsychology of depressions. *J. Neuropsychiatry Clin. Neurosci.* **2**, 202–213.
144. Burt, D. B., Zembar, M. J., and Niederehe, G. (1995). Depression and memory impairment: A meta-analysis of the association, its pattern, and specificity. *Psychol. Bull.* **117**, 285–305.
145. Hay, E. M. (1994). Psychiatric disorder and cognitive impairment in SLE. *Lupus* **3**, 145–148.
146. Dahlstrom, W. G., Welsh, G. S., and Dahlstrom, L. E. (1975). "An MMPI Handbook, Volume II: Research Applications (rev. ed)." University of Minnesota, Minneapolis.
147. Pincus, T., Callahan, L. F., Bradley, L. A., Vaughn, W. K., and Wolfe, F. (1986). Elevated MMPI scores for hypochondriasis, depression, and hysteria in patients with rheumatoid arthritis reflect disease rather than psychological status. *Arthritis Rheum.* **29**, 1456–1466.
148. Da Costa, D., Dobkin, P. L., Pinard, L., Fortin, P. R., Danoff, D. S., Esdaile, J. M., and Clarke, A. E. (1999). The role of stress in functional disability among women with systemic lupus erythematosus: A prospective study. *Arthritis Care Res.* **12**, 112–119.

149. Beers, S. R., Morrow, L. A., Morgan, M. C., and Manzi, S. (1995). Cognitive function in systemic lupus erythematosus (SLE): Relationship to mood state (abstract). *J. Int. Neuropsychol. Soc.* **1**, 137.
150. Robins, L. N., Helzer, J. E., Weissman, M. M., Orvaschel, H., Gruenberg, E., Burke, J. D., and Regier, D. A. (1984). Lifetime prevalence of specific psychiatric disorders in three sites. *Arch. Gen. Psychiatry* **41**, 949–958.
151. Schneebaum, A. B., Singleton, J. D., West, S. G., Blodgett, J. K., Allen, L. G., Cheronis, J. C., and Kotzin, B. L. (1991). Association of psychiatric manifestations with antibodies to ribosomal P proteins in systemic lupus erythematosus. *Am. J. Med.* **90**, 54–62.
152. Utset, T. O., Golden, M., Siberry, G., Kiri, N., Crum, R. M., and Petri, M. (1994). Depressive symptoms in patients with systemic lupus erythematosus: Association with central nervous system lupus and Sjögren's syndrome. *J. Rheumatol.* **21**, 2039–2045.
153. Wekking, E. M. (1993). Psychiatric symptoms in systemic lupus erythematosus: An update. *Psychosom. Med.* **55**, 219–228.
154. Lim, L., Ron, M. A., Ormerod, I. E. C., David, J., Miller, D. H., Logsdail, S. J., Walport, M. J., and Harding, A. E. (1988). Psychiatric and neurological manifestations in systemic lupus erythematosus. *Q. J. Med.* **66**, 27–38.
155. Rodin, G., Craven, J., and Littlefield, C. (1991). "Depression in the Medically Ill: An Integrated Approach." Brunner/Mazel, New York.
156. Denburg, S., Brasch, J., Carbotte, R., and Denburg, J. (1996). Depression in systemic lupus erythematosus—primary or coincidental? (abstract). *Abstr. Soc. Neurosci.* **22**, 237.
157. Reif, A. E., and Allen, J. M. B. (1964). The AKR thymic antigen and its distribution in leukemias and nervous tissues. *J. Exp. Med.* **120**, 413–433.
158. Wernet, P., and Kunkel, H. G. (1973). Antibodies to a specific surface antigen of T cells in human sera inhibiting mixed leukocyte culture reactions. *J. Exp. Med.* **138**, 1021–1026.
159. Bresnihan, B., Oliver, M., Grigor, R., and Hughes, G. R. V. (1977). Brain reactivity of lymphocytotoxic antibodies in systemic lupus erythematosus with and without cerebral involvement. *Clin. Exp. Immunol.* **30**, 333–337.
160. Bluestein, H. G., and Zvaifler, N. J. (1976). Brain-reactive lymphocytotoxic antibodies in the serum of patients with systemic lupus erythematosus. *J. Clin. Invest.* **57**, 509–516.
161. Huntley, A. C., Fletcher, M. P., Ikeda, R. M., and Gershwin, M. E. (1977). Shared antigenic determinants between rabbit antihuman brain and rabbit antihuman thymocyte sera: Relationship to the lymphocytotoxic antibodies of systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **7**, 269–280.
162. Bluestein, H. G. (1988). Nervous system disease in systemic lupus erythematosus. *Immunol. Allergy Clin. North Am.* **8**, 315–329.
163. Bresnihan, B., Grigor, R. R., and Hughes, G. R. V. (1979). Lymphocytotoxic antibodies in systemic lupus erythematosus: Their clinical significance. *J. Clin. Pathol.* **13**, 112–115.
164. Bluestein, H. G. (1978). Autoantibodies to lymphocyte membrane antigens: Pathogenetic implications. *Clin. Rheum. Dis.* **4**, 643–659.
165. Long, A. A., Denburg, S. D., Carbotte, R. M., Singal, D. P., and Denburg, J. A. (1990). Serum lymphocytotoxic antibodies and neurocognitive function in systemic lupus erythematosus. *Ann. Rheum. Dis.* **49**, 249–253.
166. Temesvari, P., Denburg, J., Denburg, S., Carbotte, R., Bensen, W., and Singal, D. (1983). Serum lymphocytotoxic antibodies in neuropsychiatric lupus: A serial study. *Clin. Immunol. Immunopathol.* **28**, 243–251.
167. Denburg, S. D., Carbotte, R. M., Long, A. A., and Denburg, J. A. (1988). Neuropsychological correlates of serum lymphocytotoxic antibodies in systemic lupus erythematosus. *Brain Behav. Immun.* **2**, 222–234.
168. Denburg, S. D., Denburg, J. A., Carbotte, R. M., Fisk, J. D., and Hanly, J. G. (1993). Cognitive deficits in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **19**, 815–831.
169. Denburg, J. A., Denburg, S. D., Carbotte, R. M., Sakic, B., and Szechtman, H. (1995). Nervous system lupus: Pathogenesis and rationale for therapy. *Scand. J. Rheumatol.* **24**, 263–273.
170. West, S. G., Emlen, W., Wener, M. H., and Kotzin, B. L. (1995). Neuropsychiatric lupus erythematosus: A 10-year prospective study on the value of diagnostic tests. *Am. J. Med.* **99**, 153–163.
171. Singh, V. K., and Fudenberg, H. H. (1986). Can blood immunocytes be used to study neuropsychiatric disorders? *J. Clin. Psychiatry* **47**, 592–595.
172. Steinberg, A. D., and Klinman, D. M. (1988). Pathogenesis of systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **14**, 25–41.
173. Dalgleish, A., Beverley, P., Clapham, P., Crawford, D., Greaves, M., and Weiss, R. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**, 763–773.
174. Klatzman, D., Champagne, E., Chamaret, S., Cruest, J., Guetard, D., Hercend, T., Gluckman, J. C., and Montagnier, L. (1984). T-lymphocyte T4 molecule behaves as a receptor for human retrovirus LAV. *Nature* **312**, 767–768.
175. Cheng-Mayer, C., Rutka, J. T., Rosenblum, M. L., McHugh, T., Stites, D. P., and Levy, J. A. (1987). The human immunodeficiency virus (HIV) can productively infect cultured human stain cells. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3526–3530.
176. Price, R. W., and Brew, B. (1988). Infection of the central nervous system by human immunodeficiency virus: Role of the immune system in pathogenesis. *Ann. N. Y. Acad. Sci.* **540**, 162–175.
177. Ziegler, J. L., and Stites, D. P. (1986). Hypothesis: AIDS is an autoimmune disease directed at the immune system and triggered by a lymphotropic retrovirus. *Clin. Immunol. Immunopathol.* **41**, 305–313.

178. Kopelman, R. G., and Zolla-Pozner, S. (1988). Association of human immunodeficiency virus infection and autoimmune phenomena. *Am. J. Med.* **84**, 82–88.
179. Query, C. C., and Keene, J. D. (1987). A human autoimmune protein associated with U1 RNA contains a region of homology that is cross-reactive with retroviral p30^{gag} antigen. *Cell* **51**, 211–220.
180. Abramsky, O., Lisak, R. P., Silberberg, D. H., and Pleasure, D. E. (1977). Antibodies to oligodendroglia in patients with multiple sclerosis. *N. Engl. J. Med.* **297**, 1207–1211.
181. Waksman, B. H. (1988). Autoimmunity in demyelinating diseases. *Ann. N. Y. Acad. Sci.* **540**, 13–24.
182. Winfield, J. B. (1985). Anti-lymphocyte antibodies in systemic lupus erythematosis. *Clin. Rheum. Dis.* **11**, 523–549.
183. Asherson, R. A., Khamashta, M. A., Gil, A., Vazquez, J.-J., Chan, O., Baguley, E., and Hughes, G. R. V. (1989). Cerebrovascular disease and antiphospholipid antibodies in systemic lupus erythematosus, lupus-like disease, and the primary antiphospholipid syndrome. *Am. J. Med.* **86**, 391–399.
184. Wang, Y., Schrieber, L., Cohen, M. G., Furphy, L., Webb, J., Chivers, T., and Pollard, K. M. (1990). Antiphospholipid antibodies in systemic lupus erythematosus: Clinical and laboratory associations in 111 patients. *Rheumatol. Int.* **10**, 75–80.
185. Kushner, M., and Simonian, N. (1989). Lupus anticoagulants, anticardiolipin antibodies, and cerebral ischemia. *Stroke* **20**, 225–229.
186. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. *Ann. Intern. Med.* **112**, 682–698.
187. Belmont, H. M., Abramson, S. B., and Lie, J. T. (1996). Pathology and pathogenesis of vascular injury in systemic lupus erythematosus. Interactions of inflammatory cells and activated endothelium. *Arthritis Rheum.* **39**, 9–22.
188. Minota, S., and Winfield, J. B. (1987). Identification of three major target molecules of IgM antilymphocyte autoantibodies in systemic lupus erythematosus. *J. Immunol.* **139**, 3644–3651.
189. Minota, S., and Winfield, J. B. (1987). IgG antilymphocyte antibodies in systemic lupus erythematosus react with surface molecules shared by peripheral T cells and a primitive T cell line. *J. Immunol.* **138**, 1750–1756.
190. Minota, S., and Winfield, J. B. (1988). Nature of IgG antilymphocyte autoantibody-reactive molecules shed from activated T cells in systemic lupus erythematosus. *Rheumatol. Int.* **8**, 165–170.
191. Denburg, J. A. (1990). Clinical and subclinical involvement of the central nervous system in systemic lupus erythematosus. In "Immunologic Mechanisms in Neurologic and Psychiatric Disease" (B. H. Waksman, Ed.), pp. 171–178. Raven, New York.
192. Hanly, J. G., Rajaraman, S., Behmann, S., and Denburg, J. A. (1988). A novel neuronal antigen identified by sera from patients with systemic lupus erythematosus. *Arthritis Rheum.* **31**, 1492–1499.
193. How, A., Dent, P. B., Liao, S.-K., and Denburg, J. A. (1985). Antineuronal antibodies in neuropsychiatric systemic lupus erythematosus. *Arthritis Rheum.* **28**, 789–795.
194. Kelly, M. C., and Denburg, J. A. (1987). Cerebrospinal fluid immunoglobulins and neuronal antibodies in neuropsychiatric systemic lupus erythematosus and related conditions. *J. Rheumatol.* **14**, 740–744.
195. Hanson, V. G., Horowitz, M., Rosenbluth, D., Spiera, H., and Puszkin, S. (1992). Systemic lupus erythematosus patients with central nervous system involvement show autoantibodies to a 50-kD neuronal membrane protein. *J. Exp. Med.* **176**, 565–573.
196. Moore, P. M., Vo, T., and Carlock, L. R. (1998). Identification and cloning of a brain autoantigen in neurobehavioral SLE. *J. Neuroimmunol.* **82**, 116–125.
197. Denburg, J. A. (1996). Neuronal antibodies. In "Autoantibodies" (J. B. Peter and Y. Schoenfeld, Eds.), pp. 546–550. Elsevier Science, Amsterdam.
198. Bluestein, H. G. (1993). Antibodies to neurons. In "Dubois' Lupus Erythematosus" (D. J. Wallace and B. H. Hahn, Eds.), 4th ed. pp. 260–263. Lea & Febiger, Philadelphia.
199. Denburg, S. D., Behmann, S. A., Carbotte, R. M., and Denburg, J. A. (1994). Lymphocyte antigens in neuropsychiatric systemic lupus erythematosus: Relationship of lymphocyte antibody specificities to clinical disease. *Arthritis Rheum.* **37**, 369–375.
200. Sanna, G., Piga, M., Terryberry, J. W., Peltz, M. T., Giagheddu, S., Satta, L., Ahmed, A., Cauli, A., Montaldo, C., Passiu, G., Peter, J. B., Shoenfeld, Y., and Mathieu, A. (2000). Central nervous system involvement in systemic lupus erythematosus: Cerebral imaging and serological profile in patients with and without overt neuropsychiatric manifestations. *Lupus* **9**, 573–583.
201. Posner, J. B., and Furneaux, H. M. (1990). Paraneoplastic syndromes. In "Immunologic Mechanisms in Neurologic and Psychiatric Disease" (B. H. Waksman, Ed.), pp. 187–219. Raven, New York.
202. Schnider, A., Bassetti, C., Gutbrod, K., and Ozdoba, C. (1995). Very severe amnesia with acute onset after isolated hippocampal damage due to systemic lupus erythematosus (letter). *J. Neurol. Neurosurg. Psychiatry* **59**, 644–646.
203. Rodgers-Johnson, P. E. B., Ono, S. G., Asher, D. M., and Gibbs, C. J. (1990). Tropical spastic paraparesis and HTLV-1 myelopathy: Clinical features and pathogenesis. In "Immunologic Mechanisms in Neurologic and Psychiatric Disease" (B. H. Waksman, Ed.), pp. 117–130. Raven, New York.
204. Husby, G., van de Rijn, I., Zabriskie, J. B., Abdin, Z. H., and Williams, R. C. (1976). Antibodies reacting with cytoplasm on subthalamic and caudate nuclei neurons in chorea and acute rheumatic fever. *J. Exp. Med.* **144**, 1094–1100.
205. Latov, H., Sherman, W. H., Nemni, R., Galassi, G., Shyong, J. S., Penn, A. S., Chess, L., Olarte, M. R., Rowland, L. P., and Osserman, E. F. (1980). Plasma cell dyscrasia and peripheral neuropathy with a monoclonal

antibody to peripheral nerve myelin. *N. Engl. J. Med.* **303**, 618–621.
206. Ginsberg, J. S., Demers, C., Brill-Edwards, P., Bona, R., Johnston, M., Wong, A., and Denburg, J. A. (1995). Acquired free protein S deficiency is associated with antiphospholipid antibodies and increased thrombin generation in patients with systemic lupus erythematosus. *Am. J. Med.* **98**, 379–383.
207. Andrade, F., Casciola-Rosen, L., and Rosen, A. (2000). Apoptosis in systemic lupus erythematosus. Clinical implications. *Rheum. Dis. Clin. North Am.* **26**, 215–227.
208. Casciola-Rosen, L., Nicholson, D. W., Chong, T., Rowan, K. R., Thornberry, N. A., Miller, D. K., and Rosen, A. (1996). Apopain/CPP32 cleaves proteins that are essential for cellular repair: A fundamental principle of apoptotic death. *J. Exp. Med.* **183**, 1957–1964.
209. Casciola-Rosen, L. A., Anhalt, G. J., and Rosen, A. (1995). DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J. Exp. Med.* **182**, 1625–1634.
210. Casciola-Rosen, L. A., Miller, D. K., Anhalt, G. J., and Rosen, A. (1994). Specific cleavage of the 70-kDa protein component of the U1 small nuclear ribonucleoprotein is a characteristic biochemical feature of apoptotic cell death. *J. Biol. Chem.* **269**, 30757–30760.
211. Casciola-Rosen, L. A., Anhalt, G., and Rosen, A. (1994). Autoantigens targeted in systemic lupus erythematosus are clustered in two populations of surface structures on apoptotic keratinocytes. *J. Exp. Med.* **179**, 1317–1330.
212. Bonfa, E., and Elkon, K. B. (1986). Clinical and serologic associations of the antiribosomal P protein antibody. *Arthritis Rheum.* **29**, 981–985.
213. Bonfa, E., Golombek, S. J., Kaufman, L. D., Skelly, S., Weissbach, H., Brot, N., and Elkon, K. B. (1987). Association between lupus psychosis and anti-ribosomal P protein antibodies. *N. Engl. J. Med.* **317**, 265–271.
214. Vaishnaw, A. K., Toubi, E., Ohsako, S., Drappa, J., Buys, S., Estrada, J., Sitarz, A., Zemel, L., Chu, J.-L., and Elkon, K. B. (1999). The spectrum of apoptotic defects and clinical manifestations, including systemic lupus erythematosus, in humans with CD95 (Fas/APO-1) mutations. *Arthritis Rheum.* **42**, 1833–1842.
215. Herrmann, M., Voll, R. E., and Kalden, J. R. (2000). Etiopathogenesis of systemic lupus erythematosus. *Immunol. Today* **21**, 424–426.
216. Rood, M. J., Keijsers, V., van der Linden, M. W., Tong, T. Q. T., Borggreve, S. E., Verweij, C. L., Breedveld, F. C., and Huizinga, T. W. J. (1999). Neuropsychiatric systemic lupus erythematosus is associated with imbalance in interleukin 10 promoter haplotypes. *Ann. Rheum. Dis.* **58**, 85–89.
217. Forster, M. J., Retz, K. C., and Lal, H. (1988). Learning and memory deficits associated with autoimmunity: Significance in aging and Alzheimer's disease. *Drug Dev. Res.* **15**, 253–273.
218. Sakic, B., Szechtman, H., and Denburg, J. A. (1997). Neurobehavioral alterations in autoimmune mice. *Neurosci. Biobehav. Rev.* **21**, 327–340.
219. Hoffman, S. A., Narendran, A., Shucard, D. W., and Harbeck, R. J. (1988). Autoantibodies, immune complexes, and behavioral disorders: Neuropsychiatric involvement in systemic lupus erythematosus. *Drug Dev. Res.* **15**, 237–251.
220. Fillit, H., Foley, P., Bradford, H. F., Bucht, G., Winblad, B., McEwen, B., Luine, V., and Hardy, J. (1988). Autoimmunity to cholinergic-specific antigens of the brain in senile dementia of the Alzheimer's type. *Drug Dev. Res.* **15**, 143–151.
221. McRae-Degueurce, A., Haglid, K., Rosengren, L., Wallin, A., Blennow, K., Gottfries, C.-G., and Dahlstrom, A. (1988). Antibodies recognizing cholinergic neurons and thyroglobulins are found in the cerebrospinal fluid of a subgroup of patients with Alzheimer's disease. *Drug Dev. Res.* **15**, 153–163.
222. Schwegler, H., Lipp, H.-P., and Crusio, W. E. (1988). NZB mouse: Hippocampal mossy fiber patterns and behavioral profiles of young and older animals. *Drug Dev. Res.* **15**, 297–305.
223. Dunn, A. L., and Crnic, L. S. (1993). Repeated injections of interferon-α A/D in Balb/c mice: Behavioral effects. *Brain Behav. Immun.* **7**, 104–111.
224. Schrott, L. M., Waters, N. S., Boehm, G. W., Sherman, G. F., Morrison, L., Rosen, G. D., Behan, P. O., Galaburda, A. M., and Denenberg, V. H. (1993). Behavior, cortical ectopias, and autoimmunity in BXSB-Yaa and BXSB-Yaa$^+$ mice. *Brain Behav. Immun.* **7**, 205–223.
225. Schrott, L. M., Denenberg, V. H., Sherman, G. F., Waters, N. S., Rosen, G. D., and Galaburda, A. M. (1992). Environmental enrichment, neocortical ectopias, and behavior in the autoimmune NZB mouse. *Brain Res. Dev. Brain Res.* **67**, 85–93.
226. Schrott, L. M., and Crnic, L. S. (1998). Attenuation of behavioral abnormalities in autoimmune mice by chronic soluble interferon-γ receptor treatment. *Brain Behav. Immun.* **12**, 90–106.
227. Sakic, B., Szechtman, H., Keffer, M., Talangbayan, H., Stead, R., and Denburg, J. A. (1992). A behavioral profile of autoimmune lupus-prone MRL mice. *Brain Behav. Immun.* **6**, 265–285.
228. Sakic, B., Szechtman, H., Talangbayan, H., Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1994). Disturbed emotionality in autoimmune MRL-lpr mice. *Physiol. Behav.* **56**, 609–617.
229. Sakic, B., Szechtman, H., Talangbayan, H., Denburg, S., Carbotte, R., and Denburg, J. A. (1994). Behavior and immune status of MRL mice in the postweaning period. *Brain Behav. Immun.* **8**, 1–13.
230. Sakic, B., Szechtman, H., Stead, R., and Denburg, J. A. (1996). Joint pathology and behavioral performance in autoimmune MRL-lpr mice. *Physiol. Behav.* **60**, 901–906.
231. Sakic, B., Denburg, J. A., Denburg, S. D., and Szechtman, H. (1996). Blunted sensitivity to sucrose in autoimmune MRL-lpr mice: A curve-shift study. *Brain Res. Bull.* **41**, 305–311.
232. Sakic, B., Szechtman, H., Braciak, T., Richards, C., Gauldie, J., and Denburg, J. A. (1997). Reduced prefer-

ence for sucrose in autoimmune mice: A possible role of interleukin-6. *Brain Res. Bull.* **44**, 155–165.
233. Szechtman, H., Sakic, B., and Denburg, J. A. (1997). Behaviour of MRL mice: An animal model of disturbed behaviour in systemic autoimmune disease. *Lupus* **6**, 223–229.
234. Maric, D., Millward, J. M., Ballok, D., Szechtman, H., Denburg, J. A., Barker, J., and Sakic, B. (2001). Neurotoxic properties of cerebrospinal fluid from behaviorally impaired autoimmune mice. *Brain Res.* **920**, 183–193.
235. Hanly, J. G., Behmann, S., Denburg, S. D., Carbotte, R. M., and Denburg, J. A. (1989). The association between sequential changes in serum antineuronal antibodies and neuropsychiatric systemic lupus erythematosus. *Postgrad. Med. J.* **65**, 622–627.
236. Hanly, J. G., Walsh, N. M., Fisk, J. D., Eastwood, B., Hong, C., Sherwood, G., Jones, J. V., Jones, E., and Elkon, K. (1993). Cognitive impairment and autoantibodies in systemic lupus erythematosus. *Br. J. Rheumatol.* **32**, 291–296.
237. Hanly, J. G., Hong, C., Smith, S., and Fisk, J. D. (1999). A prospective analysis of cognitive function and anticardiolipin antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 728–734.
238. Menon, S., Jameson-Shortall, E., Newman, S. P., Hall-Craggs, M. R., Chinn, R., and Isenberg, D. A. (1999). A longitudinal study of anticardiolipin antibody levels and cognitive functioning in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 735–741.
239. Whitelaw, D. A., Spangenberg, J. J., Rickman, R., Hugo, F. H., and Roberts, M. (1999). The association between the antiphospholipid antibody syndrome and neuropsychological impairment in SLE. *Lupus* **8**, 444–448.
240. Felten, D. L., and Felten, S. Y. (1987). Immune interactions with specific neural structures. *Behav. Immun.* **1**, 279–283.
241. Neuwelt, C. M., Lacks, S., Kaye, B. R., Ellman, J. B., and Borenstein, D. G. (1995). Role of intravenous cyclophosphamide in the treatment of severe neuropsychiatric systemic lupus erythematosus. *Am. J. Med.* **98**, 32–41.
242. Valesini, G., Priori, R., Francia, A., Balestrieri, G., Tincani, A., Airo, P., Cattaneo, R., Zambruni, A., Troianello, B., Chofflon, M., *et al*. (1994). Central nervous system involvement in systemic lupus erythematosus: A new therapeutic approach with intrathecal dexamethasone and methotrexate. *Springer Semin. Immunopathol.* **16**, 313–321.
243. Barile, L., and Lavalle, C. (1992). Transverse myelitis in systemic lupus erythematosus—the effect of IV pulse methylprednisolone and cyclophosphamide. *J. Rheumatol.* **19**, 370–372.
244. Rao, S. M., Leo, G. J., Ellington, L., Nauertz, T., Bernardin, L., and Unverzagt, F. (1991). Cognitive dysfunction in multiple sclerosis. II. Impact on employment and social functioning. *Neurology* **41**, 692–696.
245. Heaton, R. K., Velin, R. A., McCutchan, J. A., Gulevich, S. J., Atkinson, J. H., Wallace, M. R., Godfrey, H. P., Kirson, D. A., Grant, I., and the HNRC Group (1994). Neuropsychological impairment in human immunodeficiency virus-infection: Implications for employment. *Psychosom. Med.* **56**, 8–17.
246. Rao, S. M., Ed. (1990). "Neurobehavioral Aspects of Multiple Sclerosis." Oxford Univ. Press, New York.
247. Levin, H. S., Eisenberg, H. M., and Benton, A. L., Eds. (1989). "Mild Head Injury." Oxford Univ. Press, New York.
248. Sohlberg, M. M., and Mateer, C. A. (1989). "Introduction to Cognitive Rehabilitation: Theory and Practice." Guilford, New York.
249. Ewing, R., McCarthy, D., Gronwall, D., and Wrightson, P. (1980). Persisting effects of minor head injury observable during hypoxic stress. *J. Clin. Neuropsychol.* **2**, 147–155.
250. Satz, P. (1993). Brain reserve capacity on symptom onset after brain injury: A formulation and review of evidence for threshold theory. *Neuropsychology* **7**, 273–295.
251. Martin, E. M., Pitrak, D. L., Pursell, K. J., Mullane, K. M., and Novak, R. M. (1995). Delayed recognition memory span in HIV-1 infection. *J. Int. Neuropsychol. Soc.* **1**, 575–580.
252. Martin, E. M., Robertson, L. C., Edelstein, H. E., Jagust, W. J., Sorensen, D. J., San Giovanni, D., and Chirurgi, V. A. (1992). Performance of patients with early HIV-1 infection on the stroop task. *J. Clin. Exp. Neuropsychol.* **14**, 857–868.
253. Kujala, P., Portin, R., Revonsuo, A., and Ruutiainen, J. (1994). Automatic and controlled information processing in multiple sclerosis. *Brain* **117**, 1115–1126.
254. Scheffers, M. K., Johnson, R., Grafman, J., Dale, J. K., and Straus, S. E. (1992). Attention and short-term memory in chronic fatigue syndrome patients: An event-related potential analysis. *Neurology* **42**, 1667–1675.
255. Grafman, J., Schwartz, V., Dale, J. K., Scheffers, M., Houser, C., and Straus, S. E. (1993). Analysis of neuropsychological functioning in patients with chronic fatigue syndrome. *J. Neurol. Neurosurg. Psychiatry* **56**, 684–689.
256. Peavy, G., Jacobs, D., Salmon, D. P., Butters, N., Delis, D. C., Taylor, M., Massman, P., Stout, J. C., Heindel, W. C., Kirson, D., Atkinson, J. H., Chandler, J. L., Grant, I., and the HNRC Group (1994). Verbal memory performance of patients with human immunodeficiency virus infection: Evidence of subcortical dysfunction. *J. Clin. Exp. Neuropsychol.* **16**, 508–523.
257. Becker, J. T., Caldararo, R., Lopez, O. L., Dew, M. A., Dorst, S. K., and Banks, G. (1995). Qualitative features of the memory deficit associated with HIV infection and AIDS: Cross-validation of a discriminant function classification scheme. *J. Clin. Exp. Neuropsychol.* **17**, 134–142.
258. Massman, P. J., Delis, D. C., Butters, N., Dupont, R. M., and Gillin, J. C. (1992). The subcortical dysfunction hypothesis of memory deficits in depression: Neuropsychological validation in a subgroup of patients. *J. Clin. Exp. Neuropsychol.* **14**, 687–706.
259. Ryan, L., Clark, C. M., Klonoff, H., and Paty, D. (1993). Models of cognitive deficit and statistical hypotheses: Multiple sclerosis, an example. *J. Clin. Exp. Neuropsychol.* **15**, 563–577.

260. Beatty, W. W. (1992). A strategy for studying memory disorders in multiple sclerosis. In "Neuropsychology of Memory" (L. R. Squire and N. Butters, Eds.), 2nd ed., pp. 285–289. Guilford, New York.
261. Ryan, L., Clark, C. M., Klonoff, H., Li, D., and Paty, D. (1996). Patterns of cognitive impairment in relapsing-remitting multiple sclerosis and their relationship to neuropathology on magnetic resonance images. *Neuropsychology* **10**, 176–193.
262. Von Feldt, J. M., Zeit, B., Bulbul, R., Callegari, P. E., and Gur, R. (1994). A new brief cognitive screen in systemic lupus erythematosus (SLE): Correlation with formal neuropsychiatric testing (abstract). *Arthritis Rheum.* **37**, S178.

29

LUPUS ERYTHEMATOSUS AND THE SKIN

Daniel Mimouni
Carlos H. Nousari

INTRODUCTION

The term *lupus* (Latin for wolf) was used in the medieval period to describe several skin disorders. Some disorders were related to wolflike facies and others to skin lesions resembling wolf bites. These dermatoses included *lupus vulgaris*, a form of cutaneous tuberculosis; *lupus pernio*, a nasal form of cutaneous sarcoidosis, and *lupus erythematosus* (LE), an autoimmune disease.

Cazenave, a French dermatologist and Kaposi, a Hungarian dermatologist, respectively, were the pioneers in describing the cutaneous and systemic involvement of LE. See Rowell [1] for a comprehensive review and discussion of the historical aspects of cutaneous LE.

The importance of thorough dermatologic evaluation for a correct diagnosis and management of LE has been indisputably established since the original description of the disease. This chapter is intended to provide the practicing physician with a practical, yet comprehensive approach for the diagnosis and management of patients with cutaneous manifestations of LE (CLE).

CUTANEOUS FORMS OF LUPUS ERYTHEMATOSUS

Clinical and Immunopathologic Correlation

In addition to a complete history, a methodical physical examination of the skin is mandatory in LE patients. "A literal head-to-toes" screening is a practical and reliable approach to the skin examination. For example, one should start at the head, with a careful examination of the scalp searching for various forms of alopecia and discoid lesions of the ear canals. The exam should extent out to the acral areas such as the hands where periungual telangiectasia or palmar discoid lesions are common. We will discuss in more details more specific findings throughout this chapter where we illustrate a practical, location-oriented approach (Table 1).

To complete the evaluation of the patient, a skin biopsy for routine histology and direct immunofluorescence examination as well as pertinent serologic studies are mandatory. The routine histologic examination of skin biopsies requires a careful reading of a representative specimen, deep incisional sampling in subcutaneous LE, or erythematous, scaly areas in discoid LE (DLE).

The direct immunofluorescence evaluation (DIF) is an extremely helpful diagnostic tool in the assessment of autoimmune skin diseases. This technique consists of an evaluation of bound immunoglobulin and complement on frozen sections using fluoresceinated antisera. This technique is extremely helpful in the interpretation of lesional LE. In the past, the identification of immunoreactants using DIF in nonlesional, non sun-exposed areas was named Lupus band test (LBT) [2, 3]. This LBT was used as predictor of systemic disease. However, with a better understanding of the disease and more reliable tests including antinuclear antibodies,

TABLE 1 Skin Examination, Location, Lesion in Lupus Erythematosus

Head
Scalp
Alopecia: scarring (DLE) vs nonscarring (telogen effluvium)
Ear
Helix—DLE, lupus perniosis (earlobe)
Ear canal—DLE
Cheek
Butterfly (malar rash)—SLE
DLE
Lupus panniculitis
Photosensitivity (in all photodistributed areas)
Mouth
DLE
Aphthous ulcers
Trunk and proximal extremities
Psoriasiform and annular SCLE plaques
Photosensitivity
Poikiloderma
Urticarial vasculitis
Lupus panniculitis
DLE
Distal extremities
Small (palpable and nonpalpable purpura) and medium-sized vasculitis (nodules, ulcers, livedo reticularis [MVV and APLsx])
Palms and soles
DLE
Periungual telangiectasia
Chilblain—lupus perniosis
Small and medium sized vasculitis (digital infarcts)

and complement activation products, among others, the LBT has been abandoned.

In addition to the immunopathologic assessment, a pertinent serologic evaluation is of major importance. For example; the presence of anti-Ro antibodies in neonatal LE or subacute CLE or ANA in the setting of a butterfly eruption are powerful adjunctive diagnostic tools [4]. From a practical perspective, the clinical forms of cutaneous LE are classically divided into: (1) chronic cutaneous (CCLE), including DLE, lupus tumidus and lupus profundus, (2) subacute cutaneous lupus erythematous (SCLE), with annular and psoriasiform variants, and (3) acute systemic LE (ACLE), demonstrating a diffuse, erythematous butterfly eruption. The latter two are more commonly associated with systemic disease. However, all forms of cutaneous LE fall into a spectrum where overlapping skin manifestation is the most common clinical scenario. For didactic purposes we will continue this nomenclature throughout the chapter.

CHRONIC CUTANEOUS LUPUS ERYTHEMATOSUS

Discoid Lupus Erythematosus

Definition

Disk-like plaque lesions.

Clinical Findings

Classic DLE lesions, the most common form of CCLE, are present in 15–30% of SLE (data reviewed in Sontheimer and Provost [2]). Approximately 5% of patients presenting with isolated localized DLE will subsequently develop overt and fulminant systemic lupus erythematosus (SLE) [5]. DLE can present in infants and the elderly; however, it is most common between 20 and 40 years of age. It has a female: male ratio of 3:1, significantly lower than that of SLE and other forms of CLE such as SCLE. An increased prevalence in African-Americans has been well established.

Significant increases of human leukocyte antigen (HLA) B7, B8, Cw7, DR2, DR3, DQW1, and a significant decrease in HLA-A2 has been reported for DLE patients [6]. The combinations of HLA-Cw7, DR3, DQW1, and HLA-B7, Cw7, DR3 conferred the maximum relative risk (7.4) for DLE.

Immunodeficiency states are known to have an increased incidence of autoimmune disorders. DLE also occurs with increased frequency in female carriers of x-linked chronic granulomatous disease, common variable immunodeficiencies, and C1q, C2, and C4 deficiencies [7, 8].

Discoid lupus erythematosus lesions are discrete, erythematous well-defined plaques covered by an adherent scale that extends into dilated hair follicles. Hyperpigmentation is often present in the initial phases; however, eventual loss of pigment occurs in the center of older, scarred lesions. The plaques slowly expand, with active indurated erythema at the periphery, leaving depressed scars, telangiectasias, and depigmentation that are often permanent. The central atrophic scarring is very characteristic. These lesions occur most often on the head and neck areas, in particular the ear canal (Fig. 1) and scalp (Fig. 2) should always be examined. It should be noted that lichen planopilaris of the scalp may mimic both the clinical and histopathological picture of DLE.

The term *localized DLE* is used to describe those patients who have lesions only on the head or neck. *Generalized DLE* is the preferred designation when lesions are present both above and below the neck (Figs. 3, 4, 5). Of the patients with localized DLE, 90–95% will continue to have disease limited only to the skin [5,

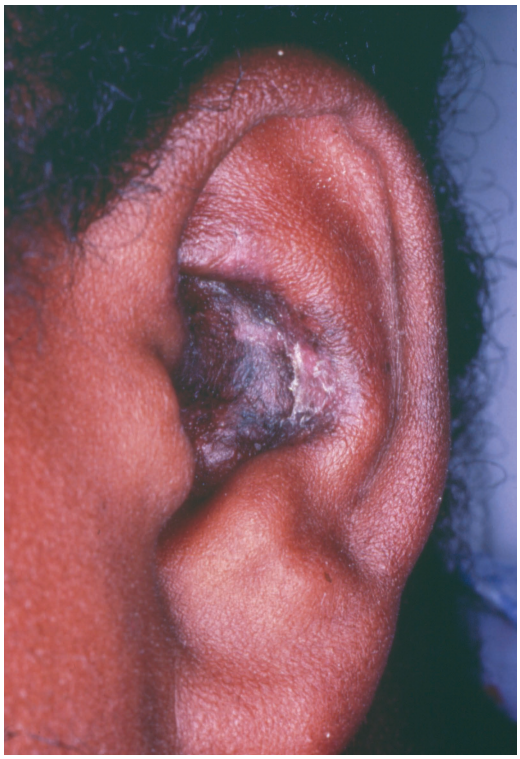


FIGURE 1 Discoid lupus erythematosus lesion of ear canal showing atrophic erythematous plaques with postinflammatory hyperpigmentation.

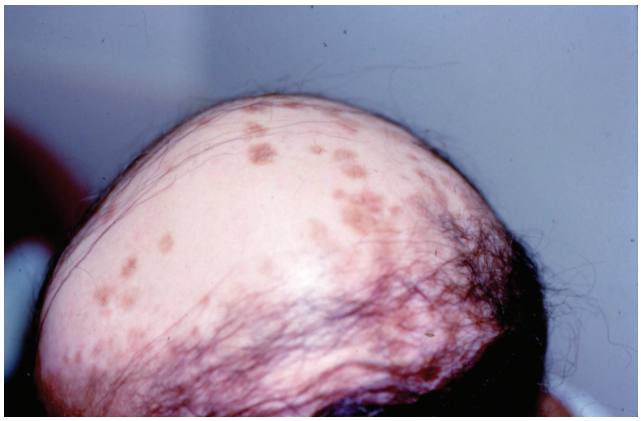


FIGURE 2 Burnt out discoid lupus erythematosus lesions of the scalp showing scarring alopecia with postinflammatory hypo- and hyperpigmentation.

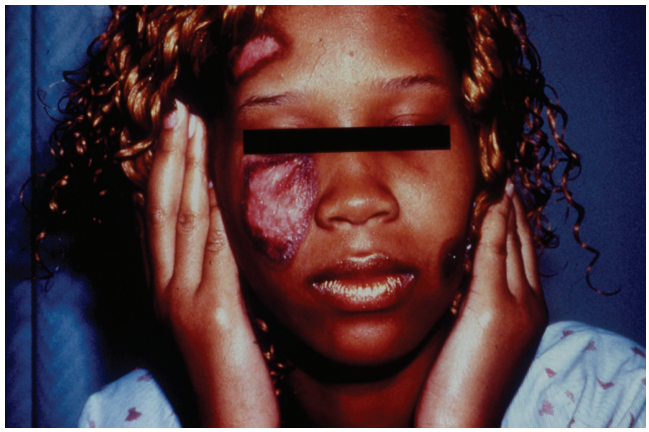


FIGURE 3 Generalized discoid lupus erythematosus with extensive disfiguring facial involvement.

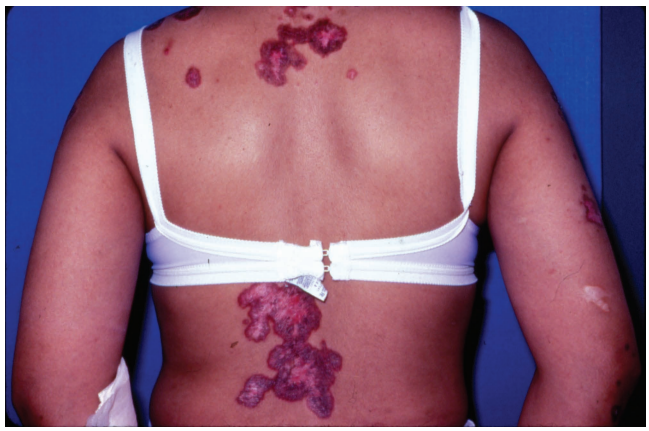


FIGURE 4 Generalized discoid lupus erythematosus with trunkal lesions.

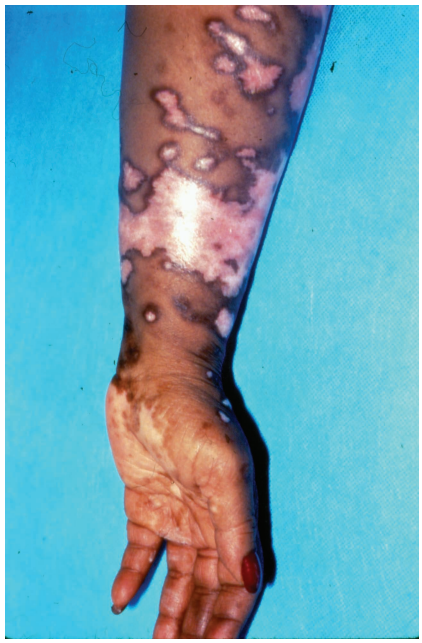


FIGURE 5 Generalized discoid lupus erythematosus with large lesions affecting arms and hands.

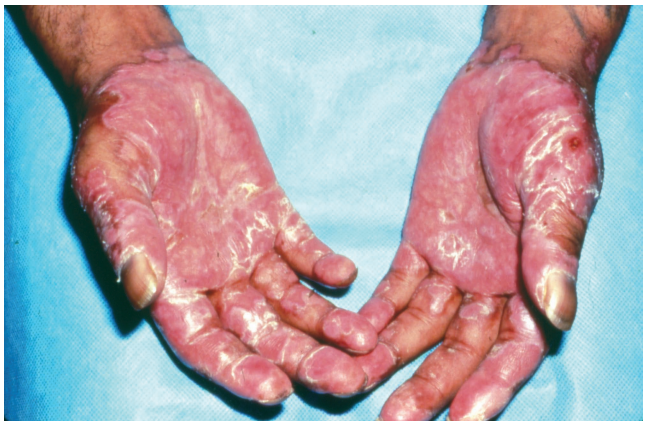


FIGURE 6 Palmoplantar discoid lupus erythematosus with diffuse atrophic erythematous scaling lesions affecting the palms.

9–15]. Although laboratory abnormalities such as positive anti-nuclear antibody (ANA), false positive tests for syphilis, leukopenia, and increases in the erythrocyte sedimentation rate suggest low-grade systemic disease, aggressive or life-threatening systemic LE rarely develops. Patients with generalized DLE appear to be at somewhat higher risk for having or developing SLE compared with localized DLE [14–16].

Palmoplantar and mucosal DLE lesions lack specific clinical features (Fig. 6). One of the main reasons is the lack of hair follicles at those sites, hence, the absence of follicular plugging as a cardinal clinical sign. One the most common clinical and histologic misdiagnoses given to patients with palmoplantar and mucosal DLE is lichen planus. Patients with palmoplantar DLE commonly have periungual involvement leading to nail dystrophy. The lack of pruritus in these cutaneous LE lesions attests firmly against a diagnosis of lichen planus.

The clinical and even histologic similarities between LE and lichen planus in mucous membranes are more striking and challenging. However, a few clinical tips often help in the differentiation: (1) Primary mucosal DLE is extremely rare, and is virtually always occurring in the setting of cutaneous DLE. (2) Oral DLE is most commonly seen in the buccal mucosa [17]. The vast majority of these patients have concomitant labial DLE. Similarly, conjunctival DLE often complicates patients with eyelid DLE.

Relationship with SLE

Patients tend not to develop clinical and/or serologic evidence of SLE; however, approximately 5% of patients presenting with isolated localized DLE will subsequently develop full-blown SLE [5]. Table 2 summarizes the predictors of associated systemic disease in patients with DLE.

TABLE 2 Predictors of Systemic Disease in Patients with DLE Lesions

Generalized DLE lesions
Palmoplantar DLE
DLE in setting of genetic complement deficiencies
DLE associated with constitutional symptoms
DLE associated with acute phase reactants or complement activation
DLE setting with a nonspecific ANA at significant titers (fine speckled >1:320)
DLE setting with a specific ANA (Anti Sm, Ro)
DLE in male patients?

Immunopathology

Routine Histology

All of the clinical presentations of DLE have a common dermatopathologic finding: A lymphocytic dominant, bandlike infiltrate at the dermal–epithelial junction (also referred to as interface infiltrate or lichenoid infiltrate). This infiltrate usually disrupts the basement membrane and epithelial basal cell layer (vacuolar degeneration). Other findings include periadnexal infiltrates, thickening of the basement membrane, dermal mucin deposition, and epidermal atrophy. All of these findings may be valuable but are not constant features.

DIF

Virtually all DLE lesions have deposition of immunoreactants at the basement membrane zone (BMZ) of the epidermal–dermal junction [18]. However, false negative and positive results are sometimes observed, primarily due to technical factors.

Any immunoreactant can be observed at the BMZ, however, by far the most common in DLE is granular "ribbon-like," coarse IgM deposits along the BMZ. IgG, IGA, and C3 are occasionally observed (Fig. 7). The immunologic findings of early lesions are more prominent at the adnexal BMZ pilosebaceous unit than at the epidermal BMZ.

Prognosis

The natural history is variable; however, in most of the cases the DLE will eventually resolve without therapy [19–21]. Therefore, the necessity of therapy is dictated by (1) The prevention of permanent atrophy and potential disfiguring sequellae (2) The potential benefit of systemic therapy in preventing new lesions.

It has been suspected that around 15% of patient with DLE would develop or already have systemic disease [19]. However, generalized DLE and palmo-

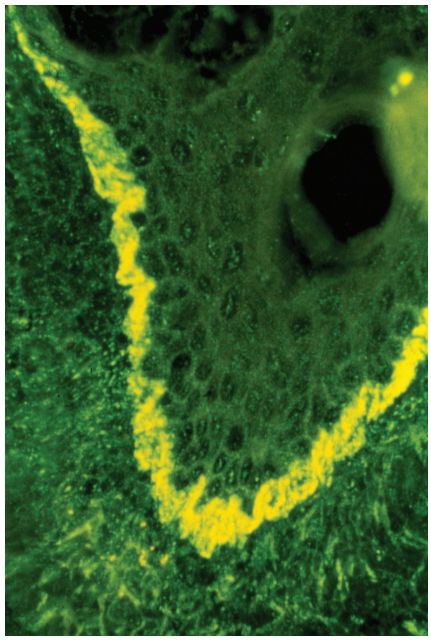


FIGURE 7 Direct immunofluorescence examination of a discoid lupus erythematosus lesion. There is a thick granular band of IgM deposited along the dermal–epidermal junction.

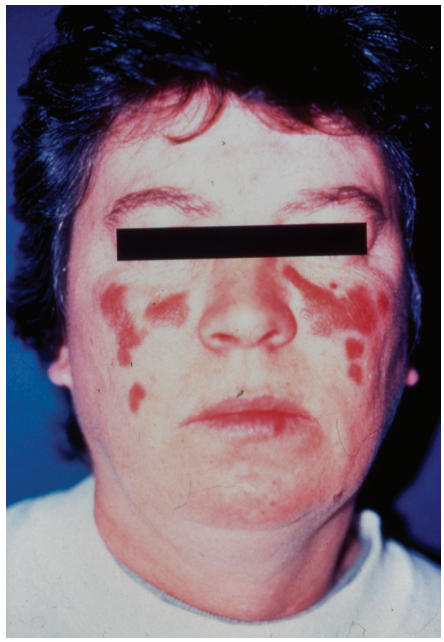


FIGURE 8 Face of a patient with lupus tumidus showing erythematous, edematous, infiltrative plaques on the cheeks.

plantar DLE have a higher frequency of constitutional symptoms and evidence of overt systemic disease than other subsets of DLE. Oral mucosal DLE lesions can degenerate into squamous cell carcinoma, as can be seen in long-standing cutaneous DLE lesions [22]. Any degree of nodular asymmetry within a mucosal DLE lesion should be evaluated for the possibility of malignant degeneration.

LUPUS Tumidus

Definition

Swollen, puffy lesions.

Clinical Findings

This frequently underrecognized subset of LE is characterized by urticaria-like papules, nodules, or plaques favoring head and neck areas, upper trunk, and proximal extremities. Plaques are commonly 2–4 cm with rounded or annular configuration (Fig. 8).

In contrast to classic urticaria, lesions in lupus tumidus (LT) are nonpruritic and last longer than 24–48 h [23]. As opposed to DLE and SCLE, lupus tumidus lesions lack the epidermal changes of hyperkeratosis, atrophy, depigmentation, and follicular plugging. The prevalence of this subset of LE is unknown; however, it seems to be higher in the Asian population. Several conditions included under the rubric of benign lymphocytic infiltrates of the skin may represent misdiagnosed cases of lupus tumidus.

Relationship with SLE

Patients tend not to develop clinical and/or serologic evidence of overt SLE; however, nonspecific findings such as low titer ANA, myalgias, intermittent mild cytopenias, and elevated ESR are not uncommon.

The sun-exposed location, higher prevalence in summertime, and benefit of some patients with sunblocks are indirect evidence of a sun-related variant in at least some of these patients. However, despite the higher association of anti-Ro antibodies in Asians, this feature has not been consistently found in non-Asians [24–26].

Immunopathology

Good clinicopathologic correlation and high index of suspicion of this variant is necessary to achieve a correct diagnosis. The histologic features are dominated by a perivascular and periadnexal lymphocytic infiltrate with minimal interface dermatitis. The presence of mucin in these specimens is an important histologic clue for the diagnosis [27, 28]. Although increased mucin is not a specific finding, it supports the diagnosis in the context of the more specific clinical findings.

Lupus tumidus may be interpreted as a variant of LE with dermal predominance. Benign lymphocytic infiltration, Jessner's lymphocytic infiltration, polymorphous light eruption, and reticular erythematous

mucinosis should all be included in the differential diagnosis [23]. However, It is noteworthy to mention that several cases of the so-called Jessner's lymphocytic infiltration and other "benign" lymphocytic infiltrates of the skin may have been misdiagnosed cases of LT.

DIF

Due to the scant or even lack of interface dermatitis in these lesions, deposition of immunoreactants at the BMZ is not usually observed. However, DIF remains an important tool in excluding other conditions that may clinically mimic LT, for example, urticarial vasculitis and annular lesions of SCLE.

Prognosis

Generally good. The lesions tend to resolve with or without treatment over years. Most of the patients respond to antimalarials and sunblocks.

Lupus (Profundus) Panniculitis

Definition

Lupus erythematosus of the skin with primarily and predominantly involvement of the subcutaneous fat (panniculus) [29–32].

Clinical Findings

The lesions of lupus panniculitis (LP) are deep, firm nodules and plaques with or without overlying DLE features. They occur primarily in areas prone to trauma, namely, on the cheeks, shoulders, thighs, and breast; however, any area can eventually be involved (Fig. 9). LP may produce breast nodules that mimic carcinoma clinically and radiologically (i.e., lupus mastitis) [33].

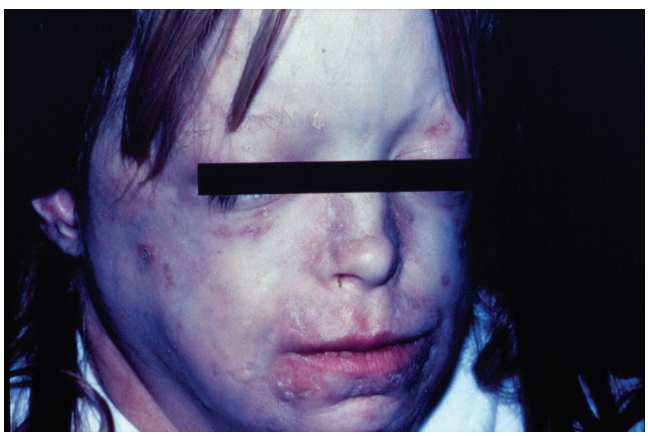


FIGURE 9 Girl with extensive atrophic plaques of lupus panniculitis (profundus) affecting the cheeks, and discoid lupus erythematosus plaques in the nose and eyes.

As older lesions of LP resolve, deep depressions and dystrophic calcification that at times can produce extensive ulcerations are not uncommonly encountered.

Most of the patients present with one or two lesions. However, generalized cases can also be seen, especially in the setting of hereditary complement deficiencies [34]. As opposed to patients with localized disease, most, if not all, patients with generalized LP have concomitant DLE overlying or elsewhere [35]. The lack of this feature should prompt the reevaluation of the diagnosis of LP in the setting of a lobular lymphocytic panniculitis.

It has been shown that other connective tissue diseases including morphea, dermatomyositis, and overlap syndrome may have inflammatory panniculitis lesions that are both clinically and histologically indistinguishable from LP [36]. The differential diagnosis includes other causes of panniculitis: (1) neoplastic; (2) inflammatory; and (3) infectious.

1. In the neoplastic category, the main disease for consideration is the subcutaneous panniculitis T-cell lymphoma. The vast majority of cases have been initially misdiagnosed primarily in the setting of hemophagocytosis-associated cytopenias and constitutional symptoms.

Several clinical clues aid in the differentiation: (A) absence of DLE lesion overlying the panniculitis or elsewhere in the setting of numerous panniculitic plaques, (B) the presence of significant constitutional symptoms (e.g.: fever, myalgias), (C) absence of significant serologic findings, (D) severe cytopenias in the setting of LE, (E) significant increase of LDH in absence of evidence of hemolytic anemia, and (F) atypical histopathology for LP.

2. In the inflammatory category: alpha 1 anti-trypsin deficiencies, pancreatitic panniculitis, sarcoidosis, factitial disorders, the so-called Weber Christian disease, and /or cytophagic panniculitis (the two last disorders have been definitively proved to be reclassified as LP, subcutaneous lymphomas, or any of the aforementioned inflammatory panniculitides) [37]. All these diagnoses require careful clinicopathologic correlation.

3. Infectious: deep-seated common bacterial pathogens, mycobacteria, and fungal pathogens are the most common mimickers of LP.

Relationship with SLE

Like in DLE and LT, most of the patients with LP do not have overt SLE symptoms. However, patients with generalized lesions have an increased incidence of smoldering or even overt SLE.

Immunopathology

The cardinal histologic feature of LP is lobular-predominant lymphocytic panniculitis. There are no specific features; however, follicular aggregates with germinal center formation, significant plasma cell infiltrates, and hyalinization of fat lobules and vessels, with variable interface dermatitis of the adnexal and/or epidermal BMZ are very characteristic. Only the few patients with significant interface dermatitis of the epidermal and/or adnexal BMZ would have granular deposits of immunoreactants.

The presence of the following features strongly argue against the diagnosis of LP and favor the most serious differential diagnosis, namely: subcutaneous panniculitic lymphoma: (1) cytologic atypia, (2) karyorrhexis, (3) lymphocyte rimming around adipocytes, (4) histiocytes with cytophagia, and (5) evidence of monoclonality in the T/NK cell infiltrate [38]. As in any inflammatory skin disease, to achieve a correct final diagnosis, it is mandatory to conduct a thorough clinicopathologic correlation.

Prognosis

The lesions of LP as a rule resolve spontaneously. In most patients this occur within 3–4 years. Because of the visible locations and cosmetic implications, effective immunosuppressive and/or immunomodulatory drugs should be started promptly. It is imperative that any kind of surgical or reconstructive procedure be done after complete and sustained remission.

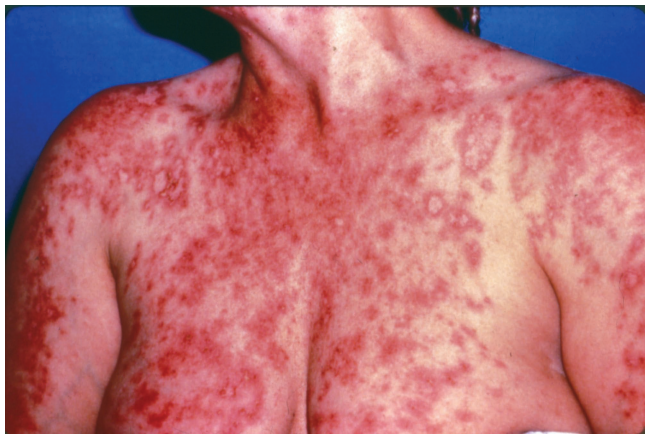


FIGURE 10 Chest and proximal upper extremities of a patient with annular form of subacute cutaneous lupus erythematosus.

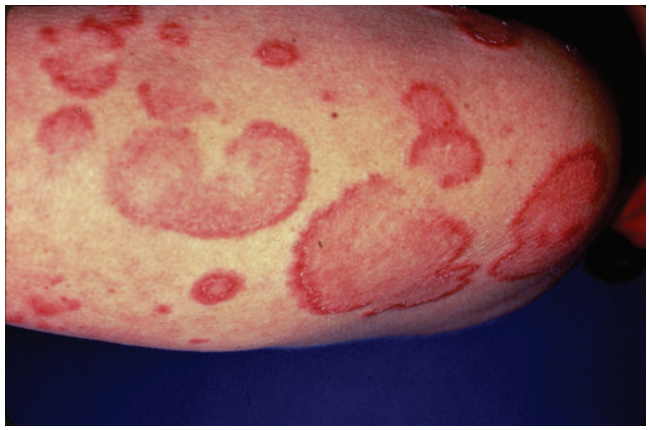


FIGURE 11 Close up of annular subacute cutaneous lupus erythematosus lesions showing arcuate erythematous plaques with discreet scaling and atrophy.

SUBACUTE CUTANEOUS LUPUS ERYTHEMATOSUS

Definition

Cutaneous form of the disease that clinically and immunopathologically oscillates between DLE and SLE. It is strongly associated with photosensitivity and anti-Ro antibodies.

Clinical Findings

Clinical findings are ill-defined, noninfiltrative plaques favoring trunk and proximities, with predilection for, but not limited to sun-exposed areas. Two clinical subsets have been described: (1) annular and, (2) psoriasiform. The former tend to have less epidermal involvement and occasionally an urticarial configuration (Figs. 10 and 11). Frequently, the annular form will have a centripetal location as opposed to the psoriasiform which often presents with significant scales and more centrifugal distribution (Fig. 12). It is mandatory to include the psoriasiform SCLE subset in the differential of psoriasiform dermatitis, especially before enrolling a patient for phototherapy. The "Rowell syndrome" (erythema multiforme-like lesion in LE) represent in the majority of the cases the annular variant of SCLE.

A subset of drug-induced, Ro positive LE has been described in the literature (Table 3 illustrated the cutaneous LE variants in the presence of anti-Ro). As opposed to the classic anti-histone induced LE these patients have striking cutaneous findings. These lesions are the same as SCLE lesions and/or diffuse photosensitive erythema. The most common implicated drugs are: anti hypertensives (hydrochlorothiazide, angiotensin converting enzyme inhibitors, calcium

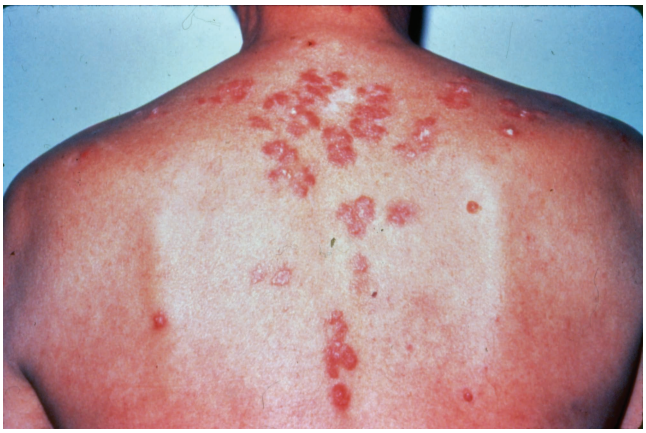


FIGURE 12 Papulosquamous lesions of subacute cutaneous lupus erythematosus. These lesions frequently involve the upper chest, upper back, shoulders, extensor surfaces of the arms, and dorsum of the hands and fingers and have a psoriasiform configuration.

TABLE 3 Different Clinical Settings of Cutaneous LE with Anti-Ro Antibody

Neonatal LE
Subacute cutaneous LE
Cutaneous LE with Sjögren's syndrome
Drug-induced cutaneous LE
Cutaneous LE in elderly
Cutaneous LE and photosensitivity

TABLE 4 Drugs Associated with Cutaneous Ro LE

Antihypertensive: hydrochlorothiazides, calcium blockers, ACE inhibitors
Antifungals: terbinafine, griseofulvin
Others; statins, interferons, minocyclin

channel blockers), antifungals (terbinafine, griseofulvin), and interferon (Table 4) [39–43].

Genetic deficiencies of various complement components, including C2, C3, C4, and C5, as well C1 esterase inhibitor, have been associated with both SCLE and DLE.

Relationship with SLE

Approximately one-half of these patients can be classified as having SLE by the American College of Rheumatology revised criteria; however, serious central nervous system disease or progressive renal disease is uncommon. Most of these patients are anti-Ro antibody positive. A significant number of these patients have smoldering systemic disease. Interestingly, the incidence of overt Sjögren's syndrome, another subset of LE strongly associated with anti-Ro, is not commonly seen in SCLE patients. Women with SCLE and anti-Ro antibodies represent the highest risk group for the development of neonatal LE in their offspring [44].

Immunopathology

The interface dermatitis in the annular variant is often patchy, without significant periadnexal infiltrates, mucin deposition, or follicular plugging. More obvious histologic evidence of the disease is seen in the psoriasiform variant.

Direct immunofluorescence evaluation has a highly characteristic immunofindings: granular IgG with fine specking "dustlike" [45] deposits along the BMZ and throughout the lower epidermis [29]. Although this is a very sensitive finding, it is not specific, and can be seen in SLE patients. Variable granular C3 or another immunoreactant can also be seen.

Prognosis

A quarter of these patients will develop overt SLE. However, the vast majority will have variable sign and symptom throughout the course of the disease. Although complete resolution is common with or without therapeutic intervention, relapses are almost the rule in these patients. It is imperative to identify the drug-induced variant since discontinuation of the drug is commonly associated with complete resolution of the disease. As will be discussed in the treatment section, photoprotection is the most important therapeutic measure.

Neonatal LE

Definition

Neonatal LE (NLE) is defined by the presence of cutaneous LE lesions in the newborn with or without systemic involvement.

Clinical Findings

Neonates from mothers with anti-Ro and/or RNP1 SLE develop cutaneous disease. The most common and characteristic eruption is a periorbital "owl-eye" appearance (heliotrope-like) with erythematous and violaceous, often-scaly patches. However, other lesions,

including annular scaly plaques and cutis marmorata, are also commonly seen in these newborns. Overall, the cutaneous lesions in NLE favor head and neck areas and extremities. The cutaneous manifestations of NLE as a rule would resolve spontaneously in less than a year. Systemic involvement is seen in less than 20% of NLE and includes: (1) cardiac disease, namely heart block. When this complication occurs it is permanent; (2) transient hematologic findings including cytopenias; and (3) increased liver enzymes [46].

Relationship with SLE

Virtually all mother and neonates are anti-Ro positive. About a third of these patients may have concomitant anti-RNP1. There is no clear association between the autoantibodies and the clinical features of NLE patients. It is not clear whether children with past medical history of NLE are at higher risk for developing any form of LE in their adulthood.

Immunopathology

Histologic examination shows interface dermatitis, and DIF demonstrate granular IgG deposits along the BMZ and throughout the lower epidermis. These immunofindings are identical to those seen in SCLE lesions.

Prognosis

Cutaneous, hematologic, and hepatic complications resolve spontaneously and do not impose a significant clinical challenge. Conversely, high index of suspicion about this diagnosis is required since, the cardiac complications are permanent and potentially life threatening. The influence of treating the mother to decrease the incidence of NLE is unknown; however, effective management including sun-protection, antimalarials, and immunosuppressive therapy throughout pregnancy is highly recommended.

ACUTE CUTANEOUS LUPUS ERYTHEMATOSUS

Definition

Cutaneous form of LE in the setting of active systemic disease.

Clinical Findings

These cutaneous lesions occur always in the setting of "overtly" active systemic disease. The most common presentation is usually overlapping cutaneous lesions. The typical lesions are classified as follow: (1) Butterfly malar rash, (2), erythematous maculopapular scaly rash (3) mucosal apthae, (4) photosensitivity, (5) diffuse nonscarring alopecia (telogen effluvium), and (6) vasculitis

1. **Butterfly eruption** (Figs. 13 and 14) has been traditionally underscored as cutaneous finding of LE. One of the most common misdiagnoses in a butterfly eruption is rosacea. The following clinical features help distinguishing these two entities: (1) butterfly malar LE eruption rarely occurs as a sole manifestation of active cutaneous LE. This type of eruption has a strong association with photosensitivity, and therefore virtually all of these cases would have other cutaneous photosensitive findings. (2) Butterfly malar LE eruption is a manifestation of SLE and always occurs in the setting of "systemic disease." In the past other distinguishing features were overemphasized including: involvement of the nasolabial fold, significant telangiectasias without poikiloderma, and presence of follicular pustules, more features that favor rosacea or seborrheic dermatitis over LE, However, these "clinical pearls" often lead to misdiagnoses, and should be utilized with great caution.
2. **Diffuse maculopapular eruption** (Fig. 15) is often scaly and more prominent in photoexposed areas;

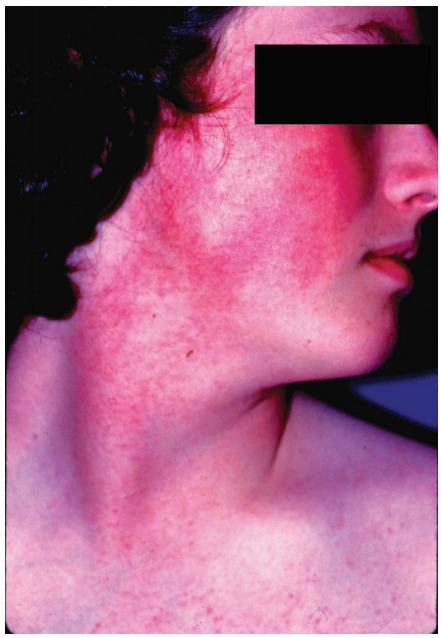


FIGURE 13 Localized acute cutaneous lupus erythematosus in a patient with a flare of systemic lupus erythematosus showing a photosensitive diffuse erythema of the face with a butterfly configuration.

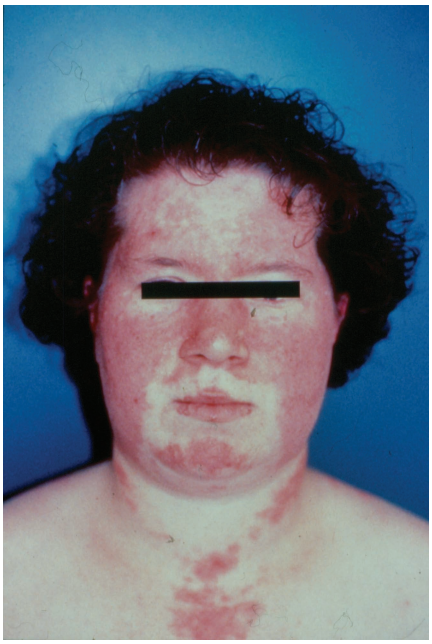


FIGURE 14 Acute cutaneous lupus erythematosus in a patient with a flare of systemic lupus erythematosus showing a photosensitive diffuse erythema of the face with a butterfly configuration with periorbital, perioral, and nasolabial fold sparing.

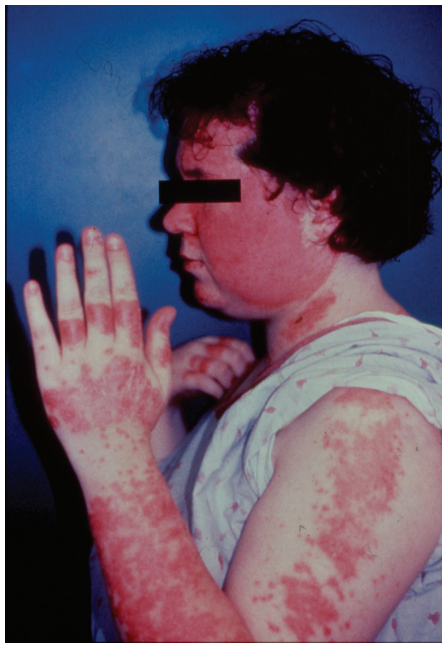


FIGURE 15 Same patient as in Fig. 14 with a diffuse maculopapular and scaly eruption affecting the face, trunk, and upper extremities. Note the sparing of the dorsal aspects of the interphalangeal and metacarpophalangeal joints.

however, covered areas are commonly involved. This manifestation often but not always occurs in the presence of the butterfly eruption.

3. **Recurrent aphthous stomatitis** (RAS) in the setting of SLE is a common feature. These ulcers have a variable degree of pain, the size varies from a few millimeters (minor) to centimeters (major). Since, RAS affects up to 20% of normal people, this mucosal finding should be taken cautiously. The presence of active SLE, atypical location for aphthae, for example, palate, and absence of family history of RAS, favor SLE-associated RAS over idiopathic aphthae.

Photosensitivity, **alopecia**, and **vasculitis** will be discussed in other sections of this chapter.

Immunopathology

The immunopathology is interface dermatitis without significant hyperkeratosis or epidermal atrophy, without significant mucin deposition or periadnexal infiltrates. DIF is positive in all patients and is manifested by granular IgG and C3 along the BMZ (Fig. 16). Other immunoreactants can also be seen. Often *in vivo* ANA is seen in these specimens.

In the past the term LBT was used to predict or diagnose SLE. This test should be abandoned due to the highly frequency of erroneous results and availability of more accurate tests to diagnose SLE.

Prognosis

Acute cutaneous lupus erythematosus has no relationship with any specific ANA. However, anti-Ro and

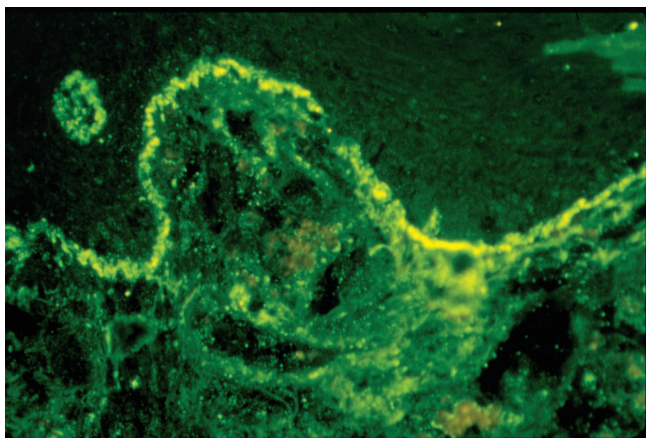


FIGURE 16 Direct immunofluorescence evaluation of lesional skin in a patient with acute cutaneous lupus erythematosus showing granular IgG deposition along the basement membrane zone.

anti-DsDNA and anti-RNP are commonly found in these patients. Photoprotection and antimalarials are helpful as adjuvant therapy. Topical steroids are pointless since an immune complex mediated disease should be treated by systemic immunosuppressive therapy.

PATHOGENESIS OF CUTANEOUS LUPUS ERYTHEMATOSUS

Increasing evidence suggests that autoantigen-specific T cells play a role in the pathogenesis of other organ-specific autoimmune disorders such as autoimmune thyroiditis and multiple sclerosis. Autoreactive T cells could also play a role in the pathogenesis of forms of CLE such as DLE that are not associated with specific autoantibodies. Evidence suggests that T cells in CLE lesions are oligoclonal and are predominately of the $CD4^+$ Th1 phenotype, supporting the idea that antigen-driven T-cell proliferation is occurring within such lesions [47]. T cells recognizing autoantigens being presented by epidermal Langerhans cells might help explain the decreased numbers of Langerhans cells in areas of active CLE inflammation. Epidermal keratinocytes in CLE lesions often express class II histocompatibility antigens as well as adhesion molecules involved in T-cell binding interactions such as intercellular adhesion molecule 1 (ICAM-1). Epidermal basal cell CD40 ligation by infiltrating T cells could contribute to the altered epidermal kinetics that have been observed in CLE lesions (hyperproliferation, normal early differentiation, premature terminal differentiation).

The pathogenic effects of UV (ultraviolet) light in cutaneous LE are poorly understood. Proposed mechanisms include neoantigen formation and autoantigen modulation, exaggerated release of immune mediators, and perturbation of cutaneous immunoregulatory circuits [48, 49]. Ultraviolet light has been suggested to induce the expression of "neoantigens" that could become the target of a dysregulated immune attack. It has also been shown that UVB can displace autoantigens such as Ro/SSA and the related autoantigens, La/SSB and calreticulin, from their normal locations inside epidermal keratinocytes to the cell surface. Evidence suggests that UV-induced keratinocyte apoptosis may be a major mechanism responsible for this aberrant pattern of cell-surface autoantigen expression [50]. Cell-surface expression would allow Ro/SSA, La/SSB, and calreticulin autoantibodies present in the circulation to bind to the autoantigens that are normally sequestered from the humoral immune response inside cells. Autoantibody binding to the exposed antigens could result in tissue injury through complement-mediated lysis or antibody-dependent cell-mediated cytotoxicity. Infection by some viruses also appears to be capable of inducing cell-surface expression of Ro/SSA, La/SSB, calreticulin, and related autoantigens in cells undergoing viral-induced apoptosis. It is possible that cellular mechanisms other than UV-induced apoptosis are responsible for perturbed autoantigen expression in epidermal keratinocytes and that humoral factors other than anti-Ro/SSA might also be involved in the pathogenesis of LE photosensitivity.

Ultraviolet light may also cause an exaggerated release of immune mediators such as interleukin-1 (IL-1), tumor necrosis factor (TNF-α), prostaglandin E, proteases, oxygen-free radicals, and histamine in genetically predisposed individuals with LE. IL-1 receptor antagonist and TNF-α gene polymorphism has been implicated as a genetic factor in CLE. Aberrant expression of adhesion molecules, such as ICAM-1, could also play a role in the pathogenesis of LE photosensitivity. Ultraviolet light may also directly affect immunoregulatory cells, such as cutaneous T cells that normally help suppress "abnormal" patterns of cutaneous inflammation. These and related issues have been discussed elsewhere in greater depth than is possible here [48, 49, 51–53].

Other Clinical Presentations

Photosensitivity

Photosensitivity has been defined by the American College of Rheumatology within its 11 classification criteria set for SLE as "Unusual skin reaction from exposure to sunlight, by patient's history or physician's observation [54]." This rather broad definition could include a heightened response to UV-induced erythema (i.e., a decreased minimal erythema dose), an abnormally prolonged UV-induced erythema phase (i.e., the sunburn that does not resolve at a normal rate), the elicitation or exacerbation of CLE skin lesions, or the elicitation or exacerbation of completely unrelated photosensitive skin conditions (e.g., acne rosacea, polymorphous light eruption, photoallergic contact dermatitis). In fact, work has suggested that as many as 50% of SLE patients also suffer from polymorphous light eruption, a benign photodermatosis without systemic manifestations [55]. The subjective aspect of the American College of Rheumatology's definition of photosensitivity and its lack of specificity raise serious questions as to its usefulness in either clinical or experimental studies, and it has been argued that some type of quantifiable end point determined by phototesting should be included in the definition of LE photosensitivity [56, 57]. Exposure to sunlight or artificial sources

of UV irradiation (unshielded fluorescent lighting, some photocopiers) is a well-recognized and frequent precipitating factor for both the systemic and cutaneous manifestations of LE (see Sontheimer [48] for a comprehensive review of all aspects of LE photosensitivity). Patients with SCLE and ACLE are especially photosensitive, whereas those with DLE show less photosensitivity. The presence of anti-Ro/SSA autoantibody is an additional risk factor for LE photosensitivity, thus the high rate of photosensitivity in patients with SCLE lesions. Early studies demonstrated that CLE lesions could be provoked in the clinically normal skin of patients with both SLE and CLE by repeated delivery high doses of UVB to the same test site. More recent studies have argued that UVA can also induce CLE lesions, especially SCLE [58–60]. In addition, long wave UVA-I has been reported to be capable of playing a role in SLE photosensitivity [56], an observation that is somewhat contradictory to the more recent work suggesting that UVA-I phototherapy might be of therapeutic value in CLE and SLE. Mononuclear cell accumulation in dermal perivascular areas has been the first recognizable pathologic change in UV-induced LE skin lesions. Deposition of Ig and complement components at the dermal–epidermal junction has been observed to follow the appearance of perivascular cellular inflammation in UV-challenged skin arguing against a primary, initiating role for such immune deposits in the pathogenesis of LE-specific skin disease.

From a practical perspective it is wise and prudent to assume that all CLE patients have variable degrees of photosensitivity most commonly to UVB, but also to UVA and visible light. A good history and physical examination is reliable and more practical than phototesting for the diagnosis and management in CLE patients.

Cutaneous Vasculitis

The definition of cutaneous vasculitis has remained a challenge for the clinician as well as for the dermatopathologist. A more comprehensive and practical definition is the following: "*cutaneous vasculitis is an idiopathic, complex, and dynamic inflammatory disorder in which the architecture of the cutaneous blood vessels is disrupted by inflammatory cells and the clinical presentation correlates with the size of the affected blood vessels.*" Each concept in this definition has important clinical application and will be developed in some extent throughout this section. However, we will concentrate on the certain subsets of cutaneous vasculitis that LE patients may develop.

Small Vessel Vasculitis (SVV)

The most useful classification in cutaneous vasculitis is that based on size of the predominantly affected vessels. Small vessels in the skin are venules, capillaries, and arterioles. They are commonly located in the upper dermis, have a poorly developed muscular layer, and they measure less than 50 μm in diameter.

When these vessels are involved, patients will present with purpuric and/or urticarial lesions. The purpuric lesion can be palpable (papules, vesicles, pustules, small superficial ulcer) or nonpalpable (macules and patches). They commonly affect dependent areas, for example, lower extremities as opposed to urticarial vasculitis (UV) lesions which tend to favor trunk and proximal extremities. Unlike classic urticaria, UV lesions are nonpruritic, last longer than 48–72 h, often leave postinflammatory hyperpigmentation, and have associated constitutional symptoms and acute phase reactants [61, 62].

Medium Sized Vasculitis (MVV)

Medium-sized vasculitis affects larger muscular vessels and is commonly located in the deep dermis, dermal subcutaneous, and subcutaneous tissue. There are four clinical presentations in this variant: nodules, large ulceration, livedo reticularis, and digital infarcts.

Large vessel vasculitis such as giant cells arteritis does not often complicate the skin. Some of the pure cutaneous SVV can complicate LE patients, such as Henoch-Schonlein purpura (IgA vasculitis), and cutaneous leukocytoclastic angiitis (hypersensitivity vasculitis). The most common triggers in these vasculitides are infections and drugs, thus LE patients may be at higher risk. However, the discussion of these vasculitides is beyond the scope of this chapter.

Specific Subsets of Cutaneous SVV in LE Patients

Urticarial vasculitis (UV) is the only entity in this group of pure SVV. Urticarial vasculitis is traditionally classified in normocomplementemic UV (NUV) and hypocomplementemic UV (HUV) variants. It is worth mentioning that if more sensitive complement tests than C3, C4, CH50, such as C3a, C5a, and C3bi, are used in UV, several cases formerly classified as NUV would be switched to HUV. NUV is a subset of cutaneous leukocytoclastic angiitis, is triggered by drugs, infection, or chronic inflammatory processes, does not often have systemic involvement, and resolves with or without treatment. Therefore, chronic cases of NUV without known trigger should prompt the revision of the diagnosis of vasculitis [62].

Hypocomplementemic UV on the other hand, is thought by many authorities to be a subset of SLE [63, 64]. Unlike NUV, HUV is commonly associated with systemic involvement, acute phase reactant, positive ANA serology, and distinctive immunopathologic features on the skin.

Both NUV and HUV are a neutrophil-predominant SVV of the skin. DIF is commonly negative in NUV; however, it is positive in virtually all HUV patients. The immunofindings of HUV are characterized by heavy granular IgG and C3 in and around small dermal vessels and also along the BMZ (LBT-like feature) (Fig. 17). It is believed that all patients with HUV have anti-C1q antibodies that appear to be pathogenic [65]. HUV patients should be treated with systemic immunosuppressive therapy.

Lymphocytic Vasculitis (LV)

This vasculitis is a rare SVV that often presents as pigmented purpura resembling Schamberg's disease. The so-called benign hypergammaglobulinemic purpura of Waldenstrom and LV fall into the category of cryoglobulinemia type 2 or 3. Biopsies of very early lesions in these patients will show leukocytoclastic vasculitis. As the lesions evolve, lymphocytes rapidly replace neutrophils. In these patients it is very common to find anti-Ro positive, rheumatic factor positive, polyclonal gammopathy, and occasional monoclonal spike. Lesions rapidly disappear without the need of any intervention [66].

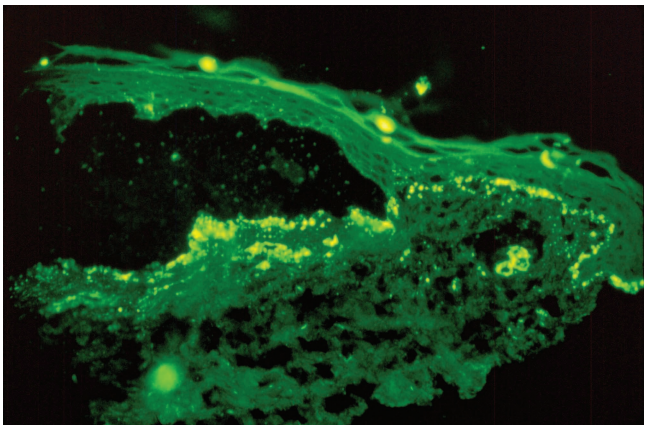


FIGURE 17 Direct immunofluorescence evaluation of an urticarial lesion in a patient with hypocomplementemic urticarial vasculitis syndrome revealing a characteristic granular IgG deposition around superficial dermal vessels as well as at the basement membrane zone (lupus band testlike feature).

The Previously Identified Connective Tissue Disease (CTD)-Associated Vasculitis

These can present as SVV (purpuric lesion) with or without MVV (PAN-like, nodules, ulcers, livedo, digital infarcts) (Fig. 18). This vasculitis is always seen in the setting of systemic and overt SLE.

Systemic involvement due to CTD vasculitis is also common. Peripheral and central nervous system, gastrointestinal, renal, and cardiovascular are not uncommonly affected systems. The histologic features correlate with the size of the clinically affected blood vessel leukocytoclastic SVV for purpuric lesions, and necrotizing MVV for PAN-like, nodules, ulcers, livedo, and digital infarcts. DIF shows IgG and C3 in and around small and medium dermal vessels, usually with a strong *in vivo* ANA (Fig. 19).

Another vasculitis that affects small and medium vessels is cryoglobulinemia type 2 and 3, often seen in LV as previously mentioned. This cryovasculitis syndrome is commonly associated with chronic infections like viral hepatitis, HIV, and lymphoproliferative diseases. CTD are rare causes of cryoglobulinemia. Therefore, the presence of well-documented cryoglobulin in LE patients should prompt the exclusion of underlying

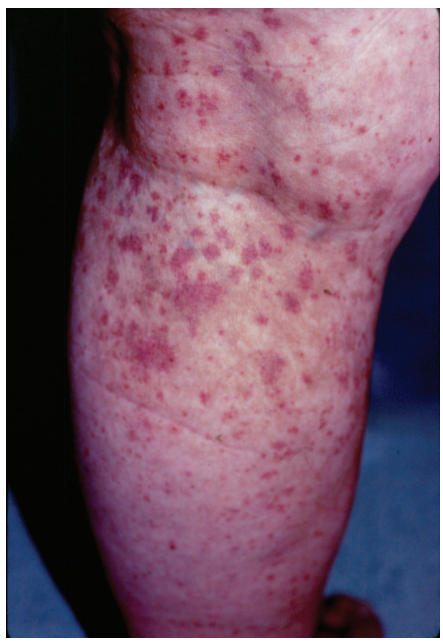


FIGURE 18 Patient with overtly active systemic lupus erythematosus and with CTD-associated vasculitis presented with palpable and nonpalpable purpuric lesion on the lower extremities. Cryoglobulin, rheumatoid factor, and ANCA were all negative. The patient had high titers of ANA, and anti-RNP, and severe hypocomplementemia.

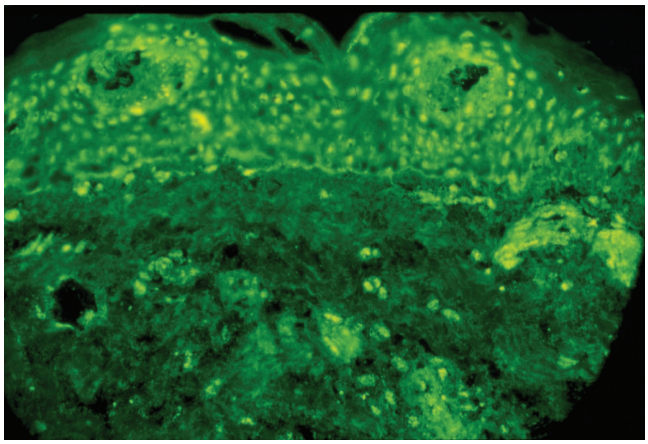


FIGURE 19 Direct immunofluorescence evaluation of the purpuric lesion of the patient in Fig. 18 showing heavy granular IgG deposition around superficial dermal vessel and strong *in vivo* ANA in keratinocytes and dermal cells.

viral infection or lymphoproliferative disease, especially in patients with Sjögren's syndrome [61].

Perniosis

Chilblains lupus perniotic LE lesions initially develop as livid patches and plaques on the toes, fingers, face, and ears that are precipitated by cold, damp climates [67, 68]. These *Chilblains lupus perniotic* lesions both clinically and pathologically overlap DLE and classic Chilblain. Chilblains lupus patients often have typical DLE lesions elsewhere. Approximately 20% of patients presenting with chilblain LE later develop SLE.

Raynaud's Phenomenon

Raynaud's phenomenon (RP) in SLE patients has ranged between 18 and 46% [2, 10, 69, 70]. Among the CLE patients, RP is more prevalent in patients with SLE, SCLE, and generalized CCLE. The presence of periungual telangiectasia, involvement of the thumbs, toes, ears, and nose by RP, ice-picked scars of the pulp, presence of high titers of ANA, and anti-RNP, and nucleolar antibodies are all predictors of LE-associated RP [71].

Degos-like Lesions

There is growing evidence that this ill-defined and controversial entity is a final common pathway. The classic clinical description of Degos is of white porcelain atrophic rounded macules surrounded by telangiectasia (Fig. 20). Patients with the Degos disease can

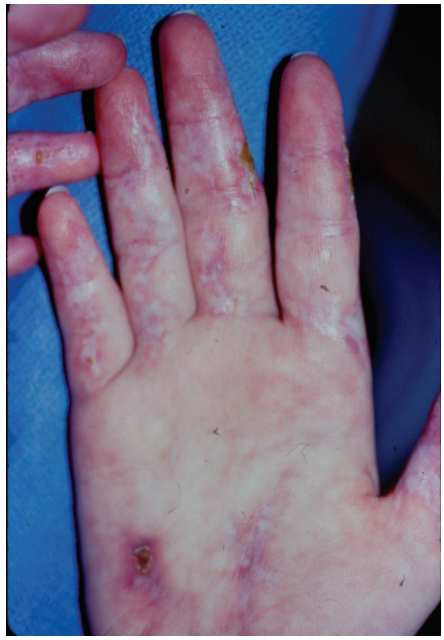


FIGURE 20 Degos-like lesions in the palms of a patient with generalized discoid lupus erythematosus. Histologic examination of these lesions revealed typical features of discoid lupus erythematosus.

have associated gastrointestinal infraction with perforation, and CNS infarcts [72].

Patients with cutaneous and systemic Degos are likely to have systemic vasculitides (polyarteritis nodosa, etc.) or thrombophilic disease (antiphospholipid syndrome, etc.) or simply SLE. Biopsies of the cutaneous lesion of Degos disease show either vasculopathy or vasculitis with a wedge of dermal necrosis and in some specimens, DLE-type interface dermatitis. Careful clinicopathologic correlation is required when the physician encounters cutaneous lesions that fit into the classic description of the so-called Degos disease.

Antiphospholipid Syndrome (APLS) [73, 74]

Patients with APLS have a primary thrombo-occlusive vasculopathy. One of the former dermatologic syndromes describing APLS was called Sneddon's syndrome describing livedo reticularis and multiple cerebral infarctions. The dermatologic presentation correlates with either arterial, venous, and capillary thrombosis phenomena.

Arterial

Arterial occlusive disease presents with: livedo reticularis (Fig. 21), arterial ulcers, digital infarcts, atrophie blanche, and Degos-like lesions. Livedo reticularis is a mottling eruption accentuated by cold, partly blanchable on pressure, or warming. Severe stage of livedo

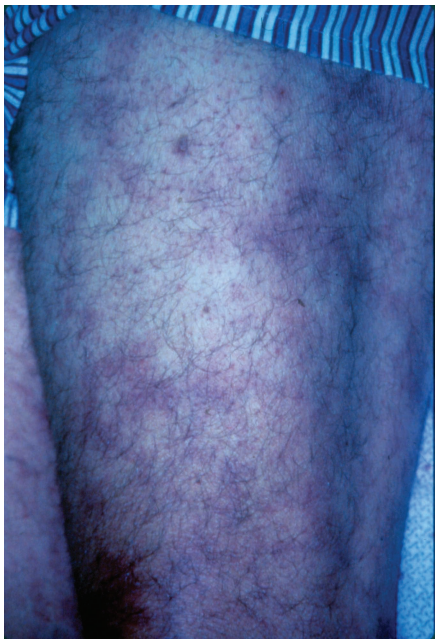


FIGURE 21 Livedo reticularis on proximal lower extremities in a patient with systemic lupus erythematosus and antiphospholipid syndrome. This mottling pattern was partially blanchable on pressure or warming.

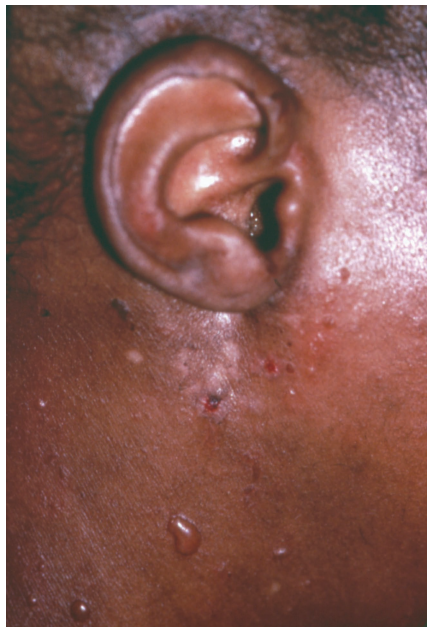


FIGURE 22 Lateral face and neck of a patient with smoldering systemic lupus erythematosus (SLE) and with bullous SLE lesions presenting as grouped tense and ruptured blisters resolving with atrophic scarring favoring flexural and head and neck areas.

reticularis is called livedoid vasculopathy and these patients present with ulcerations that heal with stellate scaring (atrophie blanche).

Leg ulcer and digital infarcts are clinically indistinguishable from MVV. Thus, ancillary diagnostic tests are mandatory for a correct diagnosis.

Venous

Venous occlusive disease presents with venous ulcers, lipodermatosclerosis, or superficial thrombophlebitis.

Capillary

A rare and severe presentation of APLS is the so-called catastrophic antiphospholipid syndrome (CAPS) where patients with APLS develop a DIC like picture with purpura fulminans after triggers such as surgeries, discontinuation of anticoagulant, or administrating procoagulant. These skin lesions demonstrate extensive areas of hemorrhagic skin necrosis [75].

Bullous Lesion in LE

Bullous lesions of several types occur in LE (data reviewed in Yell and Wojnarowska [76] and Sontheimer [77]) (Fig. 22). As mentioned, subepidermal bullae may develop in SCLE and ACLE lesions as a reflection of hyperacute injury to the epidermal basal layer that can occur as an extension of LE-specific histopathologic injury. This is the most common mechanism of bulla formation in LE patients. These are extremely photosensitive patients with extensive and active cutaneous and often systemic disease.

Histologically these bullous lesions show severe interface dermatitis with vacuolization and separation of the epidermis thru the BMZ. DIF shows heavy granular IgG, IgM, and C3 deposits along the BMZ (Fig. 23). Two rare overlap autoimmune skin syndromes can complicate LE patients: (1) epidermolysis bullosa acquisita (EBA) and, (2) pemphigus foliaceus (PF). When EBA and PF complicate LE the syndromes are called bullous SLE (BSLE) and pemphigus erythematosus (PE), respectively.

Patients with BSLE develop erythematous vesicles and bullae favoring head, neck, and flexural areas that heals with scars. Half of these patients may have scarring mucositis affecting the mouth and other mucosal membranes. Histologically, it is characterized by an often neutrophils-rich, subepidermal blister. In DIF studies, as opposed to the granular immunostaining seen in the LE interface dermatitis, these bullous SLE patients have a thick linear IgG and sometimes IgA and C3 along the BMZ. All of the patients have been shown to have circulating and tissue bound anti type VII collagen, the principal constituent of the anchoring fibrils [78].

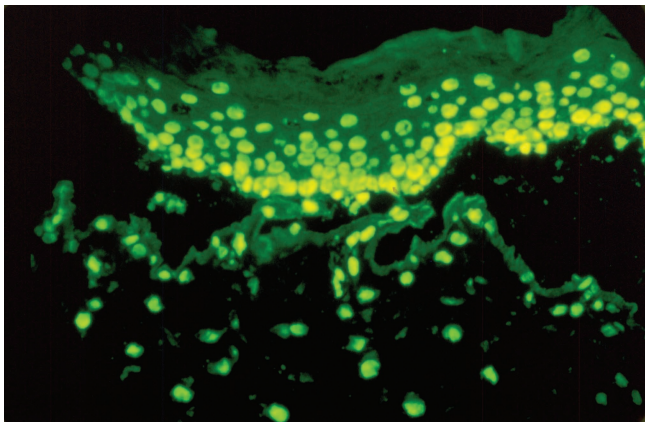


FIGURE 23 Direct immunofluorescence evaluation of the blister of patient in Fig. 22 showing thick linear IgG along the floor of the splited basement membrane and also strong *in vivo* ANA. Serologic investigation detected high titer of anti-type VII collagen (epidermolysis bullosa acquisita immunofindings) and anti-RNP (SLE finding).

Patients with PE typically present with superficial scaly lesions and occasionally blisters in a background of photosensitive erythema affecting head, neck, trunk, and proximal extremities without mucosal involvement (Fig. 24).

Histologically PE is characterized by a combination of an (often subtle) interface dermatitis, and subcorneal acantholytic blisters. DIF studies show a combination of granular immunoreactants along the BMZ (interface LE) and IgG deposits on the epidermal cell surfaces (PF-like) (Fig. 25) [76, 79–81].

Miscellaneous Lesions

Several miscellaneous lesions have and continue to be reported in LE patients. Most of those lesions are coincidental findings and not true associations with LE. We will review some of the most common dermatoses proven to be associated with LE.

Neutrophilic Dermatosis

Sweet Syndrome and Pyoderma Gangrenosum (PG)

These two dermatologic disorders fall into the group of neutrophilic dermatosis, characterized by significant neutrophilic infiltrates of the dermis and fat, without overt vasculitis. These are reactive disorders in which the underlying disease is often a chronic inflammatory disease, or autoimmune disease, or neoplasia. The most common disease associated is inflammatory bowel disease, although patients with CTD and arthritis occasionally present with neutrophilic dermatosis.

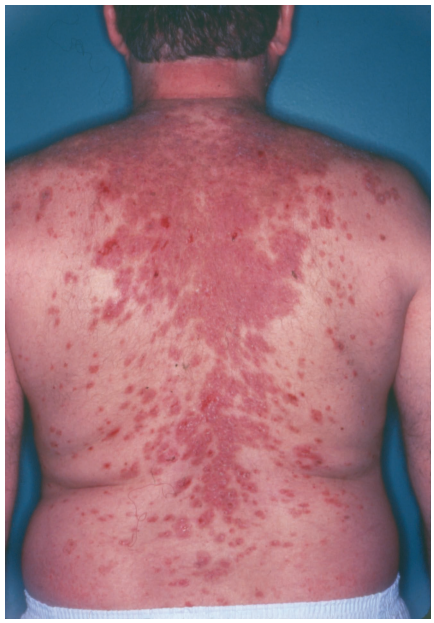


FIGURE 24 Pemphigus erythematosus showing confluent erythematous superficial blistering eruption on the upper back. The patient had smoldering photosensitive SLE. No mucous membrane lesions were identified in this patient.

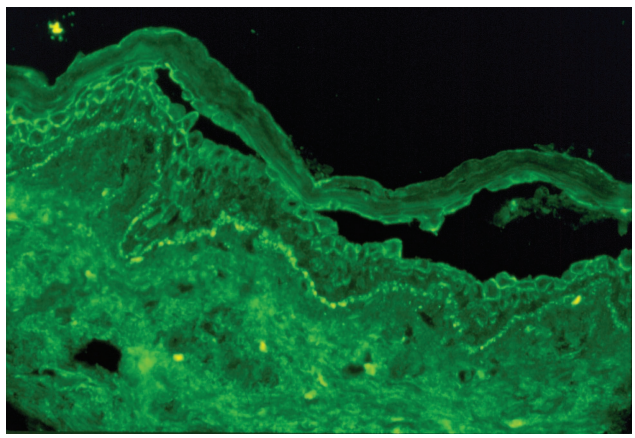


FIGURE 25 Direct immunofluorescence evaluation of the upper back of patient in Fig. 24 showing a heavy granular IgG deposits along the basement membrane zone (LE immunofinding) as well as IgG on the keratinocyte cell surfaces (Pemphigus-type). Serologic investigation detected high titer of anti-desmoglein 1 antibodies (pemphigus foliaceus) and anti-Ro (LE finding).

Pyoderma gangrenosum is characterized by painful lesions that eventually ulcerate. They have a distinct violaceous overhanging border and clean granular base. The lesions typically affect lower extremities. The clinical picture demonstrates a distinct absence of fever, or regional lymphadenopathy, unless the lesions become secondarily infected.

Sweet's lesions are urticarial and nonpruritic affecting the head, neck, and acral areas and are commonly associated with fever.

When a variant of the neutrophilic dermatoses is associated with an underlying disease, the lesion tends to be recurrent, persistent, and in an atypical location [82, 83].

Churg-Strauss Granuloma

This is also called cutaneous extravascular necrotizing granuloma (CENG) or better termed PNGD (palisade neutrophilic and granulomatous dermatosis). These are lesions that clinically and histologically mimic rheumatoid nodules.

The presence of ulceration, absence of rheumatoid factor, and significant histologic evidence of vasculitis in the specimen are features that help differentiating PNGD from rheumatoid nodules. These lesions, originally found in Churg-Strauss granuloma, have been found in different vasculitides, arthritides, and CTD [84].

Erythema Elevatum Diutinum (EED)

Erythema elevatum diutinum (EED) is a rare form of pure cutaneous vasculitis. It is characterized by chronic papule, plaques, and fibrin on extensor surfaces. Although half of the cases are idiopathic and do not have an underlying disease, three conditions have been associated with this vasculitis: (1) paraproteinemia, (2) HIV, and (3) rheumatoid arthritis and other CTD including SLE. Histologic findings include small polymorphonuclear predominant small vessel vasculitis with significant edema and later lipid deposit and fibrosis.

Alopecia

Hair loss is one of the most common nonspecific cutaneous signs of SLE. Alopecia can be classified as either scarring or nonscarring, depending on whether the scalp skin is permanently altered by the disease process. Scarring alopecia is usually caused by DLE. Diffuse, nonscarring alopecia occurs during the acute exacerbations of SLE. The nonscarring type of alopecia that is observed in lupus patients, termed lupus hair is simply a manifestation of telogen effluvium that may appear in the setting of any systemic disease. Diffuse nonscarring alopecia is usually transient; however, occasionally it can be persistent, particularly in those patients who have persistent active clinical disease [85].

Treatment

Management of patients with multisystem disease is dictated by the type of involvement. Since ACLE skin lesions are almost invariably present in patients with active SLE and respond to the same measures that are required for suppressing the systemic involvement, skin-directed treatment is usually unnecessary. The comments pertaining to therapy that follow apply especially to CCLE and SCLE. It is important to realize that these two forms of CLE usually respond to the same treatment approach, the major difference being that SCLE lesions tend to be more numerous and widespread compared with CCLE and are therefore less amenable to local therapy.

Topical Therapy

1. The potency of topical corticosteroids is based on their vasoconstrictor effect. The recommended initial topical treatment to LE lesions is medium-strength agents, such as triamcinolone acetonide 0.1% cream or ointment. However, the superpotent class I fluorinated topical corticosteroids, such as clobetasol propionate 0.05% or betamethasone dipropionate 0.05%, are often required to produce the greatest benefits. As a rule, ointments are generally more efficacious than creams in delivering the active chemical into the skin, but patients tend to find the cream-based preparations less greasy and therefore more acceptable cosmetically for regular use. A careful use of these agents is needed because of the possible atrophy effect of corticosteroids on a lesion that is already atrophying. Intralesional application of corticosteroids is highly unnecessary.
2. Sun protection: UVB and also UVA protection. Patients are advised to minimize sun exposure and to use sunblocks that protect for both UVB and UVA. This protection may be achieved with the combined use of physical blocking agents such as titanium dioxide–zinc oxide, and traditional sunscreen with a sun protecting factor of at least 45 with avobenzone [86].
3. Others: FK506 (tacrolimus) is a relatively new agent with potent immunomodulatory effect. In a more recent study, tacrolimus was effective for the treatment of some patients with cutaneous LE [87].

Systemic Therapy

The indications for systemic therapy include: extensive cutaneous disease, failure of topical therapy, associated constitutional symptoms (mainly articular symptoms), and photosensitivity. All of these manifestations should prompt the addition of antimalarials.

Hydroxychloroquine (HCQ)

The recommended dose is 6.5 mg/kg/day (usually 400 mg/day). It is crucial to keep in mind that hydroxychloroquine takes at least 3–4 months to start showing beneficial effect. It has been suspected but not fully proved that smoking may decrease its effectiveness. The major concern with the use of antimalarials is the potential retinal toxicity. However, evidence suggests that this is extremely unlikely when daily doses of hydroxychloroquine 6.5 mg/kg/day are not exceeded. The traditional approach has been to have an ophthalmologic examination carried out prior to treatment and again every 6–12 months during treatment to detect the early, potentially reversible changes of antimalarial retinopathy. However, such guidelines have been debated as being perhaps overly cautious [88–91].

Performing G6PD screening is largely unnecessary. The role of the antimalarials in the treatment of CLE is probably more important as agents used to keep patients in remission. Some practitioners claim that the addition of another antimalarial such as quinacrine may increase effectiveness. However, the benefit of this combination remains to be proved.

The significant ocular toxicity of chloroquine and similar effectiveness as HCQ lead to almost the complete discontinuation of this drug in the pharmacologic armamentarium in the treatment of CLE [92–94].

Thalidomide

The recommended dose is 1.5–5 mg/kg/day. This drug is highly effective in the treatment of DLE. The main concerns with the use of this drug are its teratogenicity and sensory neuropathy. However, the incidence of nerve damage is highly overreported and we recommend a baseline nerve conduction study (NCS) and follow up with NCS periodically. Avoidance of doses higher than 100 mg for more than 6 months largely reduces this risk. The teratogenicity is monitored by the so-called S.T.E.P.S (System for Thalidomide Education and Prescribing Safety) program that provide a strict follow-up system. Other possible and common side effects are hypothyroidism, sedation, and constipation [95–98].

Retinoids

Hyperkeratotic lesions usually present in palmoplantar DLE and may respond to the addition of topical or systemic retinoids [99].

Immunosuppressive Drugs

Immunosuppressive drugs are largely unnecessary. They may be considered in patients with extensive cutaneous disease, failure to topical and antimalarial therapy, associated constitutional symptoms, or severe articular symptoms. In this treatment group, systemic corticosteroids play a major role. For example, 1 mg/kg/day of prednisone in DLE patients is very effective. The prednisone should be tapered off over 3–4 months. Adjuvant steroid sparing agents that have proven effectiveness are azathioprine 2–3 mg/kg/day, mycophenolate mofetil (MMF) 25–35 mg/kg/day, and leflunomide 0.3 mg/kg/day [100].

The major and most worrisome side effect of azathioprine is myelosuppression, which usually manifests as leukopenia, although thrombocytopenia and anemia have been reported as well [101]. This adverse reaction is relatively rare, occurring mainly in patients with very

TABLE 5 Clinical Approach for the Management of CLE

Chronic cutaneous LE
DLE
Localized: topical corticosteroids and antimalarials and retinoids (hypertrophic LE)
Generalized: antimalarials, thalidomide, sun protection, and systemic corticosteroids, leflunomide, azathioprine or MMF (severe cases)
Lupus tumidus
Sun protection, antimalarials, thalidomide, systemic corticosteroids, leflunomide, azathioprine, or MMF (severe cases)
Lupus panniculitis
Antimalarials, thalidomide, systemic corticosteroids, leflunomide, azathioprine or MMF (severe cases)
SCLE and ACLE
Sun protection, antimalarials, thalidomide, systemic corticosteroids, leflunomide, azathioprine, or MMF (severe cases)
Cutaneous NLE
Sun protection, topical corticosteroids
Lupus perniosis: cold protection, and antimalarials, thalidomide, systemic corticosteroids, leflunomide, azathioprine or MMF (severe cases)
Others
Urticarial vasculitis
Normocomplmenetemic: usually no specific treatment required
Hypocomplemenetemic: systemic corticosteroids, leflunomide azathioprine, or MMF, alkylating agents
Lymphocytic vasculitis
Usually no specific treatment required or systemic corticosteroids, leflunomide, azathioprine, or MMF (severe cases)
Bullous SLE
Systemic corticosteroids, leflunomide, azathioprine, or MMF, alkylating agents (severe cutaneous or mucosal disease)
Erythema elevatum diutinum
Systemic corticosteroids and Dapsone, leflunomide, azathioprine, or MMF (severe cases)

low or absent thiopurine methyltransferase activity. Other possible side effects of azathioprine are toxic hepatitis, pancreatitis, hypersensitivity syndrome (azathioprine-induced shock), and drug fever [102–106]. MMF has a better safety profile than azathioprine, but is much more expensive.

Alkylating agents are largely unnecessary and paradoxically are not proved to be effective in the cutaneous forms of LE. Other immunomodulators agents including calcineurin inhibitors [107, 108], methotrexate [109], gold [110, 111], clofazimine [112], and dapsone [113–115] are inconsistently effective. The goal of immunosuppressive therapy is to achieve the safest and most effective regimen. Currently, this goal can be achieved by a synergistic combined therapy. A relatively effective combination for all forms of CLE is photoprotection, topical steroids antimalarials, and thalidomide. For severe cases, the addition of an antimetabolite to the previous regimen will achieve the highest synergistic effect.

A practical approach for the management of CLE is shown in Table 5. We believe that anti-TNF drugs such as etanercept and infliximab will have an important role in the treatment of CLE [116] as well as new analogs of thalidomide, IMiD (immunomodulatory and inhibitors drugs) drugs, that are 100–1000 times more potent than thalidomide in regulating cytokine production by peripheral blood mononuclear cells and providing a costimulatory signal for T-cell proliferation [117]. Also other biological modifiers targeting T cells harboring specific homing cell receptors that have been used in other dermatologic disorders may have an important role in the therapy of CLE.

References

1. Rowell, N. R. (1997). Some historical aspects of skin disease in lupus erythematosus. *Lupus* **6**, 76.
2. Sontheimer, R. D., and Provost, T. T. (1996). Lupus erythematosus. In "Cutaneous Manifestations of Rheumatic Diseases" (R. D. Sontheimer and T. T. Provost, Eds.), pp. 1–71. Williams & Wilkins, Baltimore.
3. David-Bajar, K. M., and Davis, B. M. (1997). Pathology, immunopathology and immunohistochemistry in cutaneous lupus erythematosus. *Lupus* **6**, 145.
4. Nousari, H. C., and Anhalt, G. J. (2002). Skin diseases. In "Manual of Clinical Laboratory Immunology" (N. R. Rose, R. G. Hamilton, and B. Detrick, Eds.), pp. 1032–1042. American Society for Microbiology Press, Washington, D.C.
5. Wallace, D. J. (1993). The relationship between discoid and systemic lupus erythematosus. In "Dubois' Lupus Erythematosus" (D. J. Wallace, B. H. Hahn, F. P. Quismorio, Jr., and J. R. Klinenberg, Eds.), pp. 310–312. Lea & Febiger, Philadelphia and London.
6. Knop, J., Bonsmann, G., Kind, P., *et al*. (1990). Antigens of the major histocompatibility complex in patients with chronic discoid lupus erythematosus. *Br. J. Dermatol*. **122**, 723.
7. Wolpert, K. A., Webster, A. D., and Whittaker, S. J. (1998). Discoid lupus erythematosus associated with a primary immunodeficiency syndrome showing features of non-X-linked hyper-IgM syndrome. *Br. J. Dermatol*. **138**, 1053.
8. Rupec, R. A., Petropoulou, T., Belohradsky, B. H., *et al*. (2000). Lupus erythematosus tumidus and chronic discoid lupus erythematosus in carriers of X-linked chronic granulomatous disease. *Eur. J. Dermatol*. **10**, 184.
9. Watanabe, T., and Tsuchida, T. (1995). Classification of lupus erythematosus based upon cutaneous manifestations. Dermatological, systemic and laboratory findings in 191 patients. *Dermatology* **190**, 277.
10. Dubois, E. L., and Tuffanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus. *JAMA* **190**, 104.
11. Rothfield, N. F., March, C., Miescher, P., and McEwen, C. (1963). Chronic discoid lupus erythematosus: A study of 65 patients and 65 controls. *N. Engl. J. Med*. **269**, 1155.
12. Prystowsky, S. D., and Gilliam, J. N. (1975). Discoid lupus erythematosus as part of a larger disease spectrum. Correlation of clinical features with laboratory findings in lupus erythematosus. *Arch. Dermatol*. **111**, 1448.
13. O'Laughlin, S., Schroeter, A. L., and Jordon, R. E. (1978). A study of lupus erythematosus with particular reference to generalized discoid lupus. *Br. J. Dermatol*. **99**, 1.
14. Millard, L. G., and Rowell, N. R. (1979). Abnormal laboratory test results and their relationship to prognosis in discoid lupus erythematosus. A long-term follow-up study of 92 patients. *Arch. Dermatol*. **115**, 1055.
15. Callen, J. P. (1982). Chronic cutaneous lupus erythematosus: Clinical, laboratory, therapeutic, and prognostic examination of 62 patients. *Arch. Dermatol*. **118**, 412.
16. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85.
17. Burge, S. M., Frith, P. A., Juniper, R. P., and Wojnarowska, F. (1989). Mucosal involvement in systemic and chronic cutaneous lupus erythematosus. *Br. J. Dermatol*. **121**, 727.
18. Prystowsky, S. D., Herndon, J. H., Jr., and Gilliam, J. N. (1976). Chronic cutaneous lupus erythematosus (DLE) Ca clinical and laboratory investigation of 80 patients. *Medicine* **55**, 183.
19. Tuffanelli, D. L., Dubois, E. L. (1964). Cutaneous manifestations of systemic lupus erythematosus. *Arch. Dermatol*. **90**, 377.
20. Tausk, F., Harpster, E., and Gigli, I. (1990). The expression of C3b receptors in the differentiation of discoid lupus erythematosus and systemic lupus erythematosus. *Arthritis Rheum*. **33**, 888.
21. Tausk, F., and Gigli, I. (1990). The human C3b receptor: Function and role in human diseases. *J. Invest. Dermatol*. **94**, 141.
22. Andreasen, J. O., and Poulsen, H. E. (1964). Oral discoid and systemic lupus erythematosus. I. Clinical Investigation. *Acta Odont. Scand*. **22**, 295.

23. Kuhn, A., Richter-Hintz, D., Oslislo, C., *et al*. (2000). Lupus erythematosus tumidus—a neglected subset of cutaneous Lupus erythematosus: Report of 40 cases. *Arch. Dermatol.* **136**, 1033.
24. Nishikawa, T., and Provost, T. T. (1991). Differences in clinical, serologic, and immunogenetic features of white versus Oriental anti-SS-A/Ro-positive patients. *J. Am. Acad. Dermatol*. **25**, 563.
25. McCauliffe, D. P., Faircloth, E., Wang, L., *et al*. (1996). Similar Ro/SS-A autoantibody epitope and titer responses in annular erythema of Sjogren's syndrome and subacute cutaneous lupus erythematosus. *Arch. Dermatol*. **132**, 528.
26. Kobayashi, T., Shimizu, H., Shimizu, S., Harada, T., and Nishikawa, T. (1993). Plaquelike cutaneous lupus mucinosis. *Arch. Dermatol*. **129**, 383.
27. Kind, P., and Goerz, G. (1987). Clinical and differentia diagnosis of cutaneous lupus erythematosus. *Z. Hautkr*. **62**, 1337.
28. Dekle, C. L., Mannes, K. D., Davis, L. S., and Sangueza, O. P. (1999). Lupus tumidus. *J. Am. Acad. Dermatol*. **41**, 250.
29. Tuffanelli, D. L. (1972). Lupus erythematosus. *Arch. Dermatol*. **106**, 553.
30. Peters, M. S., and Su, W. P. D. (1989). Lupus erythematosus panniculitis. *Med. Clin. North. Am*. **73**, 1113.
31. Watanabe, T., and Tsuchida, T. (1996). Lupus erythematosus profundus: A cutaneous marker for a distinct clinical subset? *Br. J. Dermatol*. **134**, 123.
32. Biedermann, T., Schirren, C. G., Meurer, M., and Bieber, T. (1996). Lupus erythematosus profundus Kaposi-Irgang. *Eur. J. Dermatol*. **6**, 519.
33. Harris, R. B., and Winkelmann, R. K. (1978). Lupus mastitis. *Arch. Dermatol*. **114**, 410.
34. Nousari, H. C., Kimyai-Asadi, A. and Provost, T. T. (1999). Generalized lupus erythematosus profundus in a patient with genetic partial deficiency of C4. *J. Am. Acad. Dermatol*. **41**, 362.
35. Nousari, H. C., Kimyai-Asadi, A., Santana, H. M., *et al*. (1999). Generalized lupus panniculitis and antiphospholipid syndrome in a patient without complement deficiency. *Pediatr. Dermatol*. **16**, 273.
36. Barnhill, R. L. (1998). Panniculitis and fasciitis. In "Textbook of Dermatopathology" (R. L. Barnhill, ed.), pp. 233–256. McGraw-Hill, New York.
37. White, J. W. Jr., and Winkelmann, R. K. (1998). Weber-Christian panniculitis: A review of 30 cases with this diagnosis. *J. Am. Acad. Dermatol*. **39**, 56.
38. Berg, K. D., Brinster, N. K., Huhn, K. M., *et al*. (2001). Transmission of a T-cell lymphoma by allogeneic bone marrow transplantation. *N. Engl. J. Med*. **345**, 1458.
39. Callen, J. P., Hughes, A. P., and Kulp-Shorten, C. (2001). Subacute cutaneous lupus erythematosus induced or exacerbated by terbinafine: A report of 5 cases. *Arch. Dermatol*. **137**, 1196.
40. Nousari, H. C., Kimyai-Asadi, A., and Tausk, F. A. (1998). Subacute cutaneous lupus erythematosus associated with interferon beta-1a. *Lancet* **352**, 1825.
41. Sontheimer, R. D. (1989). Subacute cutaneous lupus erythematosus: A decade's perspective. *Med. Clin. North Am.* **73**, 1073.
42. Reed, B. R., Huff, J. C., Jones, S. K., *et al*. (1985). Subacute cutaneous lupus erythematosus associated with hydrochlorothiazide therapy. *Ann. Intern. Med.* **103**, 49.
43. Crowson, A. N., and Magro, C. M. (1995). Diltiazem and subacute cutaneous lupus erythematosus-like lesions. *N. Engl. J. Med*. **333**, 1429.
44. Provost, T. T., and Watson, R. (1993). Anti-Ro(SS-A) HLA-DR3-positive women: The interrelationship between some ANA negative, SS, SCLE, and NLE mothers and SS/LE overlap female patients. *J. Invest. Dermatol*. **100**, 14.
45. Neiboer, C., Tak-Diamand, Z., and VanLeeuwen-Wallau, A. G. (1988). Dust-like particles: A specific direct immunofluorescence pattern in subacute cutaneous lupus erythematosus. *Br. J. Dermatol*. **118**, 725.
46. Weston, W. L., Morelli, J. G., and Lee, L. A. (1999). The clinical spectrum of anti-Ro-positive cutaneous neonatal lupus erythematosus. *J. Am. Acad. Dermatol*. **40**, 675.
47. Furukawa, F., Tokura, Y., Matsushita, K., *et al*. (1996). Selective expansions of T cells expressing Vbeta8 and Vbeta13 in skin lesions of patients with chronic cutaneous lupus erythematosus. *J. Dermatol*. **23**, 670.
48. Sontheimer, R. D. (1996). Photoimmunology of lupus erythematosus and dermatomyositis: A speculative review. *Photochem. Photobiol*. **63**, 583.
49. Bennion, S. D., and Norris, D. A. (1997). Ultraviolet light modulation of autoantigens, epidermal cytokines and adhesion molecules as contributing factors of the pathogenesis of cutaneous LE. *Lupus* **6**, 181.
50. Casciola-Rosen, L., and Rosen, L. (1997). Ultraviolet light-induced apoptosis: A potential mechanism for the induction of skin lesions and autoantibody production in LE. *Lupus* **6**, 175.
51. Norris, D. A. (1993). Pathomechanisms of photosensitive lupus erythematosus. *J. Invest. Dermatol*. **100**, 58S.
52. Kind, P., Lehmann, P., and Plewig, G. (1993). Phototesting in lupus erythematosus. *J. Invest. Dermatol.* **100**, 53S.
53. Walchner, M., Messer, G., and Kind, P. (1997). Phototesting and photoprotection in LE. *Lupus* **6**, 167.
54. Tan, E. M., Cohen, A. S., Fries, J. F., *et al*. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. **25**, 1271.
55. Nyberg, F., Hasan, T., Puska, P., *et al*. (1997). Occurrence of polymorphous light eruption in lupus erythematosus. *Br. J. Dermatol*. **136**, 217.
56. Nived, O., Johansen, P. B., and Sturfelt, G. (1993). Standardized ultraviolet-A exposure provokes skin reaction in systemic lupus erythematosus. *Lupus* **2**, 247.
57. Doria, A., Biasinutto, A., Ghirardello, A., *et al*. (1996). Photosensitivity in systemic lupus erythematosus: Laboratory testing of ARA/ACR definition. *Lupus* **5**, 263.
58. Wolska, H., Blaszczyk, M., and Jablonska, S. (1989). Phototests in patients with various forms of lupus erythematosus. *Int. J. Derm*. **28**, 98.
59. Velthuis, P. J., Van Weelden, H., Van Wichen, D., and De la Faille, H. B. (1990). Immunohistopathology of light-

induced skin lesions in lupus erythematosus. *Acta Derm. Venereol. (Stockh.)* **70**, 93.
60. Lehmann, P., Holzle, E., Kind, P., Goerz, G., and Plewig, G. (1990). Experimental reproduction of skin lesions in lupus erythematosus by UVA and UVB radiation. *J. Am. Acad. Dermatol*. **22**, 181.
61. Stone, J. H., and Nousari, H. C. (2001). "Essential" cutaneous vasculitis: What every rheumatologist should know about vasculitis of the skin. *Curr. Opin. Rheumatol*. **13**, 23.
62. Wisnieski, J. J. (2000). Urticarial vasculitis. *Curr. Opin. Rheumatol*. **12**, 24.
63. O'Laughlin, S., Schroeter, A. L., and Jordon, R. E. (1978). Chronic urticaria-like lesions in systemic lupus erythematosus. *Arch. Dermatol*. **114**, 879.
64. Mattarredona, J., Sendagorta, E., Rocamora, A., *et al*. (1988). Systemic lupus erythematosus appearing as an urticarial vasculitis. *Int. J. Dermatol*. **25**, 446.
65. D'Cruz, D. P., Wisnieski, J. J., Asherson, R. A., Khamashta, M. A., and Hughes, G. R. (1995). Autoantibodies in systemic lupus erythematosus and urticarial vasculitis. *J. Rheumatol.* **22**, 1669.
66. Carlson, J. A., Mihm. M. C., Jr., and LeBoit, P. E. (1996). Cutaneous lymphocytic vasculitis: A definition, a review, and a proposed classification. *Semin. Diagn. Pathol*. **13**, 72.
67. Millard, L. G., and Rowell, N. R. (1978). Chilblain lupus erythematosus (Hutchinson). A clinical and laboratory study of 17 patients. *Br. J. Dermatol*. **98**, 497.
68. Doutre, M. S., Beylot, C., Beylot, J., Pompougnac, E., and Royer, P. (1992). Chilblain lupus erythematosus: Report of 15 cases. *Dermatology* **184**, 26.
69. Pistiner, M., Wallace, D. J., Nessim, S., and Metzger, A. L. (1991). Lupus erythematosus in the 1980s: A survey of 570 patients. *Semin. Arthritis Rheum*. **21**, 55.
70. Hochberg, M. C., Boyd, R. E., Ahearn, J. M., *et al*. (1985). Systemic lupus erythematosus: A review of clinico-laboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. *Medicine* **64**, 285.
71. Sharp, G. C., Irvin, W. S., Tan, E. M., Gould, R. G., Holman, H. R. (1972). Mixed connective tissue disease: An apparent distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med*. **52**, 148.
72. Grilli, R., Soriano, M. L., Izquierdo, M. J., *et al*. (1999). Panniculitis mimicking lupus erythematosus profundus: a new histopathologic finding in malignant atrophic papulosis (Degos disease). *Am. J. Dermatopathol*. **21**, 365.
73. Sontheimer, R. D. (1987). The anticardiolipin syndrome. A new way to slice an old pie or a new pie to slice? *Arch. Dermatol.* **123**, 590.
74. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. *Ann. Intern. Med*. **112**, 682.
75. Asherson, R. A. (1998). The catastrophic antiphospholipid syndrome, 1998. A review of the clinical features, possible pathogenesis and treatment. *Lupus* **7**, 55.
76. Yell, J. A., and Wojnarowska, F. (1997). Bullous skin disease in lupus erythematosus. *Lupus* **6**, 112.
77. Sontheimer, R. D. (1997). The lexicon of cutaneous lupus erythematosus—A review and personal perspective on the nomenclature and classification of the cutaneous manifestations of lupus erythematosus. *Lupus* **6**, 84.
78. Shirahama, S., Furukawa, F., Yagi, H., *et al*. (1998). Bullous systemic lupus erythematosus: Detection of antibodies against noncollagenous domain of type VII collagen. *J. Am. Acad. Dermatol*. **38**, 844.
79. Boh, E., Roberts, L. J., Lieu, T. S., Gammon, W. R., and Sontheimer, R. D. (1990). Epidermolysis bullosa acquisita preceding the development of systemic lupus erythematosus. *J. Am. Acad. Dermatol*. **22**, 587.
80. Hall, R. P., Lawlry, T. J., Smith, H. R., and Katz, S. I. (1982). Bullous eruption of systemic lupus erythematosus, dramatic response to Dapsone therapy. *Ann. Intern. Med*. **97**, 165.
81. Barton, D. D., Fine, J. D., Gammon, W. R., and Sams, W. M., Jr. (1986). Bullous systemic lupus erythematosus: An unusual clinical course and detectable circulating antibodies to the epidermolysis bullosa acquisita antigen. *J. Am. Acad. Dermatol*. **16**, 369.
82. Cohen, P. R., and Kurzrock, R. (2000). Sweet's syndrome: A neutrophilic dermatosis classically associated with acute onset and fever. *Clin. Dermatol*. **18**, 265.
83. Powell, F. C., and Collins, S. (2000). Pyoderma gangrenosum. *Clin. Dermatol*. **18**, 283.
84. Schwartz, R. A., and Churg, J. (1992). Churg-Strauss syndrome. *Br. J. Dermatol*. **127**, 199.
85. Wysenbeek, A. J., Leibovici, L., Amit, M., and Weinberger, A. (1991). Alopecia in systemic lupus erythematosus. Relation to disease manifestations. *J. Rheumatol*. **18**, 1185.
86. Kaye, E. T., Levin, J. A., Blank, I. H., *et al*. (1991). Efficiency of opaque photoprotective agents in the visible light range. *Arch. Dermatol*. **127**, 351.
87. Yoshimasu, T., Ohtani, T., Sakamoto, T., Oshima, A., and Furukawa, F. (2002). Topical FK506 (tacrolimus) therapy for facial erythematous lesions of cutaneous lupus erythematosus and dermatomyositis. *Eur. J. Dermatol*. **12**, 50.
88. Beattie, C., Beaudet, F., and Davis, P. (1997). Antimalarial workshop. *J. Rheumatol*. **24**, 1393.
89. Shipley, M., and Silman, A. (1997). Should patients on hydroxychloroquine have their eyes examined regularly? *Br. J. Rheumatol*. **36**, 514.
90. Silman, A., and Shipley, M. (1997). Ophthalmological monitoring for hydroxychloroquine toxicity—a scientific review of available data. *Br. J. Rheumatol*. **36**, 599.
91. Levy, G. D., Munz, S. J., Paschal, J., *et al*. (1997). Incidence of hydroxychloroquine retinopathy in 1,207 patients in a large multicenter outpatient practice. *Arthritis Rheum* **40**, 1482.
92. Dubois, E. L. (1978). Antimalarials in the management of discoid and systemic lupus erythematosus. *Semin. Arthritis Rheum*. **8**, 33.

93. Furner, B. B. (1990). Treatment of subacute cutaneous lupus erythematosus. *Int. J. Dermatol*. **29**, 542.
94. Costner, M., and Sontheimer, R. D. (1997). Antimalarial therapy in photosensitive dermatoses. *Dermatol. Ther.* **4**, 86.
95. Naafs, B., Bakkers, E. J. M., Flinterman, J., and Faber, W. R. (1982). Thalidomide treatment of subacute cutaneous lupus erythematosus. *Br. J. Dermatol*. **107**, 83.
96. Knop, J., Bonsmann, G., Happle, R., *et al*. (1983). Thalidomide in the treatment of sixty cases of chronic discoid lupus erythematosus. *Br. J. Dermatol*. **108**, 461.
97. Volc-Platzer, B., and Wolff, K. (1983). Treatment of subacute cutaneous lupus erythematosus with thalidomide. *Der. Hautarzt*. **34**, 175.
98. Holm, A. L., Bowers, K. E., McMeekin, T. O., and Gaspari, A. A. (1993). Chronic cutaneous lupus erythematosus treated with thalidomide. *Arch. Dermatol.* **129**, 1548.
99. Green, S. G., and Piette, W. W. (1987). Successful treatment of hypertrophic lupus erythematosus with isotretinoin. *J. Am. Acad. Dermatol*. **17**, 364.
100. Goyal, S., and Nousari, H. C. (2001). Treatment of resistant discoid lupus erythematosus of the palms and soles with mycophenolate mofetil. *J. Am. Acad. Dermatol*. **45**, 142.
101. Shupack, J. L., Silverman Kitchin, J. E., Stiller, M. J., and Webster, G. F. (1999). Cytotoxic and antimetabolic agents. In "Fitzpatrick's Dermatology in General Medicine" (I. M. Freedberg, A. Z. Eisen, and K. Wolff, Eds.), 5th ed., pp. 2797–2810. 5th ed. McGraw-Hill, New York.
102. Gross, R. (1994). Hepatotoxicity of 6-mercaptopurine and azathioprine. *Mayo Clin. Proc*. **69**, 498.
103. Kawanishi, H., Rudolph, E., and Bull, F. E. (1973). Azathioprine-induced acute pancreatitis. *N. Engl. J. Med*. **289**, 357.
104. Pozniak, A. L., Ahern, M., and Blake, D. R. (1981). Azathioprine-induced shock. *Br. Med. J. (Clin. Res. Ed.)* **283**, 1548.
105. Pozniak, A. L. (1982). Azathioprine-induced pancreatitis. *Arthritis Rheum*. **25**, 1149.
106. Smak Gregoor, P. J., van Saase, J. L., Weimar, W., and Kramer, P. (1995). Fever and rigors as sole symptoms of azathioprine hypersensitivity. *Neth. J. Med*. **47**, 288.
107. Di Lernia, V., and Bisighini, G. (1996). Discoid lupus erythematosus during treatment with cyclosporine. *Acta Derm. Venereol.* **76**, 87.
108. Yell, J. A., and Burge, S. M. (1994). Cyclosporin and discoid lupus erythematosus. *Br. J. Dermatol*. **131**, 132.
109. Furner, B. B. (1990). Treatment of subacute cutaneous lupus erythematosus. *Int. J. Dermatol*. **29**, 542.
110. Haxthausen, H. (1930). Treatment of lupus erythematosus by intravenous injections of gold chloride. *Arch. Dermatol*. **22**, 77.
111. Dalziel, K., Going, G., Cartwright, P. H., *et al*. (1986). Treatment of chronic discoid lupus erythematosus with an oral gold compound (Auranofin). *Br. J. Dermatol*. **115**, 211.
112. Crovato, F. (1981). Clofazimine in the treatment of annular lupus erythematosus. *Arch. Dermatol*. **117**, 249.
113. McCormack, L. S., Elgart, M. L., and Turner, M. L. (1984). Annular subacute cutaneous lupus erythematosus responsive to Dapsone. *J. Am. Acad. Dermatol*. **11**, 397.
114. Fenton, D. A., and Black, M. M. (1986). Low-dose dapsone in the treatment of subacute cutaneous lupus erythematosus. *Clin. Exp. Dermatol*. **11**, 102.
115. Holtman, J. H., Neustadt, D. H., Klein, J., and Callen, J. P. (1990). Dapsone is an effective therapy for the skin lesions of subacute cutaneous lupus erythematosus and urticarial vasculitis in a patient with C2 deficiency. *J. Rheumatol*. **17**, 1222.
116. Balkwill, F., Foxwell, B., and Brennan, F. (2000). TNF is here to stay! *Immunol. Today* **21**, 470.
117. Stirling, D. (2001). Thalidomide: A novel template for anticancer drugs. *Semin. Oncol*. **28**, 602.

30

SYSTEMIC LUPUS ERYTHEMATOSUS AND THE KIDNEY

James E. Balow
Dimitrios T. Boumpas
Howard A. Austin III

INTRODUCTION

It is well recognized that renal involvement contributes substantively to the morbidity of patients with systemic lupus erythematosus (SLE). While patients rarely die directly from uremia or lack of access to renal replacement therapies in modern times, early treatment of lupus nephritis and prevention of end-stage renal disease are important objectives in management of patients with SLE. Premature death due particularly to cardiovascular complications is the result of several components of lupus nephritis, including protracted nephrotic syndrome, hypertension, chronic renal insufficiency, and infection diathesis.

Given the diversity in types and severity of clinical manifestations of extrarenal SLE, it should not be surprising that an equally broad range of pathologic processes affects the kidney in this disease. Unfortunately, many forms of lupus renal disease, including severe forms of glomerulonephritis, are asymptomatic, easily overlooked by the clinician, and insidiously progressive. In this context, it is imperative that clinicians who undertake the care of patients with SLE must have in-depth knowledge of the diverse clinical and pathologic manifestations of lupus renal disease. Effective treatment depends on recognition of early phases of renal disease, prior to the stages of scarring, atrophy, and fibrosis.

DEFINITIONS AND MANIFESTATIONS OF LUPUS RENAL DISORDERS

Certain forms of renal involvement contribute to the diagnosis of SLE according to the widely accepted criteria of the American College of Rheumatology [1]. The diagnosis of a renal disorder includes the presence of: (a) persistent proteinuria of greater than 0.5 g/day (or greater than 3+ urine dipstick reaction for albumin), or (b) cellular casts, including red blood cell (RBC), hemoglobin, granular, renal tubular cell, or mixed casts. These elements are important in classification of SLE, particularly as standards for inclusion of patients in research studies. It must be emphasized that, while these laboratory criteria define the minimal threshold of kidney disease, there are many other clinical features which are important in the management of lupus nephritis [2–4].

CLINICAL SYNDROMES AND LABORATORY MANIFESTATIONS

To ascertain early renal involvement, patients with known or suspected diagnosis of SLE should undergo urinalysis screening at regular intervals because the onset of lupus nephritis is frequently asymptomatic. Furthermore, they should be queried about nocturia or

foamy urine. While nonspecific, nocturia may date the onset of glomerular or tubular dysfunction. Foamy urine suggests substantial proteinuria. Both symptoms warrant standard urinalysis and renal function tests [5]. Microscopic hematuria is mostly discovered by screening urinalysis in lupus nephritis. In SLE, macroscopic hematuria is relatively rare; it usually indicates very severe renal involvement and warrants expeditious assessment of renal function and degree of proteinuria. Rapidly progressive glomerulonephritis (RPGN) is defined as doubling of serum creatinine in less than a 3-month period, usually in the context of proliferative glomerulonephritis and cellular crescents in the renal biopsy.

Proteinuria is primarily a reflection of the extent of involvement of peripheral glomerular capillary loops. Thus, the degree of proteinuria tends to increase incrementally from mesangial to focal proliferative to diffuse proliferative lupus nephritis; membranous nephropathy which by nature involves essentially all glomerular capillary loops is characteristically accompanied by heavy proteinuria. Nephrotic range proteinuria is defined as proteinuria greater than 3.5 g/day; this degree of proteinuria usually causes the nephrotic syndrome which includes hypoalbuminemia, hyperlipidemia and, in the absence of diuretics, peripheral edema.

Renal failure, by convention, refers to loss of glomerular filtration function. In SLE, renal failure is primarily caused by the hypercellularity and inflammation within the glomerulus, though nephrotoxic drugs and other prerenal and postrenal causes of azotemia must always be considered. Sudden, acute renal failure is exceptional; rapidly progressive renal failure (RPGN as defined previously) occurs in a small fraction of lupus patients. Mostly, renal function fluctuates in parallel with remissions and exacerbations of lupus nephritis; chronic renal insufficiency results from cumulative damage and loss of nephrons, ultimately producing end-stage renal disease (ESRD). Renal tubular dysfunction, such as impaired urine concentrating ability and renal tubular acidosis, is rarely clinically significant or demanding of therapy.

URINALYSIS

Examination of urine is one of the most important and effective methods to detect and monitor the activity of lupus nephritis [5, 6]. There are no simple methods to standardize completely urinalysis testing, particularly given individual patient differences in fluid intake, urine concentrating ability, urine pH, and level of renal function. Early morning, mid-stream, clean catch urine samples (preferably the second voiding of the day but obtained while the patient is still fasting) are recommended in order to ensure reasonably concentrated and acidic urine specimens. Dipstick tests generally give reliable results; however, vitamin C supplements may produce false-negative dipstick reaction for blood, emphasizing the need for complementary microscopic analysis of the urine sediment. Given the fact that the typical lupus patient is both young and female, the clinician must guard against wrong inferences that hematuria is related to menstrual bleeding. It is recommended to perform urinalysis 3 or more days prior to or after cessation of menstrual periods.

The urine specimen should be processed expeditiously, lest the urine sediment deteriorates. Bacterial overgrowth may render the urine alkaline, which in turn causes rapid deterioration of urinary casts. The specimen should not be refrigerated to avoid precipitation of crystals which greatly interferes with microscopic analysis. Given the importance of cellular casts in defining and monitoring lupus nephritis, we use 50 ml (which is larger than conventional volumes) of urine for preparation of urinary sediments. The decanted pellet of sediment is stained with one drop of Sedi-Stain (Becton-Dickinson, Franklin Lakes, NJ). Using this large volume technique, we set the threshold for microscopic hematuria at >10 RBC per high power field (hpf).

The morphology of urinary red blood cells helps to distinguish upper and lower urinary tract disorders. Dysmorphic (misshaped, fragmented) erythrocytes (Fig. 1) indicate inflammatory glomerular or tubulointerstitial disease, while monomorphic (normal) erythrocytes indicate bleeding in the lower tract (e.g., infection, urolithiasis, tumors). Erythrocytes, leukocytes, and renal tubular epithelial cells are separately counted. Polarized light is used to detect free fat (doubly refractile fat bodies) and oval fat bodies (renal tubular epithelial cells containing fat droplets); lipiduria results from abnormal glomerular permeability to lipoproteins and possibly tubular epithelial cell injury caused by resorption of "toxic" filtered proteins. Granular, and fatty casts reflect proteinuric states; RBC, white blood cells (WBC), and mixed cellular casts reflect inflammatory (nephritic) states (Fig. 2); broad and waxy casts reflect chronic renal failure (Fig. 3). A telescopic urine sediment contains the full range of cells and cast elements; it reflects elements of global nephron (glomerular and tubular) dysfunction with ongoing active disease superimposed on chronic renal damage.

Accurate urinalysis requires careful and expeditious processing of the urine sample, good quality controls, and experienced personnel. It is important to remember that community-based clinical pathology laboratories for various reasons are frequently unable to identify

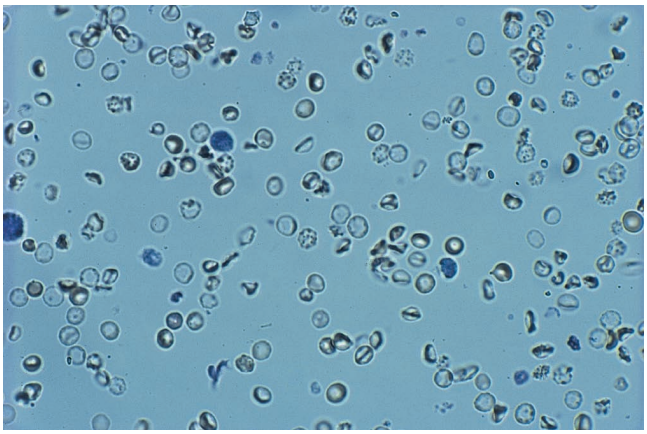


FIGURE 1 Dysmorphic red blood cells (RBC) in urine. RBC are of variable size and shape. Pattern strongly suggests hematuria of upper urinary tract origin.

pathologic casts [7]. This undoubtedly stems in part from the high volume throughput of urine samples for general screening purposes. There are two approaches to improve the reliability of urinalysis. First, the clinician should "flag" urine specimens from patients at substantial risk of lupus nephritis. Second, the clinician should, whenever feasible, personally review (and verify) the results of the microscopic urinalysis.

RENAL FUNCTION TESTS

Glomerular dysfunction is usually more prominent and clinically important than tubular dysfunction in lupus nephritis. Serum creatinine is the most widely used screening test to detect abnormalities of glomerular filtration. It is important to recognize that the normal range of serum creatinine is rather wide and that the relationship between serum creatinine and creatinine clearance, as a surrogate for glomerular filtration rate (GFR), must be determined for individual patients. Serum creatinine is affected not only by GFR, but also by age and muscle mass. The following nomograms quite reliably predict GFR from several basic clinical and laboratory parameters [8]:

Cockcroft-Gault formula

$$\mathrm{GFR}\,ml/min = \frac{(140 - \mathrm{age}\ yr)}{(\mathrm{serum\ Cr}\ mg/dL)} \times \frac{(\mathrm{weight}\ kg)}{72} \times 0.85\ (\mathrm{for\ women}) \qquad (1)$$

MDRD (four variable) formula

$$\mathrm{GFR}\,ml/min = 186.3 \times (\mathrm{serum\ Cr})^{-1.154} \times (\mathrm{age})^{-1.203} \times 1.212\ (\mathrm{if\ black}) \times 0.742\ (\mathrm{if\ female}) \qquad (2)$$

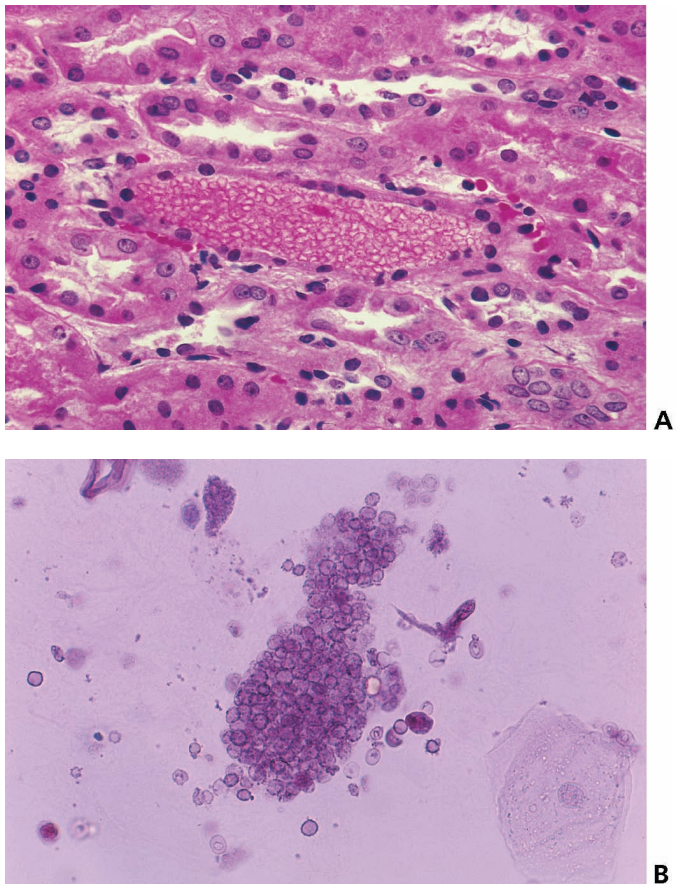


FIGURE 2 Red blood cell (RBC) casts. (**A**) RBC cast *in situ* within renal parenchyma. (**B**) RBC cast in urine sediment.

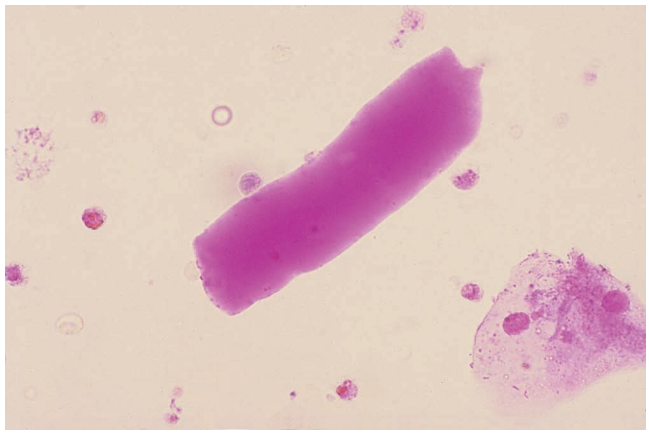


FIGURE 3 Broad, waxy cast in urine sediment. These casts indicate chronic nephron damage.

Another practical alternative for measuring changes in GFR over time is based on analysis of change in reciprocal of serum creatinine. Because creatinine excretion should be nearly constant, changes in the reciprocal of serum creatinine are directly proportional

to changes in creatinine clearance, as is evident in the following clearance formula:

$$\text{Creatinine clearance} = \frac{\text{creatinine excretion}}{\text{serum Cr}} = \text{Constant} \times \frac{1}{\text{serum Cr}} \quad (3)$$

When measuring standard creatinine clearance, one should be mindful that the major variability of the test stems primarily from errors in urine collection (volume). Patients must be explicitly instructed. Examining creatinine excretion is useful in assessing the completeness of urine collection and the reliability of the data used to calculate creatinine clearance. Creatinine excretion should be consistent on replicate collections and/or should approximate population averages for creatinine excretion, which in males is approximately 20 mg/kg/day and in females is approximately 15 mg/kg/day. Substantial deviations from either of these two standards should raise suspicion about the reliability of that particular test for estimating creatinine clearance.

Tests of true GFR (e.g., inulin, DTPA clearance), renal plasma/blood flow (e.g., para-aminohippurate, PAH, clearance), and filtration fraction (ratio of GFR to renal plasma flow) are sometimes used to enhance the accuracy and precision of measurement of changes in renal function [5]. These methods may identify substantial alterations in renal functions that are not recognized by standard methods. They are also sensitive measures of the effects of treatment on lupus nephritis. Because these tests are very labor intensive, expensive, and may involve radionuclides, they are seldom used outside the research setting.

Proteinuria is a critically important index of glomerular dysfunction. Timed collection of urine protein (usually over 24 h and in conjunction with creatinine clearance) represents the gold standard. However, test-to-test variability in proteinuria is common; this is mostly due to collection errors and differences in physical activity (bed rest tends to reduce and heavy exercise tends to increase proteinuria). Reproducibility is important before conclusions are drawn that clinically significant changes in proteinuria have occurred. We generally obtain three 24-h urine collections to document average basal proteinuria and creatinine clearance before initiating treatment.

Spot urine protein/creatinine ratio has been used as a simpler method to estimate the degree of proteinuria over time. The numeric ratio approximates the number of grams per day of proteinuria. This method has steadily gained wider application due to its simplicity and convenience [9]. Urine protein electrophoresis is of limited importance and rarely performed as part of the standard evaluation of the patient with known lupus nephritis. Proteinuria is usually of glomerular origin and unselective (mirroring serum protein concentrations). Isolated tubular proteinuria (nonalbumin) is rare. Measurement of microalbuminuria (30–300 mg/day) has not been thoroughly investigated but is generally not considered as a standard screening test in patients with suspected lupus nephritis.

Reduction in proteinuria is an important measure of favorable response to treatment in lupus nephritis [10–15]. Complete remission of abnormal proteinuria is defined as return to completely normal levels (i.e., <0.2 g/day, or urine protein/creatinine ratio <0.2). Partial remission of proteinuria is commonly defined by urine proteins of <0.5–1.0 g/day, or urine protein/creatinine ratio <0.5–1.0. Proteinuria may persist after remission of glomerular disease; this is referred to as "fixed" proteinuria due to irreversible damage to glomerular capillaries.

Laboratory abnormalities of renal tubular functions are common in lupus nephritis. Because they tend to be mild and do not warrant specific interventions, it is not usually cost-effective to document specific renal tubular cell dysfunctions in SLE. Decreased maximal urinary concentrating capacity may contribute to nocturia and may account for the tendency toward easy dehydration. Minor defects in urinary acidification (documented by acid-loading tests) are also common, but clinically significant renal tubular acidosis is rare.

RENAL BIOPSY

There are widely divergent views about the benefits of renal biopsy in lupus nephritis [16–19]. In research studies, documentation of renal pathology is considered essential. In clinical practice, neither an "always" nor a "never" approach to renal biopsy seems prudent. On the one hand, renal biopsy should *not* be considered as a method for diagnosis of SLE, nor should renal biopsy ordinarily be performed in lupus patients lacking clinical evidence of renal abnormalities. Although not absolute, there is a general consensus that most patients should have documentation of renal pathology prior to initiation of cytotoxic drug therapy. The decision to initiate cytotoxic drugs is often informed by results of a renal biopsy [20]. We agree with the opinion of Esdaile that ". . . watchful waiting is often an unwise course for management of lupus nephritis" [21]. Table 1 summarizes indications for renal biopsy and related issues in patients with lupus nephritis.

Beyond screening for a significant bleeding diathesis and urinary tract infection, renal ultrasound is

TABLE 1 Considerations Regarding Renal Biopsy in Lupus Nephritis

Renal biopsy is rarely useful in establishing a *diagnosis* of SLE in patients without laboratory evidence of renal disease, or in establishing lupus as the cause of nephritis in patients without other criteria for diagnosis of SLE
Renal biopsy may offer marginal or no incremental benefit in patients with recent onset glomerulonephritis characterized by a "full house" profile of severe disease (i.e., definitive diagnosis of SLE, nephritic syndrome, nephrotic syndrome, hypertension, and azotemia)
The standard clinical indications for renal biopsy include: nephritic urine sediment, especially cellular casts, and proteinuria >1.0 g per day
Interpretation of discordant clinical and laboratory parameters of active glomerulonephritis (e.g., discrepancies or ambiguities in urinalysis, proteinuria, lupus serologies, renal function tests) is often facilitated by the results obtained from renal biopsy
Renal biopsy findings often provide the underpinnings for describing prognosis and formulating therapeutic programs, particularly in patients deeply concerned about the risks of cytotoxic drug therapy
Studies have shown that biopsy findings, more often than clinical parameters, prompt the decision to institute cytotoxic drug therapy

TABLE 2 World Health Organization Classes of Lupus Nephritis

I.	Normal, or minimal glomerular abnormality No glomerular abnormalities on light microscopy
II.	Mesangial nephropathy Mesangial expansion, including hypercellularity, increased matrix, and immune complex deposits
III.	Focal proliferative glomerulonephritis Predominantly segmental hypercellularity ± necrosis compromising the circulatory space of capillary loops in ≤50% of glomeruli; mesangial and subendothelial immune complex deposits
IV.	Diffuse proliferative glomerulonephritis Characteristically global but irregular hypercellularity, ± necrosis, ± cellular crescents affecting >50% of glomeruli; variable sclerosis, atrophy, fibrosis; mesangial and subendothelial (± subepithelial) immune complex deposits
V.	Membranous nephropathy Generalized thickening of capillary loops; mesangial and subepithelial immune complex deposits
VI.	Sclerosing nephropathy Predominantly hyalinized glomeruli, tubular atrophy, interstitial fibrosis; no (or very rare) immune complex deposits

commonly used to assess the size of the kidneys prior to considering renal biopsy. Estimated kidney length less than three-fourths of expected normal size is associated with increased risk and decreased diagnostic yield. Percutaneous renal biopsy is usually performed under the guidance of a kidney imaging modality. Ultrasound is the most widely used technique, but computed tomography offers more precise (albeit more expensive) visualization. Operator experience is crucial to success of the procedure, because several potential shortcomings can compromise the interpretation and yield of the renal biopsy. It is important to obtain renal cortex containing 10 or more glomeruli for reliable interpretation of lupus nephritis. If biopsies are obtained by interventional radiologists (as a pure service function), it is important that the operator clearly understand the critical nature of sample size and tissue handling.

The renal biopsy specimen must be expeditiously processed in fixatives (or cooled on ice) at the biopsy table, lest autolysis of tissue ensues. In the patient with definitive diagnosis of SLE, priority for dividing and allocating samples should be given in the following order: (1) light microscopy specimen in formalin fixative, (2) electron microscopy specimen in glutaraldehyde fixative, and (3) immunofluorescence specimen on iced saline.

It is important that kidney biopsies be analyzed by experienced renal pathologists and reviewed in detail with the clinician responsible for the patient (preferably over the microscope and/or in a biopsy conference). At a minimum, renal biopsy reports should contain the World Health Organization (WHO) classification of lupus nephritis (Table 2) [22–27] and descriptions (preferably semiquantitative scores) of the elements comprising Activity and Chronicity Indexes which are further described later. A synopsis of the characteristic features and clinicopathological correlations of the different WHO classes of lupus nephritis is shown in Table 3.

PATHOLOGY OF LUPUS NEPHRITIS

There are few guidelines which can replace experience in recognizing and accurately describing renal biopsy pathology. The spectrum of pathologic changes in lupus nephritis is extremely broad, and categorization by the major WHO classes does not capture all the key features, especially degrees of activity (potentially treatable) and chronicity (irreparable damage) [27]. It is important to be able to recognize normal glomeruli; the glomerular capillary loops should appear thin and widely patent, with inconspicuous cellularity (Fig. 4). The glomerular mesangium, which seems to be the principal site of early lupus pathology, normally exhibits ≤3 contiguous nuclei and a small amount of matrix, best seen with periodic acid Schiff (PAS) stain. One can be misled about mesangial abnormalities if the microscopic

TABLE 3 Characteristic Features and Clinicopathological Correlations in Lupus Nephritis

	WHO class of lupus nephritis					
	I. Minimal	**II. Mesangial**	**III. FPLN[a]**	**IV. DPLN[b]**	**V. Membranous**	**VI. Sclerosing**
Normal urine	X					
Hematuria		X	X	X	±	
Cellular casts			X	X		
Broad, waxy casts			±	±	±	X
Proteinuria		±	X	X	X	±
Nephrotic syndrome				X	X	
Azotemia				X	±	X
Hypertension				X	±	X

[a] FPLN, focal proliferative lupus nephritis.
[b] DPLN, diffuse proliferative lupus nephritis.

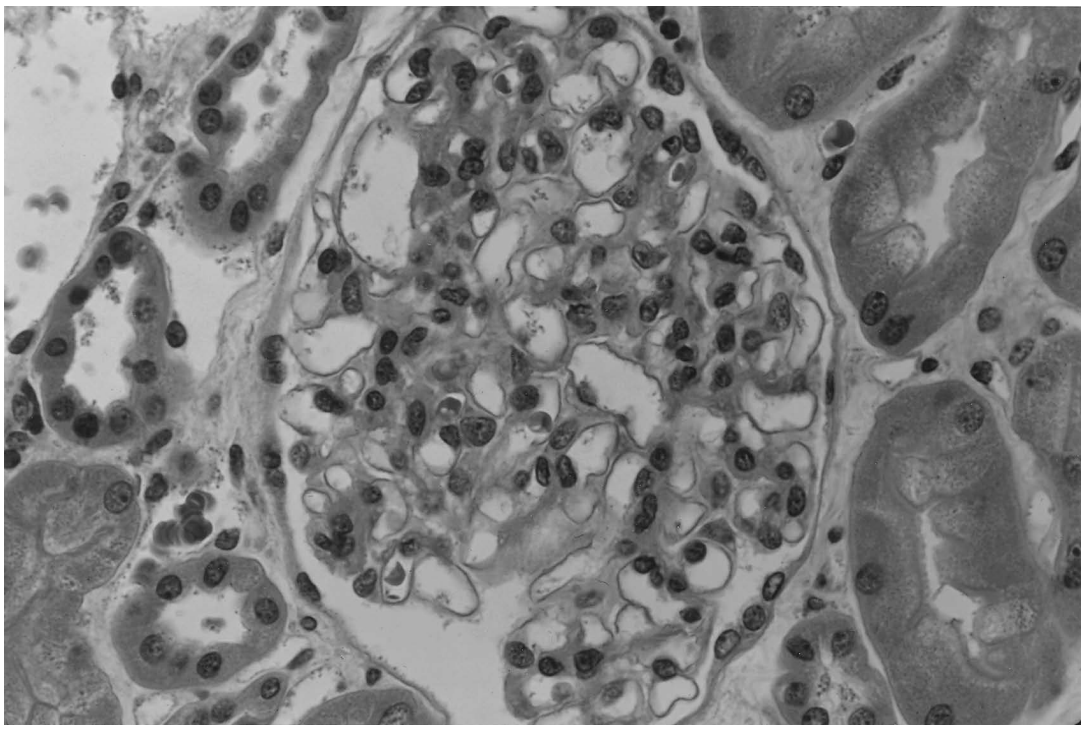


FIGURE 4 Normal glomerulus. Note that capillary loops are widely patent and that endogenous mesangial, endothelial, and epithelial cells are relatively inconspicuous (H&E stain).

sections are thick (normally tissue sections should be <4 μm).

Normal or Minimal Mesangial Abnormality (Class I)

By light microscopy, glomeruli may appear normal or show minimal mesangial expansion. Immune deposits are seen in the mesangial areas by immunofluorescence and electron microscopy. There are few cases with Class I lupus nephropathy described in published series due to the absence of clinical indications for biopsies in such patients.

Mesangial Nephropathy (Class II)

Mesangial nephropathy is diagnosed by several criteria: expansion of the mesangial stalk due to increased cellularity and matrix substance by light microscopy

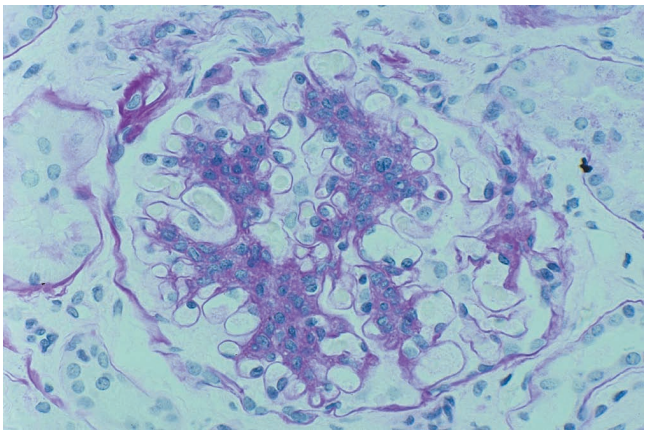


FIGURE 5 Mesangial nephropathy (WHO Class II). Note the expansion of mesangial cells and matrix without substantive encroachment on capillary loop space (PAS stain).

(Fig. 5), as well as deposition of immune complexes by immunofluorescence and electron microscopy (Fig. 6). Capillary loops remain patent and there are no other active or sclerosing lesions compromising glomerular capillary circulation and filtration. Mesangial nephropathy can be considered the least common denominator of lupus nephritis. In some cases, mesangial nephropathy represents intrinsically mild disease; in others, mesangial nephropathy is an early stage in the progression to the following more severe forms of lupus nephritis.

Focal (Class III) and Diffuse Proliferative (Class IV) Lupus Nephritis

Proliferative lupus nephritis primarily represents glomerular changes which burgeon forth from the back-

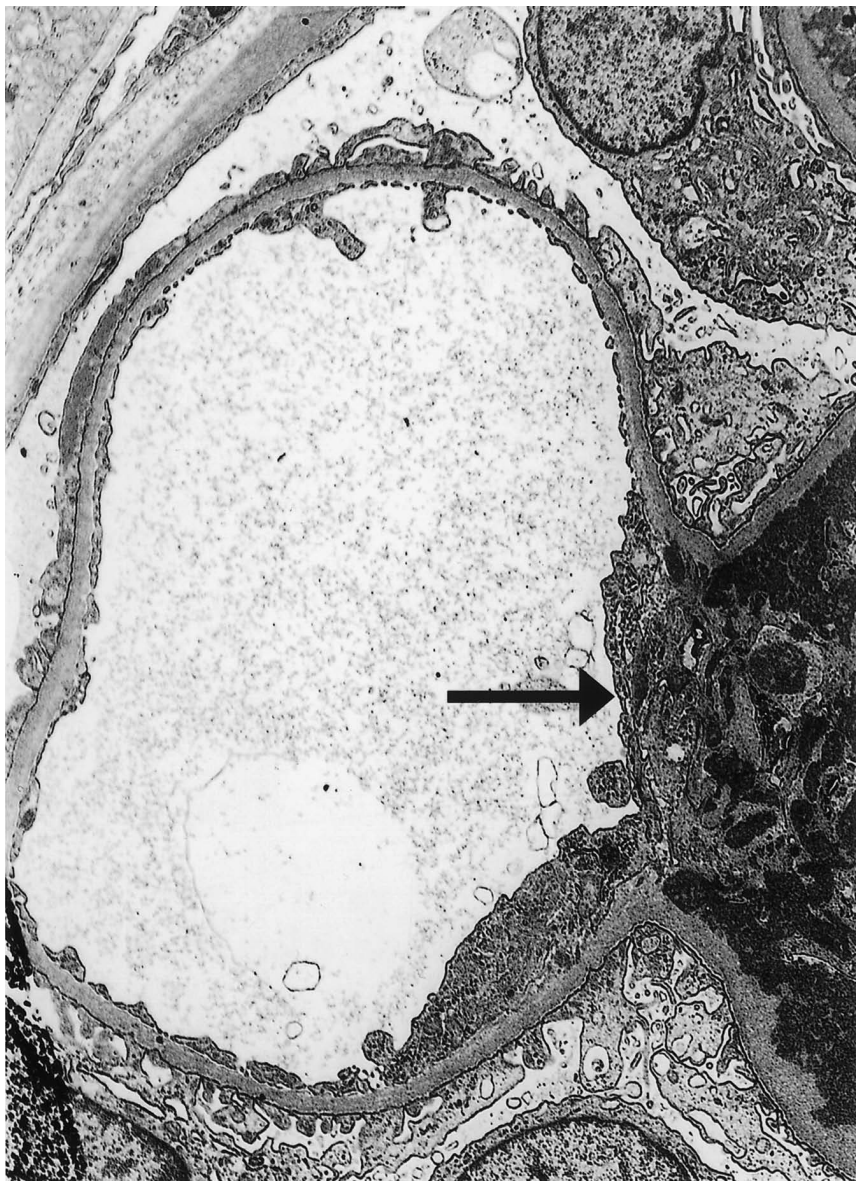


FIGURE 6 Ultrastructural appearance of mesangial deposits (arrow). The black staining deposits are admixed with gray mesangial matrix. Note the normal appearance of the peripheral capillary loops. Electron micrograph courtesy of Dr. Sharda Sabnis, Nephropathy Section, Armed Forces Institute of Pathology, Washington, D.C.

ground of mesangial nephropathy. Focal (Fig. 7) and diffuse (Fig. 8a–c) proliferative lupus nephritis are qualitatively similar and differ mostly in extent and severity of proliferative and inflammatory lesions. Conceptually, focal and diffuse proliferative lupus nephritis represent mild and severe glomerular disease, respectively. The conventional dividing line is defined by pathologic lesions that reduce glomerular capillary loop space in the minority (Class III) or majority (Class IV) of nephrons. However, it should be stressed that, even in diffuse proliferative lupus nephritis, the distribution of proliferative lesions is highly irregular, both within and among glomeruli. The increased glomerular cellularity is due to proliferation of mesangial and endothelial cells plus leukocytic infiltrates (polymorphs and macrophages). Visceral epithelial cells may enlarge and exhibit degenerative change, but are thought to have limited (if any) capacity to proliferate.

Immune complex deposits fill the mesangial interstices and extend peripherally along the subendothelial aspect of the glomerular capillaries (Fig. 9). It is not clear whether this extension of deposits is an "overflow" phenomenon, or whether focal and diffuse proliferative, in contrast to mesangial, lupus nephritis are caused by unique species of immune complexes with a special affinity for capillary basement membranes. By indirect immunofluorescence microscopy, the deposits can be shown to contain IgG, IgA, IgM, C1q, and C3 (the so-called, full house immunofluorescence of proliferative lupus nephritis). When present, fresh cellular crescents or true microvascular thrombi usually contain fibrin.

There is a subset of diffuse proliferative lupus nephritis in which there are both subendothelial deposits and extensions of mesangial matrix peripherally around glomerular capillaries. The matrix extension produces double contours in capillary loops and an overall pattern of lobulation of the glomerulus. This form of lupus nephritis is called membranoproliferative (or mesangiocapillary) glomerulonephritis after the idiopathic variety of glomerulonephritis with similar appearance.

In severe forms of proliferative lupus nephritis, heavy subendothelial deposits give rise to classic, deeply eosinophilic, and PAS-positive, "wire loop" lesions in glomerular capillaries. Massive immune deposits filling capillary loops give rise to hyaline thrombi, which are "thrombi" by appearance only. The pathogenesis of these immunoglobulin-containing lesions is uncertain, but they are particularly frequent in cases with cryoprecipitable circulating immune complexes. These hyaline thrombi have also been given the more

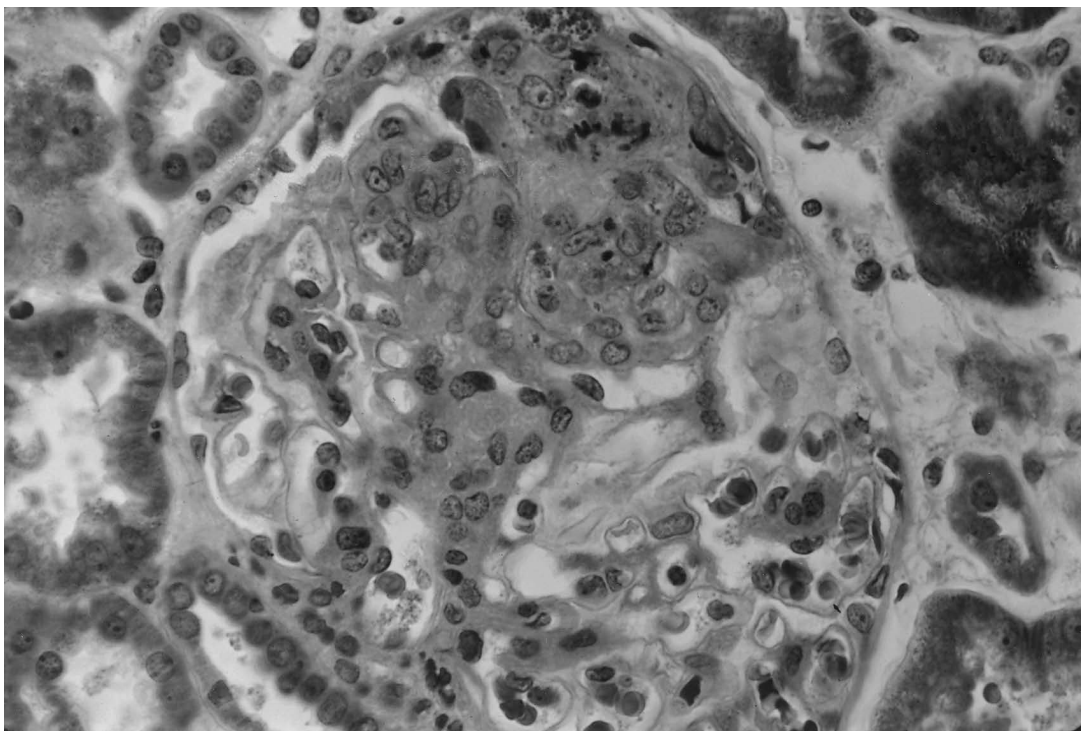


FIGURE 7 Focal proliferative lupus nephritis (WHO Class III). Glomerulus shows segmental proliferation with karyorrhexis and fibrinoid necrosis (H&E stain).

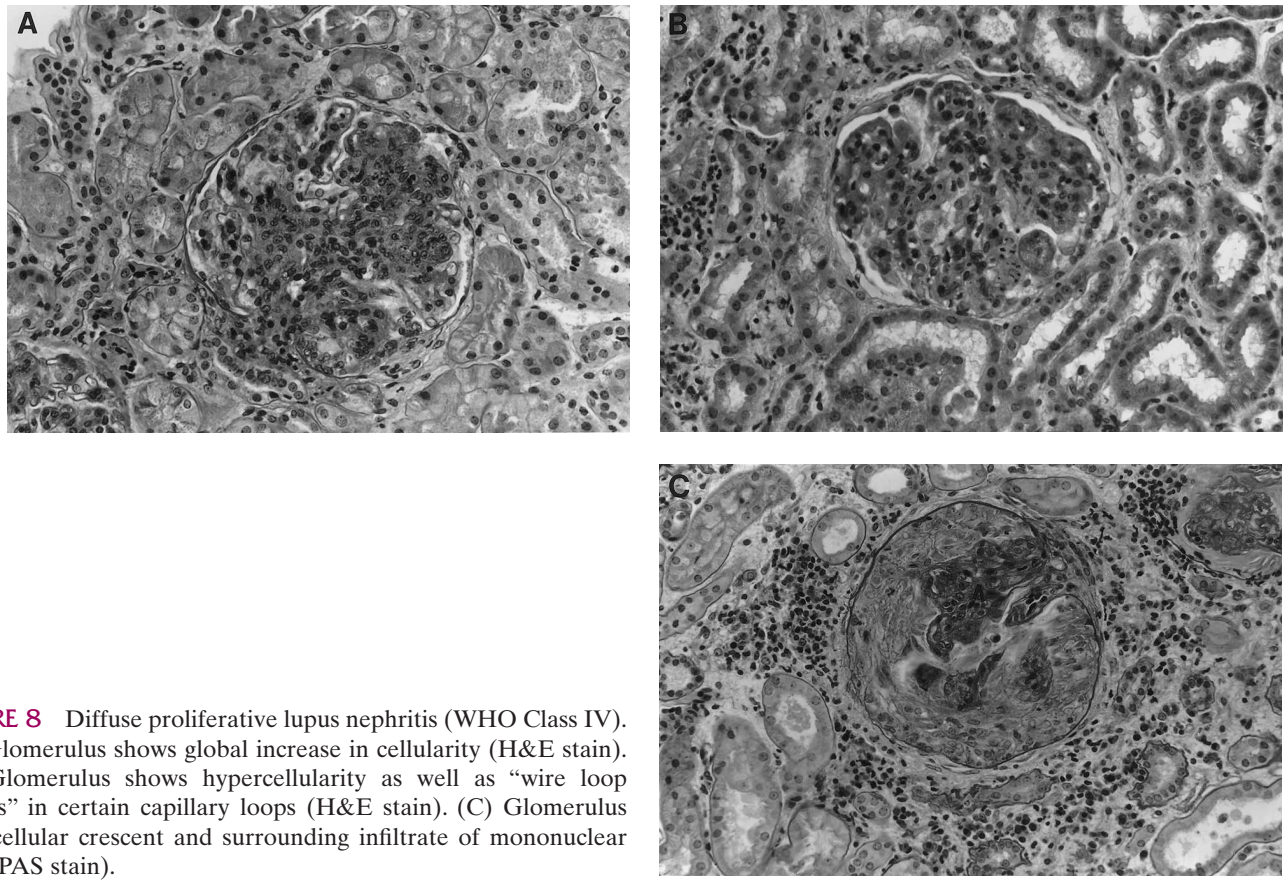


FIGURE 8 Diffuse proliferative lupus nephritis (WHO Class IV). (A) Glomerulus shows global increase in cellularity (H&E stain). (B) Glomerulus shows hypercellularity as well as "wire loop lesions" in certain capillary loops (H&E stain). (C) Glomerulus with cellular crescent and surrounding infiltrate of mononuclear cells (PAS stain).

descriptive name of immunoglobulin microvascular casts [28].

The hyaline thrombi lesions overlap and may be admixed with those of true fibrin thrombi which arise from the thrombotic diathesis seen in certain lupus patients. It is not entirely resolved whether or not antiphospholipid antibodies increase the frequency of glomerular microvascular thrombosis [29, 30]. Fibrin thrombi are seen in rare cases where the syndrome of thrombotic thrombocytopenic purpura is superimposed on SLE (see later). It is notable that in some cases of segmental necrotizing lupus nephritis, there are very sparse immune complex deposits, leading to the suggestion that this lesion may sometimes arise from a cell-mediated immune process or represent a pauci-immune glomerular disease equivalent to that of renal vasculitis [31].

Severe inflammation within glomerular capillaries may lead to cellular destruction with nuclear fragmentation (karyorrhexis) and fibrinoid necrosis (smudgy, brightly eosinophilic clumps of plasma products). Hematoxylin bodies may form when there is heavy nuclear debris, but these lupus-specific lesions are quite rare in rapidly fixed needle biopsy specimens (in contrast to surgical biopsies and autopsies). When relatively mild, segmental inflammation may produce local adhesions of glomerular capillaries to Bowman's capsule. When severe, inflammation, and particularly fibrinoid necrosis, may be followed by frank rupture of glomerular capillaries with hemorrhage into Bowman's space and formation of exuberant cellular crescents. Cellular crescents are aggregates comprised of two or more layers of cells which line one-fourth or more of the interior circumference of Bowman's capsule. Cellular crescents are comprised of a combination of proliferating epithelial cells and infiltrating macrophages [32]. Circumferential crescents are thought to doom affected glomeruli to obsolescence. In general, increasing numbers of cellular crescents have proportionally more adverse effects on prognosis.

Membranous Lupus Nephropathy (Class V)

Membranous nephropathy is defined most rigorously by the presence of subepithelial (epimembranous) deposits along peripheral glomerular capillary loops (Fig. 10). These deposits may be seen with trichrome stains under high-power light microscopy, but are more

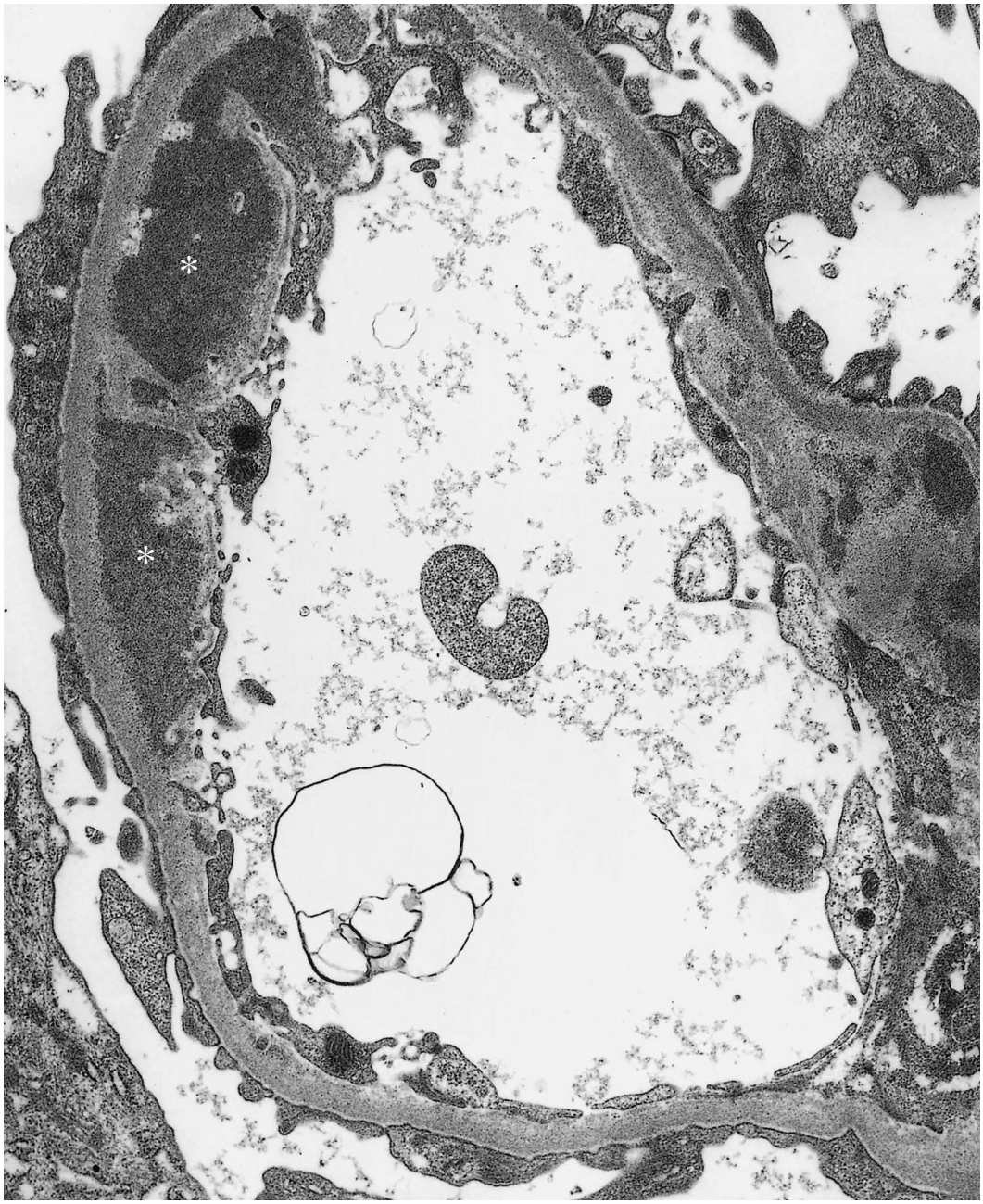


FIGURE 9 Ultrastructural appearance of subendothelial deposits (asterisks). The dark staining deposits are between the glomerular basement membrane and the endothelial lining of the capillary loop. Electron micrograph courtesy of Dr. Sharda Sabnis, Nephropathy Section, Armed Forces Institute of Pathology, Washington, D.C.

readily demonstrated by electron microscopy (Fig. 11). Early in the course, capillary loop thickening per se may not be apparent on light microscopy. In progressive stages, membranous nephropathy is characterized by capillary loop thickening and, with silver stains, the appearance of basement membrane spikes projecting outward between subepithelial immune deposits. In advanced stages, deposits may become fully incorporated within thickened basement membrane and finally undergo dissolution, leaving the membrane with a "moth-eaten" appearance. Mixed membranous and proliferative classes of lupus nephritis sometimes coexist, and membranous nephropathy is occasionally complicated by crescent formation.

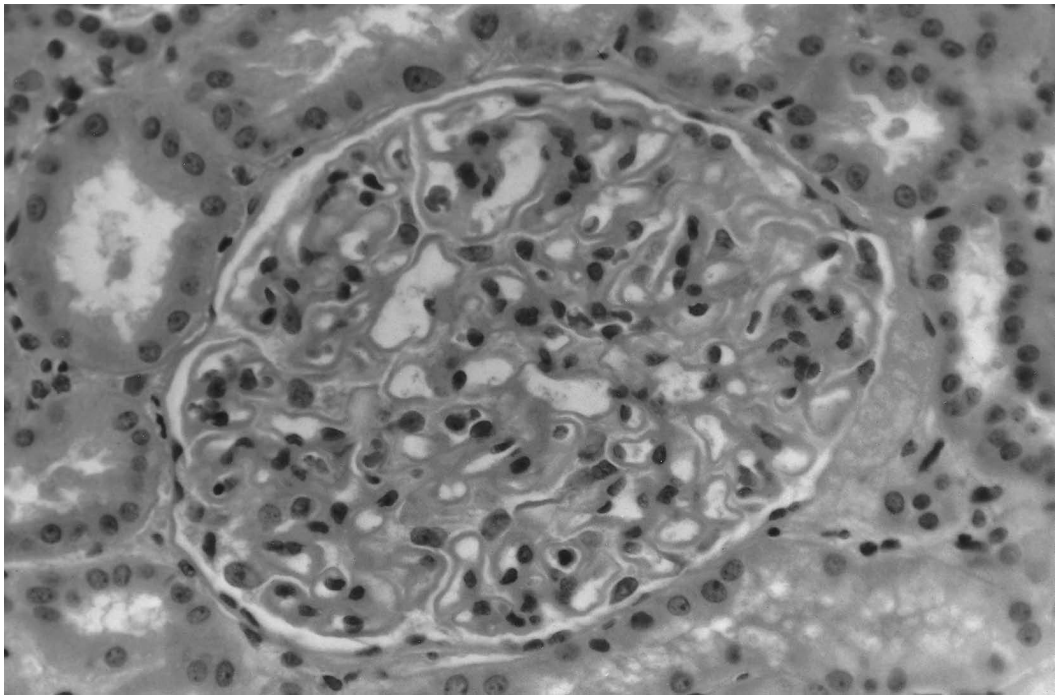


FIGURE 10 Membranous lupus nephropathy (WHO Class V). Glomerulus shows diffuse thickening but retained patency of peripheral capillary loops (H&E stain).

Sclerosing Nephropathy (Class VI)

Inclusion of this category was a late addition to the classification of lupus nephritis. It basically represents a stage rather than a specific type of lupus nephritis. Class VI reflects late stage, irreversible renal damage with advanced glomerulosclerosis, tubular atrophy, and interstitial fibrosis (Fig. 12a,b). Class VI may evolve from any prior form of severe lupus nephropathy, as well as from coincidental hypertensive, vascular, or drug-induced nephropathies. First definition of lupus nephropathy at this late stage represents neglect on the part of the patient or physician and should be exceptional.

Tubulointerstitial disease is an important component of the pathology of lupus nephritis. Indeed, interstitial scarring and atrophy are among the strongest predictors of prognosis in most glomerulonephritides, including lupus nephritis. Interstitial infiltrates of predominantly mononuclear cells (lymphocytes, plasma cells, and macrophages) are seen in the majority of cases of lupus nephritis [33]. In general, the degrees of infiltrates are proportional to the severity of the glomerular disease. Occasionally, interstitial infiltrates have been found in the absence of substantive glomerular disease. The role of mononuclear cell infiltrates in the pathogenesis of lupus nephritis is unclear. Immune complexes are frequently deposited in a peritubular location (along tubular basement membranes) and sometimes in the walls of peritubular capillaries. Whether these deposits originate from reabsorbed tubular proteins, circulating immune complexes, or locally from antibody secreting plasma cells is uncertain.

It is a reasonable hypothesis that whole nephron injury and drop out can be a bidirectional process. On the one hand, massive glomerular destruction and sclerosis could lead to downstream vascular compromise and tubular atrophy. On the other hand, primary tubular damage, either from intraluminal processes (e.g., "toxic" proteinuria) or from peritubular processes (e.g., immune complexes or cytotoxic lymphoid cells), could produce cast nephropathy, tubular dilatation, and upstream glomerular injury [34]. The relatively poor correlation of interstitial inflammation with renal outcome suggests the primacy of glomerular processes in the pathogenesis of tubulointerstitial scarring, atrophy, and fibrosis in lupus nephritis.

Assessment of the integrity of the tubulointerstitial areas is facilitated by surveying sections stained by PAS stain, which highlights tubular basement membranes. Normally, there should be little interstitial space between tubules. Thickening of tubular basement membranes is one of the best indications of impending tubular atrophy; this is followed by deposition of waxy casts, shrinkage, and drop out of tubules. Trichrome stains, which render collagen a blue color, are best for estimating interstitial fibrosis.

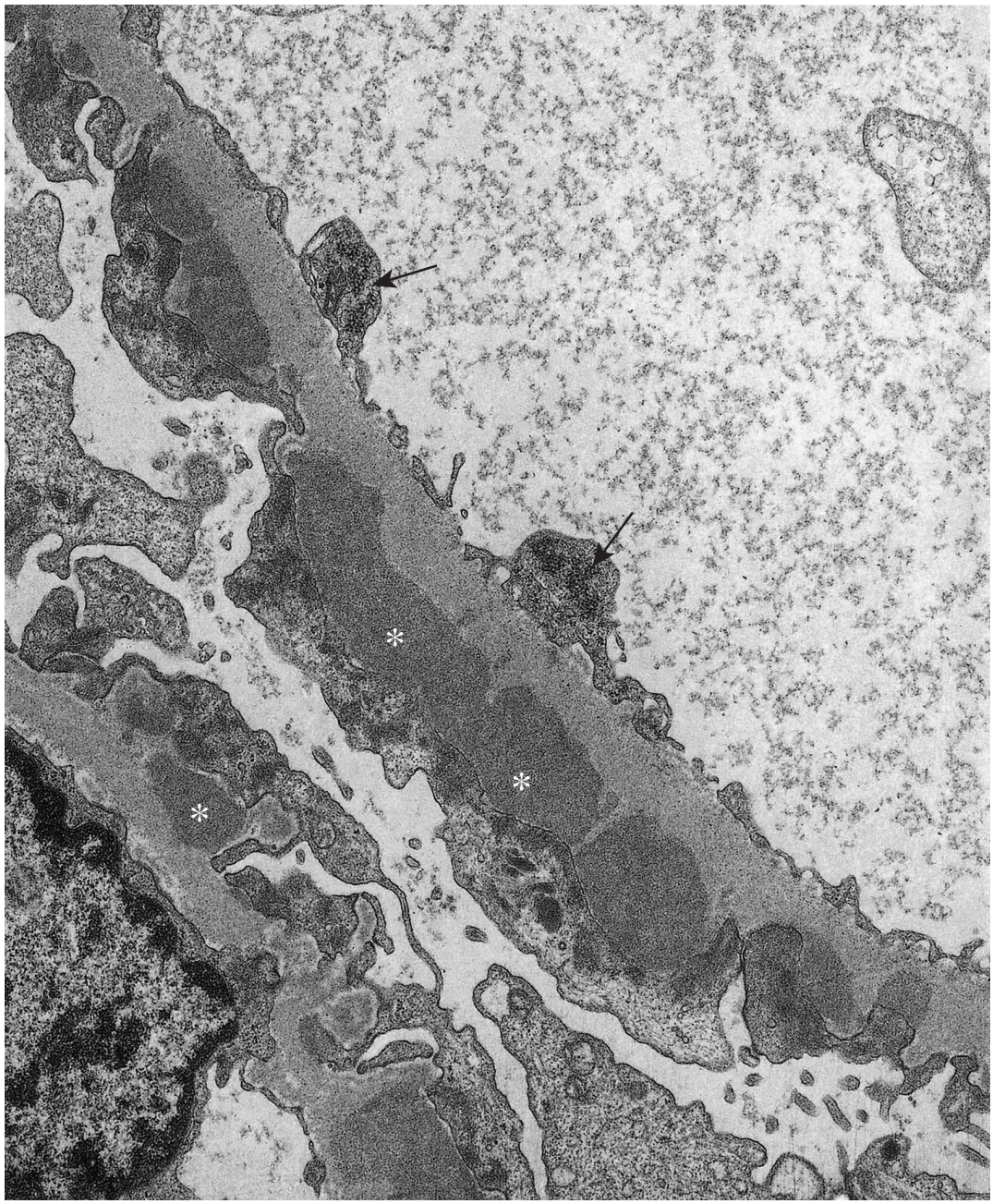


FIGURE 11 Ultrastructural appearance of subepithelial deposits (asterisks). The dark staining deposits are on the external (epimembranous) aspect of the glomerular basement membrane. The foot processes of the epithelial cells are fused. Note the presence of tubuloreticular structures in two endothelial cells (arrows). Electron micrograph courtesy of Dr. Sharda Sabnis, Nephropathy Section, Armed Forces Institute of Pathology, Washington, D.C.

Hypertensive arteriolosclerosis is relatively common in biopsies of lupus nephritis, but it is usually anticipated from clinical findings of poorly controlled blood pressure. Frank extraglomerular vasculitis is rare but well documented; the impact of necrotizing vasculitis on a prognosis in lupus has not been well studied.

ACTIVITY AND CHRONICITY INDEXES

Description of the balance of activity and chronicity in the pathology of lupus nephritis is enhanced by two related approaches. Subsets of WHO Class III focal

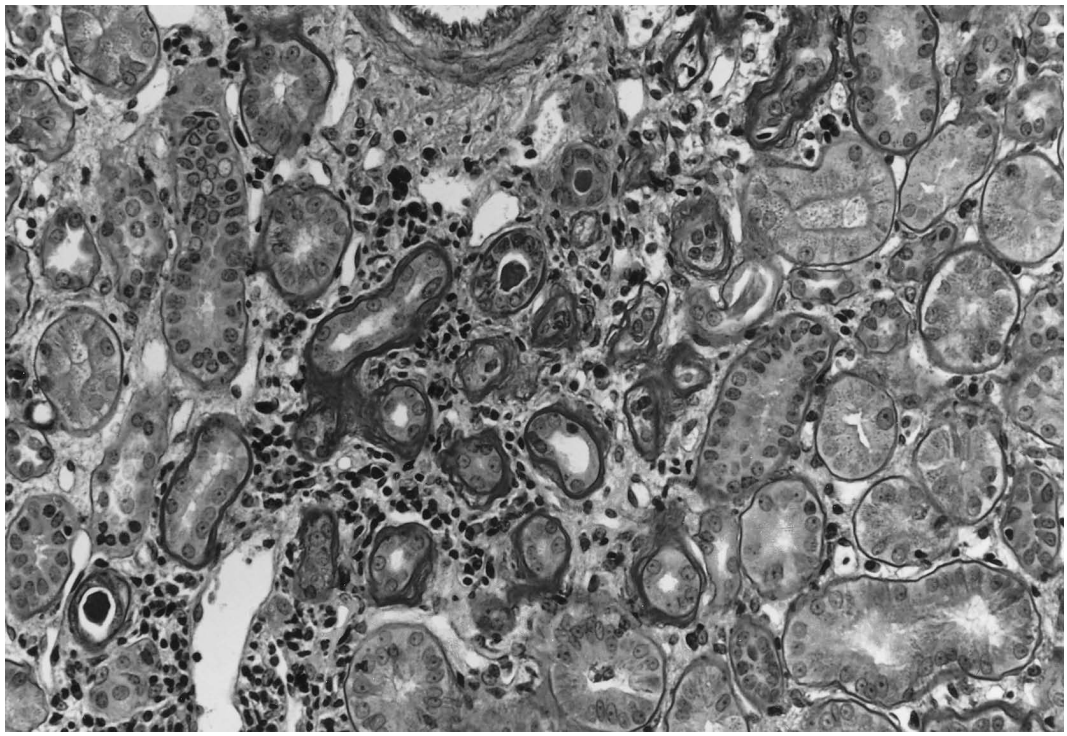


FIGURE 12 Advanced chronic lupus nephropathy. Thickened tubular basement membranes indicate tubular atrophy. Peritubular interstitial infiltrates of mononuclear cells are present (PAS stain).

proliferative lupus nephritis have been proposed which specify whether the lesions are primarily active (IIIa), mixed active and sclerosing (IIIb), or primarily sclerosing (IIIc); similarly, subsets of WHO Class IV diffuse proliferative lupus nephritis have been proposed which specify whether lesions are pure proliferative (IVa), predominantly active (IVb), mixed active and sclerosing (IVc), or predominantly sclerosing (IVd) [18, 27].

Our preferred approach is to classify the glomerular disease by major WHO class and to supplement description of the biopsy by semiquantitative scoring of disease activity and chronicity (damage) in a checklist fashion (Table 4) [35–39]. In early stages of lupus nephritis, cases fit more comfortably into one of the major WHO classes. During the evolution of lupus nephritis and under the disease-modifying influences of treatment, it becomes increasingly difficult to find a category to accommodate all the essential biopsy findings. In these cases, a synopsis of activity and chronicity features becomes particularly useful in capturing the essence of the biopsy appearance. Another utility of the activity and chronicity indexes is as a measure of change in renal pathology over time. That lupus nephritis undergoes transitions among major WHO classes is well known. However, changes in disease activity and chronicity within a given WHO class of renal pathology are even more common [40]. Here again, the activity and chronicity indexes allow one to capture the balance of pathologic changes when repeat renal biopsies are undertaken. Most, but not all, centers have found excellent reproducibility of scoring activity and chronicity and have incorporated these tools into their renal biopsy report forms.

PATHOGENESIS OF LUPUS NEPHRITIS

Detailed descriptions of the immunopathogenesis of SLE, and particularly those of murine lupus nephritis, are covered elsewhere in this volume. Clearly, cell mediated immune responses are involved in facilitating the production of autoantibodies, and possibly even for direct injury to the kidney. Studies of the genetic and immunologic basis of SLE and lupus nephritis have been greatly facilitated by murine models [41, 42]. A provocative study based on knock out of the Fc γ-chain receptor in the NZB/W model of murine lupus nephritis demonstrated an uncoupling of immune complex formation and kidney damage. Animals lacking functional Fc receptors had equivalent deposition of immune complexes in the kidney, but had markedly reduced glomerular inflammation, indicating that Fc receptors were more important in host responses to

TABLE 4 Renal Biopsy Activity and Chronicity Indexes

Activity Index (maximum score 24)
Hypercellularity: Segmental or global endocapillary hypercellularity that reduces the circulatory volume of glomerular capillary loops[a]
Leukocyte exudation: Three or more polymorphonuclear leukocytes per glomerulus[a]
Karyorrhexis/fibrinoid necrosis (weighted ×2): Karyorrhexis: pyknotic and fragmented nuclei; fibrinoid necrosis: amorphous, eosinophilic, plasma-derived debris within solidified capillaries[b]
Cellular crescents (weighted ×2): Proliferation of extracapillary epithelial cells and infiltrating macrophages creating two or more layers of cells occupying one-fourth or more of the circumference of Bowman's capsule[b]
Hyaline deposits: Wire loop lesions: eosinophilic material of a homogenous consistency along the luminal surface of capillary loops; Hyaline thrombi: more extensive globular, PAS positive material occupying the entire lumen of the capillary[a]
Interstitial inflammation: Infiltration of mononuclear cells (lymphocytes, plasma cells, macrophages) into tubulointerstitial spaces[a]
Chronicity Index (maximum score 12)
Glomerular sclerosis: Glomerular capillary collapse with attendant expansion and solidification of mesangial matrix (segmental or global pattern)[b]
Fibrous crescents: Structures composed predominantly or exclusively of fibrous tissue lining Bowman's capsule[b]
Tubular atrophy: Atrophic changes identified by thickening of tubular basement membranes, with or without tubular epithelial cell degeneration; separation of residual tubular cells is observed.[a]
Interstitial fibrosis: Deposition of periglomerular and peritubular fibrous tissue[a]

[a] Scored on a scale of 0–3+ representing absent, mild, moderate, and severe lesions, respectively.

[b] Scored on a scale of 0–3+ according to presence of lesions in none, <25%, 25–50%, and >50% of glomeruli, respectively.

TABLE 5 Overview of Major Pathogenetic Mechanisms in Lupus Nephritis

Autoantibody production
Some evidence of aberrant or dysregulated B-cell clones producing autoantibodies
Strong evidence for T-dependent, B-cell production of pathogenic autoantibodies to a limited number (~30) of autoantigens
Aberrant expression and activity of B- and T-cell costimulation molecules, CD40-CD154 (CD40L) and CD80/86-CD28, promote dysregulated autoantibody production
Immune complex injury
Deposition of preformed soluble circulating immune complexes in the kidney
Excessive circulating immune complexes is due to both overproduction and impaired reticuloendothelial system clearance
In experimental animal models, deletion of the gene for the γ-chain of the Fc receptor ameliorates nephritis, in spite of immune complex formation and complement activation in the kidney; these experiments indicate that effector mechanisms involving Fc receptors are more important in host responses to immune complexes than for the localization of complexes in the kidney
African-Americans, but not Europeans, with lupus nephritis have an increased frequency of the R131 allele of the human Fcγ receptor which less efficiently recognizes immune complexes containing IgG2 (which are potent activators of complement); however, a meta-analysis showed that Fcγ receptor polymorphisms represented risk for SLE but have no clear effect on susceptibility to lupus nephritis
In situ formation of immune complexes by antibody reaction with constitutive kidney antigens or to antigens planted in the renal parenchyma; this mechanism has been proven for the subepithelial deposits in animal models of membranous nephropathy, but remains unproven in human nephropathies
Injury produced mainly by local activation of complement components with production of leukocyte chemotactic factors (producing hypercellularity and/or necrosis) and formation of membrane attack complex, C5b-9 (increasing glomerular permeability)
Cell mediated immune responses and injury
Cellular interactions (T cells, B cells, antigen presenting cells, resident renal cells) are mediated by both cell-to-cell contact (surface activation and adhesion molecules) and soluble factors (e.g., tumor necrosis factor, interleukin-1)
T cells are characteristically present in interstitial infiltrates, but the role of direct T-cell-mediated injury in the pathogenesis of lupus nephritis is uncertain

immune complexes than to the localization of complexes in the kidney [43].

The following brief overview will focus on the role of autoantibodies and immune complexes (Table 5). This is based on the proposition that lupus nephritis is the prototype of immune complex diseases of the kidney [44–46]. SLE is a complex multisystem disease which develops from a massive overproduction of polyclonal antibodies and impaired clearance of immune complexes. Many of the antibodies are autoreactive and are used in the diagnosis of SLE. Tissue damage results from deposition of pathogenic immune complexes which triggers interacting cascades of inflammation, vasculopathy, and sclerosis.

The classic paradigm is that in disease-prone lupus patients, persistently high levels of circulating immune complexes leads to their egress into the interstices of the mesangium. It is not clear whether the low level of mesangial receptors (e.g., Fc receptors) for components of immune complexes facilitates localization of complexes, or whether the escape of complexes from the blood occurs because of the absence of a structural basement membrane barrier between the circulatory space and the mesangium interstices. Under some circumstances, a steady-state balance of deposition and

disposal (local degradation or exit through renal lymphatic channels) of complexes may produce limited mesangial reaction and no (or minor) clinical signs of renal disease. In others, either due to increasing flux and/or to particular nephritogenic potential of immune complexes, inflammation and mesangial cell activation, with proliferation and production of excessive extracellular matrix, arise to constitute the pathology of mesangial nephropathy. The details of mesangial injury and repair are complex and the subject of intense current investigation in experimental models, which are reviewed elsewhere in this volume. Clinical experience suggests that there may be two forms of mesangial nephropathy: one represents an "intrinsically" benign or compensated mesangiopathic process with little tendency to progress (stable Class II); the other represents mesangial immune complex disease in transition to pathology affecting the peripheral capillary loops (Classes III–V).

As noted previously, it is not clear whether complexes which deposit beneath the endothelium of peripheral capillary loops have qualitatively distinct properties from those with a predilection for the mesangium. One model holds that overload of mesangial clearing mechanisms leads to accumulation of complexes, first at the base or paramesangial areas of the capillary and then more peripherally. Another model holds that localization to the mesangium vs the basement membrane is determined by physicochemical properties of the complexes. Immune complexes with dominant cationic charge and affinity for the anionic basement membrane structures are found with increased frequency in subjects with more severe lupus nephritis. Charge–charge interactions with basement membrane sites may involve free antigens or antibodies or immune complexes. A hypothesis holds that free nuclear antigens released during apoptosis may be "planted" on basement membranes (perhaps via cationic histones) with formation of immune complexes by anti-nuclear antibodies *in situ*.

The nature of the nephritogenic complexes in proliferative lupus nephritis remains controversial. DNA–anti-DNA complexes have long been considered leading suspects [47]. Antibody eluates for lupus kidneys have higher anti-DNA reactivity per unit of immunoglobulin than that of circulating immunoglobulin. Among lupus patients, there are reasonable correlations between anti-DNA antibodies and nephritis activity. However, individuals without anti-DNA may have severe nephritis and others with high titers of anti-DNA may have no evidence of lupus nephritis. Antibodies to endothelial antigens have been reported, but there is no consensus that they play a pivotal role in the pathogenesis of lupus nephritis. Similarly, antiphospholipid antibodies were considered logical candidates to contribute to lupus nephritis. However, these autoantibodies do not produce nephritis in patients with primary antiphospholipid syndromes, nor do they correlate well with morphologic features of lupus nephritis.

The pathogenesis of lupus membranous nephropathy poses a further challenge. Here complexes aggregate on the outer (subepithelial) surface of the glomerular basement membrane. The access of circulating immune complexes to this site is difficult to envision. Moreover, levels of circulating immune complexes correlate poorly with the presence of subepithelial deposits. Experimental models of membranous nephropathy suggest potential mechanisms for forming subepithelial deposits. In the Heymann model, antibodies directed against constitutive antigens on the podocytes of glomerular epithelial cells leads to *in situ* formation of complexes, aggregation, capping, shedding, and finally binding of the complexes to the basement membrane [48]. Other models involve "planting" of antigens in the glomerular basement membrane to which subsequent antibody exposure leads to *in situ* formation of subepithelial immune complexes. Unfortunately, neither model has been proven applicable in human membranous nephropathy. No antibodies derived from plasma or eluted from kidneys affected by membranous nephropathy have shown clear reactivity with normal human kidney antigens. In short, the pathogenesis of human lupus membranous nephropathy remains an enigma.

It seems likely that no species of antigen or antibody is singularly responsible for nephritogenicity in lupus nephritis. Clearly, proof of nephritogenic antigens is essential for planning clinical experiments in tolerance induction, such as would be relevant to a clinical trial using artificial constructs of DNA [49]. However, current therapies focused mainly on nonspecific or polyclonal immunosuppression are not limited by lack of definitive knowledge of the hierarchy of candidate antigens and autoantibodies.

COURSE OF PROLIFERATIVE LUPUS NEPHRITIS

In contemporary clinical practice there are few opportunities to observe the true natural history of lupus nephritis. The occasional patient, unwilling or unable to access health care, may demonstrate the catastrophic consequences of untreated lupus nephritis, a situation presumably equivalent to that reported in

patients throughout the first half of the twentieth century [50]. Descriptions of the course of lupus nephritis should be recognized, not as natural history studies, but rather as the effects of treatment on the course of this disease.

The dramatic improvement in patient survival at mid-century was undoubtedly attributable to new diagnostic tests for SLE, development of corticosteroids, antihypertensives, and antibiotics. For approximately two decades into the second half of the twentieth century, improved longevity led to increases in the proportion of patients that succumbed to the renal failure of lupus nephritis. Data accumulated by the pioneers in this field showed a mean survival of less than 2 years after diagnosis of proliferative lupus nephritis [22, 23, 51]. Attempts to thwart progressive lupus nephritis with corticosteroids produced modest benefits, and often debilitating and life-threatening toxicities arose during treatment. During that era, renal biopsy led to improvement of prognostication based on recognition of the different classes of lupus nephritis. Experienced clinicians recommended intensive corticosteroid therapy for diffuse proliferative, but not for focal or membranous, forms of lupus nephritis [24–26, 52, 53]. Unfortunately, the bulk of patients with clinical evidence of renal disease had diffuse proliferative lupus nephritis and the prognosis, though modestly improved from earlier times, remained poor with corticosteroid treatment alone. Anecdotes and small series suggested additional benefit of cytotoxic drugs, recognized in oncology clinics for their (undesirable) immunosuppressive properties and in the laboratory as being effective modulators of immune reactions in experimental models.

Due to the subtle and gradual improvement in prognosis of patients, it was recognized by investigators both at the NIH and Mayo in the late 1960s that controlled trials would be necessary to ascertain the true renal risk/benefit of alternative therapies for proliferative lupus nephritis. In early studies, both short-term (months) [54–58] and long-term (years) [59–61] treatment regimens of cytotoxic drugs stabilized lupus nephritis more effectively than did corticosteroids alone (described in detail later). Continued observation in these controlled trials demonstrated objective benefit of cyclophosphamide over corticosteroid controls only in the long-term treatment protocols [40, 62, 63]; 10-year renal survival improved from approximately 50% with prednisone alone to approximately 90% with sustained cyclophosphamide treatment.

The conclusions of the NIH studies extolling the benefits of cytotoxic drugs for proliferative lupus nephritis have been challenged in two ways. Some reports have suggested that extremely favorable renal outcomes can be achieved with only brief courses of (or even without) cytotoxic drugs (Fig. 13) [28, 64–70]. As shown, the 5-year renal survival (without end-stage renal failure) ranges dramatically, from over 90% to less than 30%, in various centers within the United States and Europe. It is notable that the three series with the worse outcome include predominantly Black patients. One of the most favorable outcomes was reported from an Italian registry of over 650 patients most of whom did not receive cytotoxic drugs; the 5-year renal survival was approximately 88% [65]. At the other end of the spectrum of arguments, some reports have indicated that the intense regimen of cytotoxic drugs is ineffective in controlling lupus nephritis, at least in certain subsets of patients. The study of pediatric lupus nephritis treated with pulse cyclophosphamide showed a dismal 5-year renal survival of <30% in a population that was predominantly Black [70]. Dooley *et al.* [67] has reported substantially poorer than expected outcomes among Black patients treated with pulse cyclophosphamide. These investigators noted that the 5-year renal survival was 58% in Blacks and 95% in non-Blacks (Fig. 14). The differences were not explained by differences in frequency or severity of hypertension or in renal histopathology. Interestingly, analysis of racial subsets in our own controlled trials did not yield a difference between Black and non-Black patients in the frequency of favorable responses to cyclophosphamide [71]. These observations may be identifying important differences among patient populations and could limit the ability to extrapolate a universal benefit of cyclophosphamide therapy. This hypothesis clearly warrants further testing in therapeutic studies controlled for the effects of race [72].

Cameron has analyzed the overall trends in survival among patients with diffuse proliferative lupus nephri-

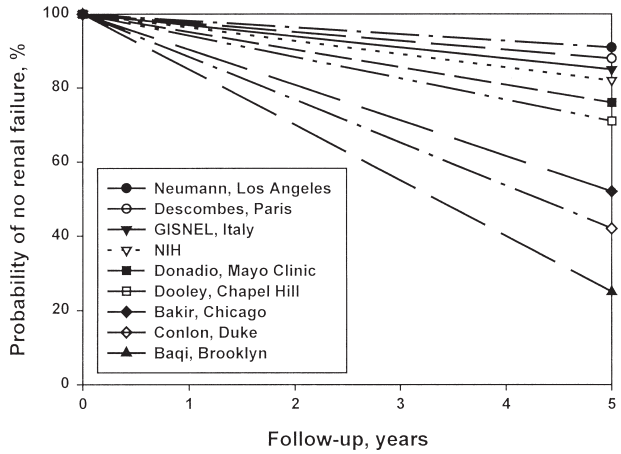


FIGURE 13 Risks of developing end-stage renal disease (ESRD) at 5 years in patients with diffuse proliferative lupus nephritis treated at nine different centers [28, 64–70].

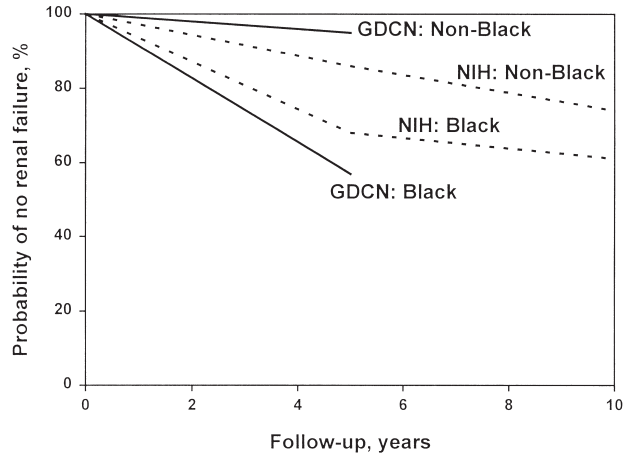


FIGURE 14 Effect of race on risk of developing end-stage renal disease (ESRD) in Black and non-Black patients with diffuse proliferative lupus nephritis treated in the Glomerular Disease Collaborative Network (GDCN) [67] and at the NIH [96].

tis [73]. Five-year patient survival estimates synthesized from several publications rose from 17% before 1970, to 55% during the period 1970–1979, and to >80% after 1980. There is general agreement that cytotoxic drugs have had less impact on patient survival than on renal survival in patients with SLE and lupus nephritis.

COURSE OF LUPUS MEMBRANOUS NEPHROPATHY

The renal prognosis of classic lupus membranous nephropathy (with no more than mesangial expansion) is generally more favorable than proliferative lupus nephritis. However, if one incorporates proliferative lupus nephritis under the rubric of lupus membranous nephropathy, as was suggested in the 1985 version of the WHO classification system (reviewed in Ref. 27), delineation of prognosis has become more complex and one must consider distinct renal prognosis for each subset. Indeed, using this earlier approach to classification, some investigators have reported that membranous subsets with diffuse proliferation (Vc) or with sclerosis (Vd) have a substantially worse five-year renal survival than patients with classic lupus membranous nephropathy [66, 74, 75]. Again, our preference for biopsies with mixed membranous and proliferative histologies is to categorize them as proliferative lupus nephritis with commensurate considerations of prognosis and treatment. Preliminary studies of the impact of race on prognosis of lupus membranous nephropathy have been reported [76]. In a comparatively large study of 60 patients with lupus membranous nephropathy, Black race was a predictor of renal failure. In a smaller study, Bakir *et al*. [68] found no apparent effect of Black race on the prognosis of lupus membranous nephropathy. As with proliferative forms of lupus nephritis, continued search for the explanation(s) of the worsened renal prognosis among Blacks is clearly warranted [77–79].

PROGNOSTIC INDICATORS

Demographic, clinical, laboratory, and pathologic features of lupus nephritis influence the course of lupus nephritis (Table 6) [44, 71, 73, 80–88]. Some parameters which correlate statistically with renal outcome may not be directly responsible for disease progression, but simply represent surrogates for less well defined pathogenic factors that determine prognosis (e.g., anemia as a surrogate for overall severity of SLE). Multivariate statistical methods are widely used to define independent prognostic factors. The predictive value of hematocrit, platelet count, serum creatinine, complement levels, hypertension, and renal biopsy data have been demonstrated in this fashion. Some of these factors are present at diagnosis and will remain static; others wax and wane during the course of lupus nephritis and reflect a changing prognosis. A composite of prognostic factors is useful in assessing severity and developing a rational plan of therapy for lupus nephritis. In general, the larger the number of factors, the more unfavorable the renal outcome and the stronger the indications for aggressive immunosuppressive drug therapy. Clinical and histologic evidence of severe, active lupus nephritis or active superimposed on chronic, irreversible parenchymal injury represent the most ominous prognostic features.

As the treatment and long-term survival of patients with lupus nephritis have improved, it should not be surprising that the impact of factors at disease onset assume less predictive value. Thus, time-dependent parameters (e.g., serologic parameters, urinary sediment, proteinuria, and serum creatinine) and those present later in the course of the disease (e.g., relapses of nephritis) may be of greater prognostic importance than those present at the onset of lupus nephritis. Finally, the challenge for the clinician is to negate, to the extent possible, the negative impact of the predictors of adverse prognosis by effective therapeutic intervention. A reanalysis of data from a NIH clinical trial provides evidence that contemporary regimens of immunosuppression can achieve this goal. Patients with extensive chronic irreversible histologic changes on renal biopsy

TABLE 6 Factors Associated with Adverse Prognosis and High Risk of Renal Progression in Lupus Nephritis

Demographic
Black race
Hispanic ethnicity
Male gender[a]
Extremes of age at onset of SLE[a]
Limited access to health care
Clinical
Hypertension
Severe extrarenal (especially neuropsychiatric) lupus activity
Failure to achieve remission or marked delay to renal remission
Multiple relapses of lupus nephritis
Pregnancy
Laboratory
Nephritic urinary sediment
Azotemia
Anemia
Thrombocytopenia
Thrombotic microangiopathy (with or without anti-phospholipid antibodies)
Hypocomplementemia (especially falling levels)
High anti-DNA (especially rising titers)
Persistent severe nephrotic syndrome (atherosclerotic and thrombotic diathesis)
Renal pathology
Contracted kidney size[b]
Proliferative glomerulonephritis (WHO Class III, IV)
Mixed membranous (V) and proliferative (III–IV) glomerulonephritis
Cellular crescents (>25% of glomeruli)
Fibrinoid necrosis (>25% of glomeruli)
Very high activity index
Moderate-to-high chronicity index[b]
Combinations of active (cellular crescents) and chronic features (interstitial fibrosis)
Extensive subendothelial deposits

[a] There is controversy regarding the level of impact of these prognostic factors.

[b] These prognostic factors per se are not indications for treatment.

Modified from: Balow *et al.* (2003). Lupus Nephritis. In "Therapy in Nephrology and Hypertension" (Brady and Wilcox, Eds.), 2nd ed., p 158. W.B. Saunders, London.

(chronicity index >3) are at increased risk for progressive deterioration of renal function. A relatively effective treatment (30 months course of pulse cyclophosphamide) modulates this risk to a greater degree than a short course (6 months) of pulse methylprednisolone (Fig. 15). Figure 15 illustrates the impact of prognostic factors and therapeutic intervention on renal function outcome.

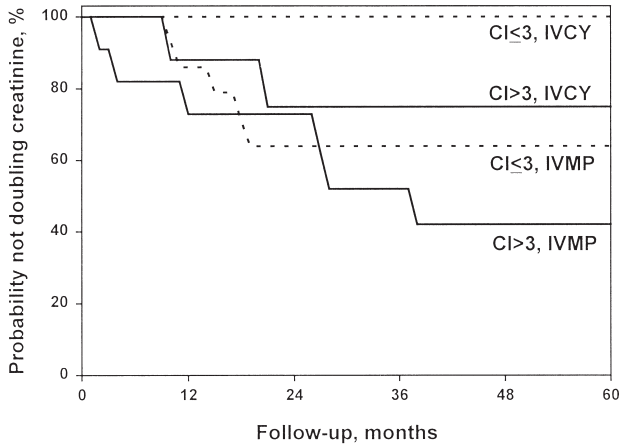


FIGURE 15 Prognostic impact of therapy and renal histology. The differential effectiveness of pulse methylpredniso lone and pulse cyclophosphamide according to the degree of chronicity on renal biopsy is shown. Substantial underlying chronic renal damage increases the probability of doubling serum creatinine with either pulse therapy, but particularly pulse methylprednisolone.

APPROACHES TO TREATMENT OF LUPUS NEPHRITIS

There is no panacea for SLE, nor for lupus nephritis. However, modern treatments offer the prospect of sustained remissions and substantial longevity. Numerous therapeutic options are available for management of lupus nephritis, including immunosuppressive drugs, adjunctive therapies for secondary complications of renal disease, and a variety of experimental agents (Table 7). While our opinions on the uses and effectiveness of each option for treatment of lupus nephritis are summarized in this table, we acknowledge that individual perspectives on these therapeutic modalities vary widely.

EVIDENCE FROM CONTROLLED TRIALS OF THERAPY FOR LUPUS NEPHRITIS

Because of the intrinsic diversity in severity and rates of progression of lupus nephritis among various patient populations, controlled clinical trials have been absolutely essential in defining the objective risk/benefits of particular therapeutic interventions in SLE and lupus nephritis. Objective proof of the value of experimental interventions depends in large measure on randomized design and comparison of outcome frequencies between experimental and concurrent (rather than historical) control groups. It is imperative to continue to use this

TABLE 7 Therapeutic Options for Management of Lupus Nephritis

- Immunosuppressive drug therapy
  - Prednisone
    - Initiate at 1.0 mg/kg/day
    - Continue for no more than 8 weeks (except when extreme clinical conditions mandate)
    - Taper to alternate day therapy in doses of 0.25 mg/kg every other day
    - Use alternative immunosuppressive drugs rather than continued daily prednisone therapy, whenever possible
    - Maintain assiduous surveillance and protection against steroid-induced osteoporosis (i.e., exercise, adequate calcium, vitamin D, bisphosphonates)
  - Methylprednisolone pulse therapy
    - Initiate at 1.0 g/m^2 daily for 3 days for severe activity (rapidly progressive renal failure, crescentic glomerulonephritis)
    - Option to repeat pulse doses at monthly intervals
    - May combine with pulse cyclophosphamide in severe or refractory cases
  - Cyclophosphamide
    - Intermittent pulse therapy has highest therapeutic index
    - Conventional daily cyclophosphamide therapy (2 mg/kg/day) is mostly avoided, or used for <3 months (see Table 9 for details on administration of pulse cyclophosphamide)
  - Azathioprine
    - Alternative agent in SLE
    - Mostly used for extrarenal disease, in mild lupus nephritis, as maintenance after period of improvement induced by cyclophosphamide, or as a steroid sparing agent in patients who require sustained high doses of prednisone or in those unwilling to accept or unable to tolerate cyclophosphamide treatment
  - Mycophenolate mofetil
    - Preliminary results suggest it may be a useful alternative to cyclophosphamide for induction therapy (controlled trial in progress)
    - Alternative to azathioprine for maintenance therapy
  - Methotrexate
    - Mostly used as adjunct or alternative anti-inflammatory therapy for extrarenal manifestations of SLE
    - Use in lupus nephritis is limited because renal dysfunction enhances risk of side effects
  - Cyclosporine
    - Mostly used in low doses (e.g., <5 mg/kg/day) in lupus membranous nephropathy
    - Limited use in proliferative lupus nephritis
  - Plasma exchange
    - Controlled trials have not demonstrated benefit of plasmapheresis in lupus nephritis
    - Mostly used for microangiopathic complications of lupus, such as vasculopathy of superimposed thrombotic thrombocytopenic purpura
  - Intravenous immunoglobulins
    - Expensive, short-term therapy
    - Mostly used in lupus for immune mediated thrombocytopenia or refractory CNS disease
- Adjunctive therapies for secondary complications of renal disease
  - Angiotensin antagonists, including angiotensin converting enzyme inhibitors (ACEI) and angiotensin receptor blockers (ARB)
    - Glomerular proteinuria persisting after control of active nephritis may be decreased by these agents (this must be balanced against their potential adverse effects on GFR and serum potassium)
  - Other antihypertensives
    - Treatment of hypertension and drug choices follow standard guidelines
    - Use blood pressure goals appropriate for age of patient
  - Lipid-lowering drugs
    - For control of hyperlipidemia of nephrotic syndrome
    - Usually start HMG-CoA-reductase inhibitors and fibric acid derivatives if nephrotic
- Experimental therapies
  - Sirolimus (rapamycin)
    - Immunosuppressive and antiproliferative properties
    - Limited observations in human SLE
  - Leflunomide
    - Anecdotal experience only
  - Adenosine analogs (cladrabine, fludarabine)
    - Limited pilot studies in SLE and refractory lupus nephritis
  - DNA toleragen (LJP-394)
    - Preliminary studies suggests marginal benefits in most patients
    - May be larger benefit in patients with high titer anti-DNA
  - Autologous stem cell transplants
    - Encouraging preliminary results
    - Intense immunoablative conditioning associated with high risk
    - Appropriate patient selection remains a major challenge
  - Monoclonal antibodies
    - Anti-T-cell: several small pilot studies (no definitive recommendations)
    - Anti-B-cell (e.g., rituximab): pilot studies in progress
    - Anti-complement C5b-9: pilot study in progress
  - Costimulation inhibitors
    - Anti-CD154 (CD40 ligand): preliminary data suggest some efficacy, but studies halted due to thromboembolic complications
    - CTLA4-Ig: effective in lupus mice, especially in combination with cyclophosphamide; pilot human studies (psoriasis) suggest good therapeutic index; no studies in human lupus

Modified from: Balow *et al.* (2003). Lupus Nephritis. In "Therapy in Nephrology and Hypertension" (Brady and Wilcox, Eds.), 2nd ed., p 159. W.B. Saunders, London.

design methodology if we are to advance the state of the art in treatment of lupus nephritis [89].

Animal models of SLE (reviewed elsewhere in this volume) have been extremely valuable in screening a wide range of new therapies for human lupus nephritis. In general, the murine models respond better and to more therapies than do human patients, but overall the responses to treatment are quite well correlated. In murine lupus, major benefit has been realized with cyclophosphamide, total lymphoid irradiation, and bone marrow transplantation. Moderate benefit has been observed with a host of interventions, including immunosuppressive drugs (e.g., corticosteroids, azathioprine, mycophenolate, methotrexate, cyclosporine, tacrolimus, rapamycin), monoclonal antibodies (particularly against activated T cells and nephritogenic idiotypes), as well as an array of cytokine antagonists, modulators of inflammation, anticoagulants, fibrinolytic agents, and dietary manipulations (Table 7).

Corticosteroids

The reader may be surprised that no controlled clinical trials have ever been performed to prove unequivocally the value of corticosteroids over simple supportive therapies, nor have there been any studies directly comparing conventional prednisone with methylprednisolone pulse therapy. Indeed, it is unlikely that prospective clinical trials comparing placebo, low-dose, and high-dose corticosteroids for lupus nephritis will ever be performed, due to the potentially confounding effects of the standard clinical practice of using high-dose corticosteroids as first line treatment for the myriad of extrarenal manifestations and complications of SLE. The best advice for the practitioner is not to withhold corticosteroids for fear of complications, but rather to test regularly the feasibility of reducing doses (preferably to alternate day) and to be willing to substitute alternative immunosuppressive drug strategies if clinical response is delayed. Physicians treating patients with SLE and lupus nephritis should always be inclined to reduce dosages of corticosteroids to the lowest necessary for disease control in order to minimize risk of their insidious complications. Indeed, the substantial toxicity of prolonged high-dose corticosteroids is one of the most compelling reasons for continued exploration of alternative immunosuppressive therapies.

Azathioprine

Early human studies, including controlled trials, showed azathioprine to be, at best, of modest benefit as an adjunct to corticosteroids in lupus nephritis [54, 55, 90–92]. At the present time, azathioprine is used as principal therapy in patients with severe lupus nephritis who decline cyclophosphamide or for treatment of milder forms of lupus nephritis. Particularly in European centers, azathioprine is typically used as maintenance therapy following an initial 2- to 3-month induction course of cyclophosphamide [44].

Cyclophosphamide

Cyclophosphamide is considered one of the most potent immunosuppressive drugs both in murine models and in human lupus nephritis. Table 8 summarizes, in a chronological fashion, a series of controlled trials addressing the benefits of various forms of cyclophosphamide in treatment of lupus nephritis. Conclusions from studies of the efficacy of cyclophosphamide depend heavily on the duration of treatment. Short courses of daily cyclophosphamide were more efficacious than prednisone alone in decreasing activity of lupus nephritis [56, 57, 59–63]. Maintenance with prednisone alone did not protect against late risk of renal failure, while continuation of cyclophosphamide with prednisone as maintenance therapy was associated with decreased risk of late renal failure [62]. The counterbalancing cumulative toxicity of prolonged daily therapy has generally constricted the use of cyclophosphamide to short courses of therapy in lupus nephritis.

Pulse Cyclophosphamide

The risk/benefit advantage of pulse cyclophosphamide over daily oral cyclophosphamide has been amply demonstrated in clinical oncology, and in a vasculitis study wherein increased complications of daily cyclophosphamide were noted [93]. Proof of a comparable advantage of pulse cyclophosphamide over corticosteroids alone in human lupus nephritis was realized incrementally in long-term studies. Several regimens of cyclophosphamide, including conventional dose oral therapy, low-dose combination of cyclophosphamide and azathioprine, and intermittent pulse cyclophosphamide were superior to prednisone alone in controlling clinical activity of nephritis, in attenuating renal pathologic changes over time [40], and ultimately in reducing the risk of developing end-stage renal failure [62]. While any regimen containing cyclophosphamide was superior to corticosteroids alone, increased toxicities of daily cyclophosphamide has rendered intermittent pulse administration the preferable approach to cyclophosphamide therapy.

TABLE 8 Chronology of Controlled Trials of Cyclophosphamide Therapy in Lupus Nephritis

Steinberg and Decker, 1974 [59]: Decker *et al*., 1975 [60]: Extended courses of oral CY (up to 4 years) produced higher rates of improvement in extrarenal lupus activity, lupus serologies, and urinary abnormalities than prednisone alone

Donadio *et al*., 1978 [57]: Donadio *et al*., 1982 [58]: Short courses (6 months) of oral CY produced higher rates of improvement and stability of lupus nephritis than did prednisone alone; the benefits of short courses of oral CY were not sustained during extended follow-up (no differences in late risk of ESRD)

Dinant *et al*., 1982 [61]: Extended courses (treatment beyond remission) of combined oral AZ and CY or quarterly pulse IV-CY produced higher rates of improvement in urine sediment, proteinuria, and lupus serologies than prednisone alone

Balow *et al*., 1984 [40]: Analysis of the evolution of renal pathology; cytotoxic drugs afforded better protection against worsening of scarring, atrophy, and fibrosis; prednisone alone was associated with worsening of chronicity features in proportion to the interval between biopsies

Austin *et al*., 1986 [62]: Extended treatment (beyond renal remission) with pulse IV-CY significantly reduced risk of progression to ESRD compared to prednisone alone

Steinberg and Steinberg, 1991 [63]: Additional observation of patients in previous study; sustained courses of oral CY, combined low-dose oral AZ and CY, or pulse IV-CY decreased the risks of ESRD compared to that of prednisone alone

Boumpas *et al*., 1992 [96]: IV-CY reduced the risks of doubling creatinine from that of a short course (6 months) of monthly IV-MP; extended course (30 months) of pulse IV-CY reduced the risk of renal relapse compared to a short course (6 months) of IV-CY

Lewis *et al*., 1992 [121]: Plasmapheresis with prednisone and short courses (2 months) of oral CY did not improve clinical outcomes compared to prednisone and oral CY alone

Sesso *et al*., 1994 [97]: Limited courses (10 months) of pulse IV-CY or pulse IV-MP (monthly ×4 and quarterly ×2) showed no differences in risks of doubling creatinine or of developing ESRD

Gourley *et al*., 1996 [98]; Illei *et al*., 2001 [99]: Extended courses (up to 3 years) of pulse IV-CY or combination pulse IV-CY and pulse IV-MP produced higher rates of renal remission than did extended courses (up to 3 years) of monthly pulse IV-MP; remission achieved slightly more rapidly with combination pulse therapy

Wallace *et al*., 1998 [122]: Plasmapheresis synchronized with pulse IV-CY offered no clinical advantages (in lupus activity scores or rates of adverse events) over pulse IV-CY alone

Chan *et al*., 2000 [13]; Chan *et al*., 2001 [118]: Comparison of mycophenolate with conversion to maintenance azathioprine at 12 months and daily oral cyclophosphamide with conversion to maintenance azathioprine at 6 months; at 12 months, there were no differences in favorable and unfavorable renal outcomes between the two treatment arms; however, after an additional year of follow-up into the azathioprine maintenance phase of treatment, there was a significantly greater risk of relapse in the subjects originally treated with mycophenolate induction therapy

Houssiau *et al*., 2002: Compared high-dose (≤1.5 g) pulse cyclophosphamide monthly for 6 months followed by 2 quarterly doses *vs* low doses (0.5 g fixed dose) of pulse cyclophosphamide fortnightly for 6 doses; after median follow-up of 3.5 years, there were no substantive differences in the proportions of favorable and unfavorable renal outcomes

Additional studies have examined whether pulse methylprednisolone [94, 95] might improve on the efficacy of standard prednisone and meet the efficacy of pulse cyclophosphamide. No differences were found in short-term (6 months) studies comparing pulse methylprednisolone and pulse cyclophosphamide [96, 97]. However, the NIH study showed that an extended course of pulse cyclophosphamide (30 months) was superior to pulse methylprednisolone in reducing the probability of doubling serum creatinine, as well as being superior to short-term cyclophosphamide in reducing the probability of relapse of lupus nephritis [96]. A subsequent NIH study compared extended courses of pulse methylprednisolone alone, pulse cyclophosphamide alone, or in combination. Renal remission occurred more rapidly with combination pulse therapy and was least likely with pulse methylprednisolone alone [98, 99]. These and other uncontrolled studies [100–106] favor the use of pulse cyclophosphamide in patients with proliferative lupus nephritis.

In summary, neither pulse methylprednisolone (of any duration) nor short-term pulse cyclophosphamide are as efficacious as extended courses of pulse cyclophosphamide. Short courses (3–6 months) of either daily or intermittent pulse cyclophosphamide tend to produce responses of limited duration. Thus, some form of maintenance therapy appears essential to reduce the risk of relapsing lupus nephritis [107–111].

Studies comparing azathioprine and cyclophosphamide as maintenance therapies are in progress. Early results (after 8 months) of an ongoing controlled trial in the Netherlands have suggested that a regimen of pulse methylprednisolone and azathioprine is comparable to a regimen of pulse cyclophosphamide [112]. Azathioprine continues to be used in many centers around the world as maintenance therapy—often after patients have had substantial improvement or achieved remission of lupus nephritis with cyclophosphamide therapy. Preliminary results of an ongoing controlled clinical trial in the United States have suggested that azathioprine may be as effective as quarterly pulse cyclophosphamide for maintenance therapy [113].

Mycophenolate Mofetil

Based principally on studies showing greater efficacy of mycophenolate over azathioprine in reducing transplant rejection, several investigators began exploring the use of mycophenolate in lupus nephritis (particularly for patients adverse or refractory to cyclophosphamide) [114–117]. There are several issues impinging on the decision to use mycophenolate. Among the most attractive attributes of mycophenolate is its lack of

gonadal and urinary bladder toxicity (compared to cyclophosphamide).

A controlled trial showed that induction therapy with mycophenolate was comparable to daily oral cyclophosphamide for achieving remission [13]. In a preliminary follow-up report, these investigators found that relapses following conversion to maintenance azathioprine therapy were more common in patients initially treated with mycophenolate than with cyclophosphamide [118]. These results suggest that cyclophosphamide induction confers a longer period of stability than does mycophenolate. Sustained treatment with mycophenolate, or a more effective alternative than azathioprine, may be necessary to reduce the tendency to early relapse. If long-term maintenance is necessary, the extremely high cost of mycophenolate could become a practical limitation. Prospective studies in progress should address the effectiveness of lower cost azathioprine as maintenance therapy following induction of remission by either cyclophosphamide or mycophenolate. It is noteworthy that a prospective, multicentered clinical trial is in progress comparing mycophenolate to standard pulse cyclophosphamide as induction therapy for proliferative lupus nephritis [119].

Plasmapheresis

Plasmapheresis has been studied in several controlled trials of SLE and lupus nephritis. The earliest study showed no clinical differences between sham and real plasmapheresis [120]. Subsequent studies showed no benefit of adding plasmapheresis to standard immunosuppression with prednisone and a short course of cyclophosphamide [121]. Preliminary results of a controlled trial involving synchronized plasmapheresis and pulse cyclophosphamide also failed to demonstrate a benefit of plasmapheresis [122–124].

TREATMENT OF PROLIFERATIVE LUPUS NEPHRITIS

Among the various immunosuppressive drugs presented, cyclophosphamide has been shown to have one of the highest therapeutic indices for proliferative lupus nephritis, based on evidence gained in controlled trials in both murine and human disease. In general, short courses (e.g., 2–6 months) of cyclophosphamide, either in conventional daily doses or in intermittent pulse doses, improve the activity of lupus nephritis, but there are high probabilities of exacerbation without some form of maintenance cytotoxic drug therapy. Prospective studies of alternative maintenance therapies are clearly needed. Because of the acknowledged cumulative risk of toxicity with conventional therapy, daily oral cyclophosphamide is no longer considered a viable option for extended maintenance therapy.

Table 9 describes our approaches to administration of pulse cyclophosphamide therapy. It is critically

TABLE 9 Recommendations for Administration of Pulse Cyclophosphamide Therapy

Estimate glomerular filtration rate (GFR) by standard methods

Calculate body surface area (m^2): $\mathrm{BSA} = \sqrt{\frac{\mathrm{Height\,(cm)} \times \mathrm{Weight\,(kg)}}{3600}}$

Cyclophosphamide (Cytoxan) (CY) dosing and administration:
- Initial dose CY is 0.75 g/m^2 (*important note*: start with 0.5 g/m^2 of CY if glomerular filtration rate is less than one-third of expected normal)
- Administer CY in 150-ml normal saline iv over 30–60 min (*alternative*: equivalent dose of pulse CY may be taken orally in highly motivated and compliant patients)

Obtain WBC at days 10 and 14 after each CY treatment (*note*: advise patient to delay taking prednisone until after blood tests are drawn to avoid transient steroid-induced leukocytosis)

Adjust subsequent doses of CY to keep nadir WBC above 1500/μl (escalate CY to maximum dose of 1.0 g/m^2 unless WBC nadir falls below 1500/μl)

Repeat CY doses monthly (every 3 weeks in patients with extremely aggressive disease) for 6 months, then quarterly for 1 year *after* remission is achieved; remission is defined by: inactive urine sediment, proteinuria <1 g/day, and a state of minimal or no activity of extrarenal lupus (also, ideally, normalization of serum complement and anti-DNA).

Protect bladder against CY-induced hemorrhagic cystitis:
- Induce diuresis with 5% dextrose and 0.45% saline (e.g., 2 liters at 250 ml/h) and encourage frequent voiding; continue high-dose oral fluids through 24 h; counsel patients to return to clinic if they cannot sustain ingestion of enteral fluids
- Give mesna (Mesnex) (each dose 20% of total CY dose) intravenously or orally at 0, 2, 4, and 6 h after CY dosing (*note*: use of mesna strongly urged whenever sustained diuresis may be difficult to achieve, or if pulse CY is given in outpatient setting)
- If patients anticipated to have difficulty with sustaining diuresis (e.g., severe nephrotic syndrome) or with voiding, insert a 3-way Foley catheter with continuous bladder flushing with standard antibiotic irrigating solution (e.g., 3 liters) for 24 h to minimize risk of hemorrhagic cystitis

Antiemetics (usually administered orally):
- Dexamethasone (Decadron) 10-mg single dose *plus*:
- Serotonin receptor antagonists: Granisetron (Kytril) 2 mg concurrent with CY dose and 1 mg after 12 h; alternative: Ondansetron (Zofran) 8-mg tid for 1–2 days (more expensive)

Monitor fluid balance during diuresis: if patient develops progressive fluid accumulation, use diuretics to reestablish fluid balance

Modified from: Balow *et al*. (2003). Lupus Nephritis. In "Therapy in Nephrology and Hypertension" (Brady and Wilcox, Eds.), 2nd ed., p 160. W.B. Saunders, London.

important to adjust the dose of pulse cyclophosphamide according to renal function [3]. Decreased glomerular filtration rate prolongs the half-life of cyclophosphamide and its metabolites; inappropriately high doses can be associated with life-threatening bone marrow suppression. It is unproven whether cyclophosphamide effectiveness is within limits proportional to the degree of induced leukopenia. We recommend that doses of cyclophosphamide should be increased gradually to achieve moderate leukopenia at the expected 10- to 14-day nadir. Use of arbitrarily low doses of cyclophosphamide, which do not affect white blood counts, often leads to ambiguity whether the lack of expected clinical response is due to cyclophosphamide resistance or to inadequate dosing.

Protection of the urinary bladder against the toxicity of cyclophosphamide metabolites is accomplished by forced diuresis, mesna treatment (to bind the toxic metabolite, acrolein), frequent voidings, and diuretics (as needed) for at least 8–12 h after pulse cyclophosphamide. Newer antiemetic drugs (serotonin receptor antagonists) have virtually revolutionized the control of nausea and greatly improved the tolerability of repeated doses of pulse cyclophosphamide. Table 10 contains a synopsis of potential side effects of pulse cyclophosphamide therapy, including ovarian toxicity [125–127]; perspectives on cancer in lupus patients is also included [128–130].

An outline of practical approaches to treatment of diffuse and severe focal proliferative lupus nephritis is shown in Table 11. These therapeutic guidelines address initial approaches to treatment based on severity of nephritis, recommendations for transition to maintenance immunosuppressive drug therapies, alternative induction and maintenance regimens, and suggestions for duration of treatment. Table 12 contains a set of guidelines for discontinuation of cytotoxic drugs in situations where treatment of lupus nephritis has failed.

TABLE 10 Potential Toxicities of Pulse Cyclophosphamide

Nausea/vomiting: Currently, minimal problems with use of serotonin receptor antagonists
Alopecia: Mild, diffuse hair thinning common; cosmetically severe alopecia is exceptional
Infection: Bacterial, fungal, and pneumocystis infections increased by excessive or prolonged leukopenia (<1500 per μl); risk of herpes zoster, papilloma infections moderately increased
Hemorrhagic cystitis: Minimal risk (compared to conventional cyclophosphamide); risk reduced by diuresis, frequent voiding, mesna
Bladder fibrosis, bladder carcinoma: Low risk with intermittent pulse cyclophosphamide
Gonadal failure: Risk proportional to age of patient and cumulative dose of cyclophosphamide; potential benefit of preemptive gonadal suppression with leuprolide (Lupron) is undefined
Teratogenesis: Rare case report with cyclophosphamide during unrecognized pregnancy; N.B.: patients should be screened for pregnancy prior to each dose cyclophosphamide
Cardiac: Myocardial toxicity with congestive heart failure is rare at cyclophosphamide doses ≤1.0 g/m^2
Pulmonary: Pulmonary fibrosis exceedingly rare at cyclophosphamide doses ≤1.0 g/m^2
Hepatic: Anecdotal cases of severe hepatocellular injury with pulse cyclophosphamide
Metabolic (SIADH): Rare complication but potential cause of water intoxication during planned diuresis; monitor intake and output following cyclophosphamide pulse therapy
Bone marrow: Rare aplastic anemia, myelodysplasia
Malignancy: Small, but apparently real, increase in incidence of malignancies (e.g., leukemia/lymphoma, gynecologic, and skin cancers); relative risk is low

TREATMENT OF LUPUS MEMBRANOUS NEPHROPATHY

Historically, lupus membranous nephropathy was considered to be an intrinsically mild form of lupus nephritis that did not merit specific treatment. Pure forms of lupus membranous nephropathy (those with only background mesangial expansion) were noted to have low risk of progression to end-stage renal failure [25, 26, 73, 74, 131, 132].

Against these comments about the generally favorable renal outcomes of lupus membranous nephropathy, it has become apparent that the typically persistent nephrotic syndrome of this disease substantially increases the risk of thromboembolic [133] and atherosclerotic [134, 135] complications, which in turn jeopardize patient survival. These issues have been analyzed in most detail in idiopathic membranous nephropathy, but similar considerations apply in lupus membranous nephropathy. Currently, the motivation to treat patients with membranous nephropathy are based, at least in part, on the goal of reducing the risks of thrombotic and atherosclerotic complications. However, the fact that the risk of end-stage renal failure approximates 20% at 10 years after diagnosis strengthens the rationale for interventions in lupus membranous nephropathy.

A perspective on the treatment of lupus membranous nephropathy involved a retrospective analysis of outcomes in 19 patients treated with prednisone alone or in combination with alternating months of pulse methylprednisolone and chlorambucil [136]. Of 8 patients treated with prednisone alone, 3 achieved complete and one partial remission; 7 subsequently relapsed

TABLE 11 Treatment of Diffuse (and Severe Focal) Proliferative Lupus Nephritis

I. Recommended Initial treatment
  A. Moderate disease: defined by limited number and severity of risk factors (Table 6)
    1. Prednisone (1.0 mg/kg/day): limited trial (up to 8 weeks)
      a. If *complete* response occurs, including clearing of cellular casts and proteinuria, normalization of complement, and minimal lupus activity, simply taper prednisone to alternate day (~0.25 mg/kg) and monitor for flares of nephritis.
      b. If no or incomplete response to prednisone, or nephritis worsens, start monthly pulse cyclophosphamide (follow protocol below for severe disease).

  N.B.: Do not delay this therapeutic decision beyond 8 weeks because of a partial response to prednisone
  B. Severe disease: defined by presence of a constellation of high-risk factors (Table 6)
    1. Prednisone (1.0 mg/kg/day tapered) plus:
    2. Monthly pulse cyclophosphamide (0.75 g/m^2) (0.5 g/m^2 if GFR is less than one-third normal) for 6 months; increase dose of cyclophosphamide by up to 0.25 g/m^2 increments, to maximum of 1.0 g/m^2 unless total leukocytes fall below 1500/μl at the 10 to 14-day nadir point.

II. Recommended transition to maintenance immunosuppressive drug therapy or early discontinuance of induction therapy (at 6 months)
  A. In selected patients, pulse cyclophosphamide may be stopped and treatment continued with alternate day prednisone (0.25 mg/kg) alone. Such patients have exquisitely responsive nephritis (defined by complete clearing of cellular casts and proteinuria, normal complement, and minimal lupus activity within the first 6 months). Limited duration of cyclophosphamide therapy is also important for patients giving high priority to maintaining fertility while accepting the risk of low-grade activity of lupus nephritis.
  B. The majority of patients with proliferative lupus nephritis will not be in full remission at 6 months. Convert this group to maintenance pulse cyclophosphamide every 3 months (doses adjusted by same guidelines used during the induction therapy phase).

  N.B.: Microscopic hematuria often does not clear for several months, even when most other clinical parameters have remitted; by itself, microscopic hematuria is usually not a sufficient reason to abandon a particular therapeutic program

III. Alternative induction and maintenance treatment regimens
  A. Induction therapy: prednisone (1.0 mg/kg/day tapered) plus:
    1. Pulse methylprednisolone 1.0 g/m^2 daily for 3 doses; may repeat pulses at monthly intervals and continue for 6–12 months if there is steady progress toward remission.
    2. Mycophenolate mofetil, 0.5–2.0 g/day; titrate dose as tolerated (GI side effects mostly dose limiting; low to moderate risk of bone marrow suppression.
    3. Daily oral cyclophosphamide, 2 mg/kg/day for 2–6 months (risks greater gonadal and urinary bladder toxicities).
    4. Oral pulse cyclophosphamide, ranging from 0.5 g weekly to 1.0 g/m^2 monthly (in highly motivated, fastidiously compliant patients).
  B. Maintenance therapy: alternate day prednisone (~0.25 mg/kg) plus:
    1. Azathioprine, 2 mg/kg/day.
    2. Mycophenolate mofetil, 0.5–2.0 g/day.

IV. Duration of therapy
  A. Cyclophosphamide: continue quarterly maintenance cyclophosphamide treatments for 1 year *after* remission of lupus nephritis is achieved [defined by: inactive urine sediment, proteinuria <1 g/day, and a state of minimal or no activity of extrarenal lupus (and, ideally, normalization of serum complement and anti-DNA)]. Patients with isolated fixed proteinuria or persistently elevated anti-DNA (i.e., without other supportive signs of active lupus nephritis) may be considered in remission. If uncertainty persists, findings on repeat renal biopsy may be extremely useful in defining status of renal disease and indications for ongoing therapy.
  B. Alternate day prednisone: tapered in very small increments to discontinuance if the patient has been in sustained complete remission for >3 years.

Modified from: Balow *et al*. (2003). Lupus Nephritis. In "Therapy in Nephrology and Hypertension" (Brady and Wilcox, Eds.), 2nd ed., p 161. W.B. Saunders, London.

and 3 doubled serum creatinine. Of the 11 patients treated with corticosteroids and chlorambucil, 7 achieved complete and 4 partial remission; only one relapsed and one doubled serum creatinine. The authors suggested that combination treatment with corticosteroids and chlorambucil is more effective than prednisone alone and that a prospective clinical trial is warranted in lupus membranous nephropathy. Other studies have supported the use of low-dose cyclosporine in lupus membranous nephropathy [137, 138].

Our group has been conducting a prospective controlled trial (now closed to accrual) comparing alternate day prednisone alone or in combination with pulse cyclophosphamide (alternate months) or with low-dose cyclosporine A for 1 year in patients with lupus membranous nephropathy and baseline of >2 g/day of proteinuria. Preliminary results show that both pulse cyclophosphamide and cyclosporine A are more effective than alternate day prednisone alone in inducing remission of proteinuria. More sustained remissions were seen after completion of pulse cyclophosphamide than after discontinuing cyclosporine A [139].

Current options for treatment of lupus membranous nephropathy are shown in Table 13. Patients with mixed

TABLE 12 Guidelines for Discontinuation of Cytotoxic Drugs if Treatment has Failed

Failure to achieve remission of nephritis
Continuous activity after protracted (e.g., 4 years) therapy
Consider that *complete* renal remission may be delayed more than 2 years
Do not abandon therapy if renal disease is active after the first 6–12 months, unless there is definitive worsening of disease
Beware of suboptimal cyclophosphamide treatment protocols, especially inadequate doses (<1.0 g/m^2) and/or lags in treatment intervals
Development of advanced chronic renal insufficiency
Prolonged (≥3 months) dialysis-dependent renal failure, not explained by other factors
Steadily rising serum creatinine ≥6 mg/dl
Inactive urine sediment with broad and/or waxy (renal failure) casts
Contracted kidney size (<3/4 of expected normal)
Renal biopsy showing exclusively scarring, atrophy, and fibrosis

TABLE 13 Treatment of Lupus Membranous Nephropathy

Mixed membranous and proliferative nephropathies
Treat as proliferative lupus nephritis (see Table 11)
Membranous nephropathy with nephrotic range proteinuria
First line treatment is usually high dose alternate day prednisone (e.g., 1–2 mg/kg) for 2 months; taper to ~0.25 mg/kg alternate days within 3–4 months
Optional adjuncts to prednisone therapy
Cyclosporine A, ≤5 mg/kg/day
Pulse cyclophosphamide, ≤1 g/m^2 every 1–3 months
Oral cyclophosphamide, 2 mg/kg/day
Methylprednisolone/cyclophosphamide given cyclically on alternate months: pulse methylprednisolone, 1.0 g/day for 3 days followed by prednisone (0.5 mg/kg/day) for the balance of the month; alternate with oral cyclophosphamide (2 mg/kg/day adjusted as needed for leukopenia) for 1 month; three cycles of each therapy are given over a 6-month period.
Membranous nephropathy with nonnephrotic proteinuria
Treat according to extrarenal disease activity; monitor carefully for evidence of progression to nephrotic syndrome or to mixed membranous and proliferative nephropathy

Modified from: Balow *et al*. (2003). Lupus Nephritis. In "Therapy in Nephrology and Hypertension" (Brady and Wilcox, Eds.), 2nd ed., p 162. W.B. Saunders, London.

membranous and proliferative nephritis should be treated the same as those with proliferative disease. As described previously, we believe there is ample justification for treatment of patients with membranous nephropathy and nephrotic range proteinuria. Alternate day prednisone is the first line treatment and may be worth a limited trial as single therapy. Patients failing to achieve remission of proteinuria should be considered for adjunctive cyclosporine or an alkylating agent. Until the results of prospective trials are definitive, the choices are optional. Indications for treatment of patients with nonnephrotic proteinuria are ambiguous and most would treat according to extrarenal manifestations, while continuing to monitor renal parameters diligently.

MONITORING PATIENTS WITH LUPUS NEPHRITIS

Selected serological tests are very useful for monitoring activity of lupus nephritis and in guiding treatment. While anti-nuclear antibodies (ANA) are cardinal markers for diagnosis of SLE, titers of these antibodies are not useful in gauging either the severity or the activity of lupus nephritis. Anti-DNA antibodies correlate better with both type and activity of lupus nephritis. Anti-DNA antibodies occur more frequently and in higher titers in proliferative (Class III or IV) than in membranous (Class V) nephropathy. However, there are many exceptions to this rule. High titers of anti-DNA can occur in patients with little nephritis, and severe nephritis can occur in patients with no or low-titers of anti-DNA antibodies. In general, *changes* in anti-DNA titers are more valuable clinically than their absolute levels. Rising anti-DNA titers are true harbingers of exacerbation of lupus nephritis [44]. However, we do not consider the correlation to be strong enough to recommend preemptive treatment for isolated changes in anti-DNA titers. Patients with only rising titers of anti-DNA warrant intensified monitoring for evidence of clinical signs of lupus flares. Other antibodies, such as antinucleosome and anti-C1q, are under study for assessment of their use in monitoring activity of lupus nephritis [140].

Monitoring serum complement components is extremely useful in management of lupus nephritis. Complement components are more readily available and reliable than measures of hemolytic complement. C3 and C4 complement components are both useful in following activity of lupus nephritis; because trends in their values are rarely discordant, C3 is the preferred choice for monitoring patients with lupus nephritis. Even more reliably than rising levels of anti-DNA antibodies, *falling* levels of serum complement components predict impending flares of lupus nephritis. Moreover, the absolute level of complement components is more predictive of immunologic activity than is the absolute level of anti-DNA antibodies. The historical practice of using serum complement levels as one of the cardinal determinants for ongoing treatment has been largely abandoned. At the present time, patients with either

rising anti-DNA antibodies and/or falling complement levels should be compulsively monitored for other early clinical signs of activity at which point preemptive treatment should be promptly instituted.

Other laboratory parameters for monitoring lupus nephritis have been examined in the research setting. Measurement of circulating immune complexes (by a wide range of tests) has generally been abandoned as a cost *ineffective* method of monitoring patients with SLE or lupus nephritis. Measurement of the late complement membrane attack complex, C5b-9, has been suggested as a method for monitoring activity of idiopathic membranous nephropathy. Its potential use in monitoring activity of proliferative or membranous lupus nephropathy has not been studied. Experimental studies have suggested that plasma and urinary cytokines (e.g., interleukin-6) or their receptors (e.g., interleukin-2 receptors) may predict lupus activity, but these tests are not widely used in the clinic at the present time [44].

REMISSION

There are no universal criteria for remission of lupus nephritis. Whether it would be useful to distinguish partial and complete remissions has not been thoroughly studied. However, it is evident in reading the literature that there is great ambiguity about the concepts of remission. Until consensus is formed on clinically validated definitions of remission, there will be wide variations in descriptions of the natural history of lupus nephritis.

Currently, the notion of *partial* remission of lupus nephritis is important in deciding the transition from cyclophosphamide (induction) therapy to maintenance (azathioprine, quarterly pulse cyclophosphamide, or prednisone alone). In severe lupus nephritis, we think there is reasonable justification to move from cyclophosphamide induction to either of the lower-intensity cytotoxic drug therapies if there is partial remission (i.e., objective improvement in urine sediment and renal function). However, we do not believe that partial remission should be accepted as a criterion to discontinue cytotoxic drug therapy unless there are other contraindications. Our studies found a very high rate of relapse with early discontinuance of cytotoxic drug therapy and we would continue therapy for several months after *complete* remission of lupus nephritis.

In proliferative lupus nephritis, we define complete remission as clearing of active elements from the urine sediment, reduction of proteinuria to <1 g/day, inactive extrarenal disease and, ideally, normalization of lupus serological tests (complement components and anti-DNA). One should be aware that up to 20% of patients may not achieve a complete remission after even 3 years of immunosuppressive drug therapy [99, 111]. We generally continue quarterly pulse cyclophosphamide for 1 year beyond complete remission as the best method of reducing risk of relapse of lupus nephritis. It is noteworthy that antinuclear antibodies (ANA) become negative in a tiny fraction of patients, including those in sustained clinical remission. Hence, we would not use the ANA in defining remission or making decisions about discontinuing treatment.

A small portion of patients develop "fixed proteinuria" as a result of severe, irreparable injury to glomerular basement membranes. Proteinuria of this cause is usually <2 g/day, but occasionally may be in the nephrotic range. In a patient with otherwise quiescent SLE and lupus nephritis (by clinical, serological, and urine sediment criteria), it may be difficult to define fixed proteinuria of a nonimmunologic cause without a renal biopsy. The typical biopsy shows marked thickening and lucency of glomerular basement membranes without substantial immune complex deposits. Failure to recognize persistent proteinuria in this context can lead to over treatment of patients and needless exposure to serious toxicities.

RELAPSE

One of the most perplexing aspects of the natural history of lupus is its remitting and relapsing course. Modern treatment neither cures lupus, nor completely prevents exacerbations. Furthermore, each major exacerbation is expected to leave residual and cumulative irreversible (often subclinical) damage. The more episodes of relapse, the greater is the likelihood of irreversible damage and progression to permanent renal failure.

Exacerbations of lupus nephritis can emerge from a state of partial remission (improvement from initial baseline disease activity) or from a state of complete and sustained remission. Approximately one-third to one-half of patients have a relapse of nephritis after achieving partial or complete remission of proliferative lupus nephritis [107–111]. In aggregate, these studies showed that the relative risk of renal functional deterioration was much greater for nephritic flares than for proteinuric flares. Thus, nephritic exacerbations clearly have adverse effects on renal prognosis, while proteinuric exacerbations have much less prognostic importance. These observations argue in support of strategies to minimize probabilities of flares of nephritis.

The role of repeat renal biopsy and the optimal therapy of exacerbations of lupus nephritis are unde-

fined. However, a study of repeat renal biopsy after 6 months of treatment indicated that stabilization and improvement of renal disease was predicted by improvement in morphologic parameters, particularly inflammation, interstitial fibrosis, and glomerular scarring [141].

Nephritic flares with modest increases in proteinuria (e.g., <2 g/day) and without concomitant rise in serum creatinine are usually managed without a renal biopsy and with an empiric trial of moderate dose prednisone (e.g., 0.5 mg/kg) for 4–6 weeks followed by gradual tapering. Failure to completely resolve within 2 months should prompt reevaluation, possibly with information from repeat renal biopsy (particularly if activity of the urinary sediment is ambiguous), and consideration of cytotoxic drug therapy. With steroid-resistant nephritic flares, we usually re-cycle therapy using the same guidelines as proposed for initial immunosuppressive drug therapy.

Management of incipient flares of lupus nephritis is controversial. One controlled trial has suggested that rises of anti-DNA activity predict impending flares which could be averted by preemptive boosts in corticosteroid therapy [107]. While many agree with the general value of monitoring anti-DNA (or other serologic) activity, most clinicians would use this information as motivation to intensify clinical screening for supportive signs of lupus activity prior to boosting therapy. Several studies have shown that cyclophosphamide is more effective than steroids alone in preventing renal flares. However, the risk/benefit of prolonged maintenance courses of cyclophosphamide remains controversial. Also, the possible equivalence of maintenance azathioprine to quarterly pulse cyclophosphamide in preventing relapses continues under study. In the interim, we advise continuation of quarterly pulse cyclophosphamide for 1 year beyond clinical and serological remission as the best method of reducing risk of relapse of lupus nephritis.

HYPERTENSION

Hypertension is a significant problem in SLE. The prevalence of hypertension is highest in patients with diffuse proliferative and sclerosing lupus nephritis and is associated with an increased risk of progressive renal failure, as well as cardiovascular disease. Severe nephritis contributes to hypertension diathesis by producing high renin and angiotensin II states, as well as by inducing salt and water retention. Angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor antagonists plus diuretics offer effective blood pressure control in most patients. There is also evidence that these agents may be renoprotective by reducing intraglomerular pressure which, in turn, reduces proteinuria and downstream injury to renal tubular cells [142, 143]. Patients starting on ACEI or angiotensin receptor antagonists must be monitored carefully for several weeks after initiation for development of hyperkalemia and azotemia which may confound interpretation of the course and activity of lupus nephritis. Calcium channel antagonists may be preferable to angiotensin inhibitors in Black lupus patients. The full range of adrenergic antagonists and vasodilators, including minoxidil, may be necessary for effective control of hypertension. Older antihypertensives, such as hydralazine and alpha methyldopa, are rarely used due to their tendency to aggravate SLE and the availability of excellent alternative medications.

The most effective monitoring of blood pressure is accomplished by the patient. Patients developing hypertension should be encouraged to keep their own logbook of blood pressure readings, with instructions on when to contact the medical team for medication adjustment. The target for effective blood pressure control is age-dependent. In adults, the traditional guideline of blood pressure of <140/90 mm Hg has been revised downward to values of <120/75 [144].

HYPERLIPIDEMIA AND CARDIOVASCULAR RISKS

Hypercholesterolemia is mostly the consequence of nephrotic syndrome, though the pathophysiology is not completely understood [145]. Hyperlipidemia appears to have two adverse consequences: increased cardiovascular risk and possibly (though unproven) enhanced renal injury. The increased risk of cardiovascular events in lupus is clearly multifactorial, including corticosteroids, hypertension, increased LDL cholesterol, reduced HDL cholesterol, elevated lipoprotein (a), anticardiolipin antibodies, and elevated homocysteine levels [146–148]. Management of cardiovascular risk factors in lupus patients is described to a limited extent in Table 7 and, in general, should follow conventional therapeutic guidelines.

THROMBOTIC DIATHESIS

Thrombotic diathesis is often multifactorial in patients with lupus nephritis. The most important factors are nephrotic syndrome and anti-phospholipid antibodies. Nephrotic syndrome is associated with increased synthesis of clotting factors (e.g., fibrinogen) and loss of fibrinolytic factors (e.g., protein S) in the

urine. The precise mechanism(s) by which anti-phospholipid antibodies produce a thrombotic diathesis is not completely understood (anti-platelet agents are used prophylactically and anticoagulant agents are used following clinically apparent thromboses). Patients with persistent nephrotic syndrome and/or anti-phospholipid antibodies should be carefully informed about the importance of early recognition of and prompt intervention for thromboembolic events. Increased levels of plasma homocysteine have been reported to increase the risk of venous thrombosis, in the general population, and of stroke and arterial thrombosis in lupus patients. Although clear practice guidelines have not been established, it seems reasonable to measure plasma homocysteine and to prescribe folate supplements (e.g., 2 mg/day) if the levels of homocysteine are substantially elevated.

RENAL VASCULOPATHIES IN LUPUS NEPHRITIS

Extraglomerular renal vascular lesions are commonly seen in lupus nephritis [28–30]. Immune complex deposition (with or without necrosis) within arterioles and small arteries is frequent in immunofluorescence studies of renal biopsies. By light microscopy, subintimal eosinophilic, PAS-positive deposits are common, even in the absence of hypertension. This process may extend to a non-inflammatory necrotizing vasculopathy and to a true necrotizing vasculitis, though there is no direct evidence that the latter is an immune complex initiated process. True vasculitis is, indeed, rare in biopsies of patients with lupus nephritis. The prognostic impact of necrotizing vasculitis in the kidney is unclear, but most would treat this condition aggressively with corticosteroids and cytotoxic drugs.

A thrombotic thrombocytopenic purpura (TTP-like) microangiopathy occasionally develops in patients with lupus nephritis. The absence of immune complexes in vessel walls underscores that the fibrin thrombi represent a true microvascular thrombotic process. It is critical to recognize this diagnosis early because it is associated with very poor prognosis. Treatment with plasma exchange is indicated as in cases of idiopathic TTP [149].

PREGNANCY AND LUPUS NEPHRITIS

A comprehensive review of the interactions between disease activity and pregnancy in lupus patients is included elsewhere in this volume. Pregnancy is normally associated with increased glomerular perfusion. Glomerular hyperfiltration is associated with fall in serum creatinine. Lack of fall in serum creatinine is abnormal and indicates significant underlying renal disease.

Patients with normal renal function and sustained remission of lupus nephritis (i.e., inactive urine sediment and proteinuria <1 g/day) incur low risks of exacerbations of either lupus or renal disease and have high probability of successful pregnancies [150–152]. At the other extreme, in patients with active nephritis and impaired renal function pregnancy is associated with substantial risks of developing exacerbations and irreversible renal failure. A study in patients with various forms of glomerular and tubulointerstitial diseases showed that a serum creatinine ≥1.4 mg/dl at the time of conception was associated with significant worsening of maternal renal function, proteinuria, and hypertension, as well as fetal prematurity and growth retardation [153]. Overall, 10% of these women developed end-stage renal failure within the first postpartum year; the risk rose to 35% in women with baseline creatinine ≥2.0 mg/dl. Patients with moderate to severe renal function impairment contemplating pregnancy should be fully informed about both maternal and fetal risks. Overall for SLE patients, there is increased risk of fetal loss, prematurity, and intrauterine growth retardation in the fetus. The risk is the same in patients with inactive lupus nephritis. With renal functional impairment, the risks increase for both the fetus and the mother. If pregnancy occurs in a patient with substantial renal involvement, consultation with high-risk obstetricians and nephrologists should be undertaken.

LUPUS NEPHRITIS IN CHILDREN

The natural history of lupus nephritis in children is controversial. Study results have varied from the course of disease in children being described as better in some instances and worse in others than comparable classes of lupus nephritis in adults. Indeed, one study showed a dismal renal survival of 29% at 10 years in a cohort of 56 pediatric patients with lupus nephritis [68]. While not specifically analyzed in this study, the high risk of renal failure may have been affected by racial factors (previously discussed); more than 64% of patients were Black and less than 10% were White.

The natural histories, indications for treatment, and outcomes are similar in children and adults with lupus nephritis (Fig. 16), though most studies have not analyzed the confounding issue of race on the outcomes of pediatric aged patients [154–157]. It is apparent that

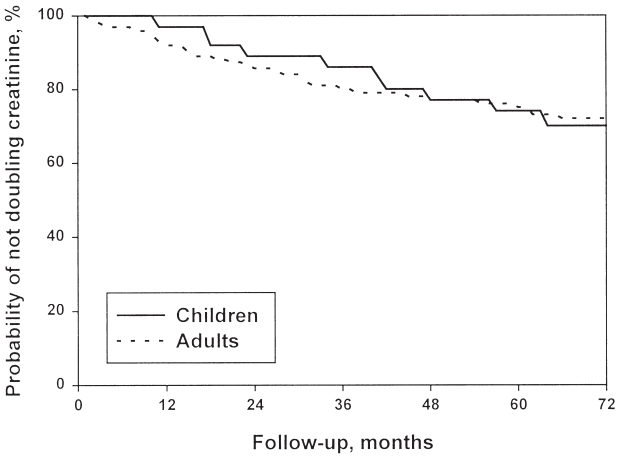


FIGURE 16 Comparison of the courses of lupus nephritis in children and adults. Probability of not doubling serum creatinine among 166 patients with lupus nephritis participating in controlled clinical trials at NIH [62, 96].

children have greater capacity to compensate for glomerular damage than do adults. Thus, children and adults with comparable proportions of obsolescent glomeruli have greater and lesser potential, respectively, for compensatory hypertrophy in less involved glomeruli (presumably with increased single nephron hyperfiltration) and in overall preservation of renal function.

CHRONIC RENAL INSUFFICIENCY

Progressive renal insufficiency in lupus evolves from two fundamentally different processes: nephron damage from the various immunopathologic lesions and from maladaptive compensatory mechanisms. By uncertain mechanisms, focal nephron loss evokes compensatory hypertrophy and hemodynamic changes in less involved glomeruli. Hypertrophy, increased blood flow, and hydrostatic pressure temporarily improve glomerular filtration, even to normal levels. It is not uncommon to find normal GFR in patients with loss of a substantial proportion of the total nephron population. Histologically, glomeruli with several-fold increased volumes are scattered among obsolescent nephrons. Experimental models of renal ablation and high protein intake have shown increased blood flow and elevated intracapillary pressures; the latter, in turn, augment proteinuria and glomerulosclerosis and culminate in progressive decline of GFR. The contributions of these maladaptive processes in progressive human diseases, including lupus nephritis, have not been proven. However, these experimental observations form the basis of clinical recommendations for stringent blood pressure control, particularly with ACEI, and low protein diet in patients with chronic renal insufficiency.

TABLE 14 Management Issues in Patients with Chronic Renal Insufficiency

Minimize exposure to nephrotoxic drugs: radiologic contrast, prostaglandin inhibiting analgesics (e.g., ketorolac), and chronic non-steroidal anti-inflammatory drugs (contribution to chronic renal failure is controversial)
Salt intake: avoid severe salt restriction (will worsen azotemia if there is renal salt wasting)
Potassium: avoid salt substitutes; carefully monitor potassium when initiating ACEI; prescribe low potassium diet as dictated by serum electrolytes
Protein: use low protein diet (0.6 g/kg/day) during chronic renal insufficiency (creatinine >2)
Phosphate: decrease intestinal absorption of phosphate by using calcium carbonate *with* each food intake (titrating dose to maintain normal serum phosphorus, without hypercalcemia)
Acidosis: metabolic acidosis is usually mild; occasionally requires $NaHCO_3$ or bicitra if serum bicarbonate is <18 mEq/liter (e.g., start with 10–20 mEq of base per day)
Anemia: correct iron or folate deficiencies; use erythropoietin (e.g., 80–100 units/kg given 2–3 times per week) for symptomatic anemia or to keep hematocrit >30%

Once chronic renal insufficiency arises in patients with advanced lupus nephritis, control of other pathophysiologic processes must be addressed, ideally in consultation with nephrology (Table 14). Control of hyperphosphatemia is critically important in order to prevent insidious secondary hyperparathyroidism. The patient should be thoroughly instructed in renal diets and nutrition, particularly regarding potassium and phosphate contents of various foods. Calcium salt supplements are a good method to simultaneously reduce dietary phosphate absorption and ensure adequate calcium intake. Calcium carbonate (e.g., starting with 650 mg taken during meals) is an effective method to control hyperphosphatemia. Multivitamins, including folate, are usually prescribed. Vitamin D supplements may also be appropriate, particularly in advanced renal insufficiency when renal production of active 1,25-dihydroxy-vitamin D may be significantly impaired. Erythropoietin production declines with progressive renal insufficiency; epogen treatment is warranted for symptomatic anemia or to maintain hematocrit above 30%.

END-STAGE RENAL DISEASE

With advancing renal failure, it is important to anticipate the need for vascular access for hemodialysis. If hemodialysis is anticipated within several weeks, protection of arm veins is critically important to facilitate creation of A-V fistulas, which are far preferable to synthetic grafts. On the other hand, too early placement of vascular access can be injudicious because of the risk of spontaneous thrombosis and the need for reoperation. Overall, it is advisable to refer the patient early for evaluation by a dialysis team. The timing depends on the rate of deterioration of renal function, as well as difficulties in fluid, blood pressure, potassium, and phosphate regulation. In the otherwise well controlled and clinically stable patient, dialysis is usually initiated when serum creatinine rises above 8–10 mg/dl.

DIALYSIS

The choice of dialysis modality is mostly dependent on patient preference. Peritoneal dialysis is an option for management of end-stage lupus nephritis and may offer the patient greater independence. Patients treated with either peritoneal dialysis or hemodialysis may experience clinically significant lupus activity, many components of which may be difficult to distinguish from complications of uremia per se.

Serological tests, including complement and anti-DNA may be useful in this regard. It has generally been held that lupus becomes inactive during sustained uremia. However, this varies considerably, depending mostly on the rate of progression to uremia. When renal failure develops rapidly, active lupus is more likely to persist for a considerable time on dialysis. When renal failure develops insidiously over an extended period, lupus activity tends to subside gradually in a process often described as "burning out."

Definitive lupus flares are generally treated with a trial of corticosteroids, but the whole armamentarium of immunosuppressive therapy may be needed to control serious complications of SLE in dialysis patients. Cyclophosphamide must be used in reduced dose due to impaired renal excretion in order to avoid severe bone marrow toxicity. Small daily cyclophosphamide doses (e.g., ≤100 mg) or pulse doses (e.g., 0.5 g/m^2 given 8–12 h before the subsequent dialysis) have been successfully used. The clinician must be particularly mindful that in the context of chronic dialysis, the constellation of active lupus requiring corticosteroids and cyclophosphamide places patients at particularly high risk of death from sepsis.

There are conflicting data regarding survival of lupus patients during chronic dialysis therapy [158–161]. Most series have suggested that survival of patients with end-stage renal failure due to lupus nephritis is equivalent to that for the overall ESRD population. Some have reported reduced survival. Pediatric patients in particular seem to have reduced survival on dialysis (155). Most series have lacked appropriate adjustments for age and sex. Thus, it remains uncertain whether a background of SLE has an adverse effect on the survival of patients on chronic dialysis.

RENAL TRANSPLANTATION

Many patients with end-stage renal failure due to lupus nephritis have undergone renal transplantation [44, 162–165]. The optimal timing for renal transplantation varies widely. Practices vary from those where patients are maintained on chronic dialysis for 12 or more months to ensure sustained quiescence of lupus activity (clinical and serological), to those where renal transplant is performed in the patient in remission without their ever being treated with dialysis therapy. The latter option is acceptable providing that the patient has been in sustained remission of SLE during the late stages of chronic renal insufficiency.

Some physicians have been reticent to recommend renal transplantation at all. This is based largely on outdated historical experience indicating decreased patient and graft survivals and, to a lesser extent, concern about recurrence of lupus nephritis in the allograft. Most contemporary series indicate that patient survival following renal transplantation is approximately the same in lupus patients and controls (there is a caveat that not all comparisons of survival were controlled for age and sex). Similarly, the preponderance of studies have suggested that renal allograft survival following transplantation for lupus nephritis is comparable to those for other renal diseases. However, some studies have presented data indicating poorer than expected graft survival in patients with underlying SLE. One study by Lochhead *et al*. in 80 lupus patients showed marked differences in allograft survival depending on the donor source [164]. With cadaver donors, 5-year graft survival in lupus patients was only 41% compared to 71% in unselected controls. Also, noteworthy in the lupus cohort was that graft survival from living related donors improved to 89% at 5 years. The authors suggested an increased role of living related over cadaver grafts in lupus patients.

Some studies indicate that lupus patients with renal transplants did not fare as well as patients with other causes of renal failure [165]. However, review of a much

larger data sets from the United States Renal Data System (USRDS) and the North American Pediatric Renal Transplant Cooperative Study has shown that both patient and graft survival are comparable for lupus and nonlupus cohorts [166, 167]. Though traditional teaching is that recurrence of lupus nephritis in the renal allograft is rare, a study suggests that pathologic evidence of recurrence is relatively common [168], but fortunately, recurrent lupus nephritis seems not to be an important cause for graft loss.

REFERRAL GUIDELINES AND USE OF CONSULTANTS

Given the typically insidious nature of renal involvement, substantial damage can easily accrue before lupus nephritis is recognized by either the patient or the nonvigilant physician. It is commonplace that subspecialists become too narrowly focused on isolated components of SLE. Checklists are useful tools for monitoring patients with SLE; they decrease the risk of failure to ascertain important (particularly subclinical) components of the disease.

A multidisciplinary approach to diagnosis and treatment of patients with SLE and lupus nephritis is optimal. Regular interchange among experienced clinicians, particularly about interpretation of ambiguous findings and if only in conference settings, offers the best opportunity to achieve comprehensive management of lupus patients. At the same time, it is critically important that one physician assumes a primary or care coordinator role in order to enhance communication with the patient, to prevent fragmentation of diagnosis and treatment, and to distill divergent opinions offered by consultants. In the case of severe lupus nephritis, it is important that rheumatologists and nephrologists share diagnostic and therapeutic decisions.

FUTURE DIRECTIONS AND EXPERIMENTAL THERAPIES

Patients and physicians alike continue to hope for "curative" treatments for patients with SLE and lupus nephritis. Many investigators envision that reconstitution of the disordered immune system is the most likely avenue to this end. Translational work has begun employing different methods aimed at overhauling the immune system of patients with severe and conventional treatment-refractory SLE. Availability of therapeutic granulocyte colony stimulating factor (G-CSF) has allowed immunoablative regimens involving extreme doses of cyclophosphamide (e.g., 200 mg/kg) with or without adjunctive fludarabine, total body irradiation, and antithymocyte globulin to be tested [169–174]. Results to date are encouraging, but these immunoablative and reconstituting regimens are associated with substantial morbidity, high costs, and not insignificant risk of failure, relapse, and death [175]. Appropriate selection of candidate patients to undergo these experimental approaches remains a major challenge.

References

1. Tan, E. M., Cohen, A. S., Fries, J. F., *et al.* (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271–1277.
2. Balow, J. E., Boumpas, D. T., and Austin, H. A. (1996). Renal disease. In "The Clinical Management of Systemic Lupus Erythematosus," (P. Schur, Ed.), 2nd ed., pp. 109–126. Lippincott-Raven, Philadelphia.
3. Balow, J. E., Boumpas, D. T., and Austin, H. A. (2003). Lupus nephritis. In "Therapy in Nephrology and Hypertension" (H. R. Brady, and C. Wilcox, Eds.), 2nd ed., W.B. Saunders, London.
4. Lewis, E. J., Schwartz, M. M., and Korbet, S. M. (Eds.) (1999). "Lupus Nephritis," pp. 1–315. Oxford Univ. Press, Oxford.
5. Lafayette, R. A., Perrone, R. D., and Levey, A. S. (1997). Laboratory evaluation of renal function. In "Diseases of the Kidney" (R. W. Schrier, and C. W. Gottschalk, Eds.), 6th ed., pp. 307–354. Little, Brown & Co., Boston.
6. Fogazzi, G. B., Ponticelli, C., and Ritz, E. (1999). "The Urinary Sediment" 2nd ed., pp. 1–188. Oxford Univ. Press, Oxford.
7. Rasoulpour, M., Banco, L., Laut, J. M., and Burke, G. S. (1996). Inability of community-based laboratories to identify pathological cast in urine samples. *Arch. Pediatr. Adolesc. Med.*, 1201–1204.
8. Manjunath, G., Sarnak, M. J., and Levey, A. S. (2001). Prediction equations to estimate glomerular filtration rate: An update. *Curr. Opin. Nephrol. Hypertens.* **10**, 785–792.
9. Chitalia, V. C., Kothari, J., Wells, E. J., *et al.* (2001). Cost–benefit analysis and prediction of 24-hour proteinuria from the spot urine protein–creatinine ratio. *Clin. Nephrol.* **55**, 436–447.
10. Appel, G. B., Cohen, D. J., Pirani, C. L., *et al.* (1987). Long-term follow-up of patients with lupus nephritis. A study based on the classification of the World Health Organization. *Am. J. Med.* **83**, 877–885.
11. Fraenkel, L., MacKenzie, T., Joseph, L., *et al.* (1994). Response to treatment as a predictor of long-term outcome in patients with lupus nephritis. *J. Rheumatol.* **21**, 2052–2057.
12. Lim, C. S., Chin, H. J., Jung, Y. C., *et al.* (1999). Prognostic factors of diffuse proliferative lupus nephritis. *Clin. Nephrol.* **52**, 139–147.
13. Chan, T. M., Li, F. K., Tang, C. S., *et al.* (2000). Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. *N. Engl. J. Med.* **343**, 1156–1162.

14. Korbet, S. M., Lewis, E. J., Schwartz, M. M., *et al.* (2000). Factors predictive of outcome in severe lupus nephritis. Lupus Nephritis Collaborative Study Group. *Am. J. Kidney Dis.* **35**, 904–914.
15. Illei, G. G., Takada, K., Parkin, D., *et al.* (2002). Renal flares are common in patients with severe proliferative lupus nephritis treated with pulse immunosuppressive therapy. *Arthritis Rheum.* **46**, 995–1002.
16. Ponticelli, C., and Moroni, G. (1998). Renal biopsy in lupus nephritis—what for, when and how often? *Nephrol. Dial. Transplant.* **13**, 2452–2454.
17. Grande, J. P., and Balow, J. E. (1998). Renal biopsy in lupus nephritis. *Lupus* **7**, 611–617.
18. Kashgarian, M. (1994). Lupus nephritis: Lessons from the path lab. *Kidney Int.* **45**, 928–938.
19. Esdaile, J. M., Mackenzie, T., Barre, P., *et al.* (1992). Can experienced clinicians predict the outcome of lupus nephritis? *Lupus* **1**, 205–214.
20. Esdaile, J. M., MacKenzie, J. L., Kashgarian, M., and Hayslett, J. P. (1994). The benefit of early treatment with immunosuppressive drugs in lupus nephritis. *J. Rheumatol.* **21**, 2046–2051.
21. Esdaile, J. M. (1998). Current role of renal biopsy in patients with SLE. *Baillieres Clin. Rheumatol.* **12**, 433–448.
22. Muehrcke, R. C., Kark, R. M., Pirani, C. L., and Pollak, V. E. (1957). Lupus nephritis: A clinical and pathologic study based on renal biopsies. *Medicine* **36**, 1–145.
23. Pollak, V. E., Pirani, C. L., and Schwartz, F. D. (1964). The natural history of the renal manifestations of systemic lupus erythematosus. *J. Lab. Clin. Med.* **63**, 537–550.
24. Baldwin, D. S., Lowenstein, J., Rothfield, N. F., *et al.* (1970). The clinical course of the proliferative and membranous forms of lupus nephritis. *Ann. Intern. Med.* **73**, 929–942.
25. Baldwin, D. S., Gluck, M. C., Lowenstein, J., and Gallo, G. (1977). Lupus nephritis: Clinical course as related to morphologic forms and their transitions. *Am. J. Med.* **62**, 12–30.
26. Appel, G. B., Silva, F. G., Pirani, C. L., *et al.* (1978). Renal involvement in systemic lupus erythematosus (SLE): A study of 56 patients emphasizing histologic classification. *Medicine* **57**, 371–410.
27. Churg, J., Bernstein, J., and Glassock, R. J. (1995). Lupus nephritis. In "Renal Disease: Classification and Atlas of Glomerular Diseases," 2nd ed., pp. 151–179. Igaku-Shoin, New York.
28. Descombes, E., Droz, D., Drouet, L., *et al.* (1997). Renal vascular lesions in lupus nephritis. *Medicine* **76**, 355–368.
29. Appel, G. B., Pirani, C. L., and D'Agati, V. (1994). Renal vascular complications of systemic lupus erythematosus. *J. Am. Soc. Nephrol.* **4**, 1499–1515.
30. Daugas, E., Nochy, D., Huong, L. T., *et al.* (2002). Antiphospholipid syndrome nephropathy in systemic lupus erythematosus. *J. Am. Soc. Nephrol.* **13**, 42–52.
31. Akhtar, M., al-Dalaan, A., and el-Ramahi, K. M. (1994). Pauci-immune necrotizing lupus nephritis: Report of two cases. *Am. J. Kidney Dis.* **23**, 320–325.
32. Tipping, P. G., Kitching, A. R., Cunningham, M. A., and Holdsworth, S. R. (1999). Immunopathogenesis of crescentic glomerulonephritis. *Curr. Opin. Nephrol. Hypertens.* **8**, 281–286.
33. Massengill, S. F., Goodenow, M. M., and Sleasman, J. W. (1998). SLE nephritis is associated with an oligoclonal expansion of intrarenal T cells. *Am. J. Kidney Dis.* **31**, 418–426.
34. Eddy, A. A. (2000). Molecular basis of renal fibrosis. *Pediatr. Nephrol.* **15**, 290–301.
35. Pirani, C. L., Pollak, V. E., and Schwartz, F. D. (1964). The reproducibility of semiquantitative analyses of renal histology. *Nephron* **1**, 230–237.
36. Morel-Maroger, L., Mery, J. P., Droz, D., *et al.* (1976). The course of lupus nephritis: Contribution of serial renal biopsies. *Adv. Nephrol.* **6**, 79–118.
37. Austin III, H. A., Muenz, L. R., Joyce, K. M., *et al.* (1984). Diffuse proliferative lupus nephritis: Identification of specific pathologic features affecting renal outcome. *Kidney Int.* **25**, 689–695.
38. Gamba, G., Reyes, E., Angeles, A., *et al.* (1991). Observer agreement in the scoring of the activity and chronicity indexes of lupus nephritis. *Nephron* **57**, 75–77.
39. Hill, G. S., Delahousse, M., Nochy, D., *et al.* (2000). A new morphologic index for the evaluation of renal biopsies in lupus nephritis. *Kidney Int.* **58**, 1160–1173.
40. Balow, J. E., Austin III, H. A., Muenz, L. R., *et al.* (1984). Effect of treatment on the evolution of renal abnormalities in lupus nephritis. *N. Engl. J. Med.* **311**, 491–495.
41. Hahn, B. H. (2001). Lessons in lupus: The mighty mouse. *Lupus* **10**, 589–593.
42. Peutz-Koostra, C. J., DeHeer, E., Hoedemaeker, P. J., *et al.* (2001). Lupus nephritis: Lessons from experimental animal models. *J. Lab. Clin. Med.* **137**, 244–260.
43. Clynes, R., Dumitru, C., and Ravetch, J. V. (1998). Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* **279**, 1052–1054.
44. Berden, J. H. (1997). Lupus nephritis (Nephrology Forum). *Kidney Int.* **52**, 538–558.
45. Kewalramani, R., and Singh, A. K. (2002). Immunopathogenesis of lupus and lupus nephritis: Recent insights. *Curr. Opin. Nephrol. Hypertens.* **11**, 273–277.
46. Oates, J. C., and Gilkeson, G. S. (2002). Mediators of injury in lupus nephritis. *Curr. Opin. Rheumatol.* **14**, 498–503.
47. Hahn, B. H. (1998). Antibodies to DNA. *N. Engl. J. Med.* **338**, 1359–1368.
48. Kerjaschki, D. (2000). Megalin/GP330 and the pathogenetic concepts of membranous glomerulopathy (MGN). *Kidney Blood Press. Res.* **23**, 163–166.
49. Wallace, D. J. (2001). Clinical and pharmacological experience with LJP-394. *Expert Opin. Invest. Drugs* **10**, 111–117.
50. Klemperer, P., Pollack, A. D., and Baehr, G. (1941). Pathology of disseminated lupus erythematosus. *Arch. Pathol.* **32**, 569–631.

51. Pollak, V. E., Pirani, C. L., and Kark, R. M. (1961). Effect of large doses of prednisone on the renal lesions and life span of patients with lupus glomerulonephritis. *J. Lab. Clin. Med.* **57**, 495–511.
52. Cameron, J. S., Turner, D. R., Ogg, C. S., *et al.* (1979). Systemic lupus with nephritis: A long-term study. *Q. J. Med.* **48**, 1–24.
53. Ponticelli, C., Zucchelli, P., Moroni, G., *et al.* (1987). Long-term prognosis of diffuse lupus nephritis. *Clin. Nephrol.* **28**, 263–271.
54. Donadio, J. V., Jr. Holley, K. E., Wagoner, R. D. *et al.* (1972). Treatment of lupus nephritis with prednisone and combined prednisone and azathioprine. *Ann. Intern. Med.* **77**, 829–835.
55. Donadio, J. V., Jr., Holley, K. E., Wagoner, R. D., *et al.* (1974). Further observations on the treatment of lupus nephritis with prednisone and combined prednisone and azathioprine. *Arthritis Rheum.* **17**, 573–581.
56. Donadio, J. V., Jr., Holley, K. E., Ferguson, R. H., and Ilstrup, D. M. (1976). Progressive lupus glomerulonephritis: Treatment with prednisone and combined prednisone and cyclophosphamide. *Mayo. Clin. Proc.* **51**, 484–494.
57. Donadio, J. V., Jr., Holley, K. E., Ferguson, R. H., and Ilstrup, D. M. (1978). Treatment of diffuse proliferative lupus nephritis with prednisone and combined prednisone and cyclophosphamide. *N. Engl. J. Med.* **299**, 1151–1155.
58. Donadio, J. V., Jr., Holley, K. E., and Ilstrup, D. M. (1982). Cytotoxic drug treatment of lupus nephritis. *Am. J. Kidney Dis.* **2** (Suppl.), 178–181.
59. Steinberg, A. D., and Decker, J. L. (1974). A double-blind controlled trial comparing cyclophosphamide, azathioprine and placebo in the treatment of lupus glomerulonephritis. *Arthritis Rheum.* **17**, 923–937.
60. Decker, J. L., Klippel, J. H., Plotz, P. H., and Steinberg, A. D. (1975). Cyclophosphamide or azathioprine in lupus glomerulonephritis: A controlled trial: results at 28 months. *Ann. Intern. Med.* **83**, 606–615.
61. Dinant, H. J., Decker, J. L., Klippel, J. H., *et al.* (1982). Alternative modes of cyclophosphamide and azathioprine therapy in lupus nephritis. *Ann. Intern. Med.* **96**, 728–736.
62. Austin III, H. A., Klippel, J. H., Balow, J. E., *et al.* (1986). Therapy of lupus nephritis: Controlled trial of prednisone and cytotoxic drugs. *N. Engl. J. Med.* **314**, 614–619.
63. Steinberg, A. D., and Steinberg, S. C. (1991). Long-term preservation of renal function in patient with lupus nephritis receiving treatment that includes cyclophosphamide versus those treated with prednisone only. *Arthritis Rheum.* **34**, 945–950.
64. Neumann, K., Wallace, D. J., Azen, C., *et al.* (1995). Lupus in the 1980s: III. Influence of clinical variables, biopsy, and treatment on the outcome in 150 patient with lupus nephritis seen at a single center. *Semin. Arthritis Rheum.* **25**, 47–55.
65. Gruppo Italiano per lo Studio della Nefrite Lupica (GISNEL). (1992). Lupus nephritis: Prognostic factors and probability of maintaining life-supporting renal function 10 years after the diagnosis. *Am. J. Kidney Dis.* **19**, 473–479.
66. Donadio, J. V., Jr., Hart, G. M., Bergstralh, E. J., and Holley, K. E. (1995). Prognostic determinants in lupus nephritis: A long-term clinicopathologic study. *Lupus* **4**, 109–115.
67. Dooley, M. A., Hogan, S., Jennette, J. C., and Falk, R. J. (1997). Cyclophosphamide therapy for lupus nephritis: Poor renal survival in black Americans. *Kidney Int.* **51**, 1188–1195.
68. Bakir, A. A., Levy, P. S., and Dunea, G. (1994). The prognosis of lupus nephritis in African-Americans: A retrospective analysis. *Am. J. Kidney Dis.* **24**, 159–171.
69. Conlon, P. J., Fischer, C. A., Levesque, M. C., *et al.* (1996). Clinical, biochemical and pathological predictors of poor response to intravenous cyclophosphamide in patients with proliferative lupus nephritis. *Clin. Nephrol.* **46**, 170–175.
70. Baqi, N., Moazami, S., Singh, A., *et al.* (1996). Lupus nephritis in children: A longitudinal study of prognostic factors and therapy. *J. Am. Soc. Nephrol.* **7**, 924–929.
71. Austin III, H. A., Boumpas, D. T., Vaughan, E. M., and Balow, J. E. (1995). High-risk features of lupus nephritis: Importance of race and clinical and histological factors in 166 patients. *Nephrol. Dial. Transplant.* **10**, 1620–1628.
72. Lea, J. P. (2002). Lupus nephritis in African Americans. *Am. J. Med. Sci.* **323**, 85–89.
73. Cameron, J. S. (1997). The long-term outcome of glomerular diseases. In "Diseases of the Kidney" (R. W. Schrier, and C. W. Gottschalk, Eds.), 6th ed., pp. 1965–1969. Little, Brown & Co., Boston.
74. Sloan, R. P., Schwartz, M. M., Korbet, S. M., and Borok, R. Z. (1996). Long-term outcome in systemic lupus erythematosus membranous glomerulonephritis. *J. Am. Soc. Nephrol.* **7**, 299–305.
75. Najafi, C. C., Korbet, S. M., Lewis, E. J., *et al.* (2001). Significance of histologic patterns of glomerular injury upon long-term prognosis in severe lupus glomerulonephritis. *Kidney Int.* **59**, 2156–2163.
76. Appel, G. B. (1997). Cyclophosphamide therapy of severe lupus nephritis. *Am. J. Kidney Dis.* **30**, 872–876.
77. Freedman, B. I., Wilson, C. H., Spray, B. J., *et al.* (1997). Familial clustering of ESRD in blacks with lupus nephritis. *Am. J. Kidney Dis.* **29**, 729–732.
78. Salmon, J., Millard, S., Schacter, L., *et al.* (1996). Fc-gamma-RIIA alleles are heritable risk factors for lupus nephritis in African-Americans. *J. Clin. Invest.* **97**, 1348–1354.
79. Karassa, F. B., Trikalinos, T. A., and Ioannidis, J. P. (2002). Role of Fcγ receptor IIa polymorphism in susceptibility to systemic lupus erythematosus and lupus nephritis: A meta-analysis. *Arthritis Rheum.* **46**, 1563–1571.
80. Austin III, H. A., Boumpas, D. T., Vaughan, E. M., and Balow, J. E. (1994). Predicting renal outcomes in severe lupus nephritis: Contributions of clinical and histologic data. *Kidney Int.* **45**, 544–550.

81. Austin III, H. A., Muenz, L. R., Joyce, K. M., *et al.* (1983). Prognostic factors in lupus nephritis. Contribution of renal histologic data. *Am. J. Med.* **75**, 382–391.
82. Banfi, G., Mazzucco, G., Barbiano di Belgiojoso, G. B., *et al.* (1985). Morphological parameters in lupus nephritis: Their relevance for classification and relationship with clinical and histological findings and outcome. *Q. J. Med.* **55**, 153–168.
83. Derksen, R. H., Hene, R. J., and Kater, L. (1992). The long-term clinical outcome of 56 patients with biopsy-proven lupus nephritis followed at a single center. *Lupus* **1**, 97–103.
84. Esdaile, J. M., Levinton, C., Federgreen, W., *et al.* (1989). The clinical and renal biopsy predictors of long-term outcome in lupus nephritis: A study of 87 patients and review of the literature. *Q. J. Med.* **72**, 779–833.
85. McLaughlin, J., Gladman, D. D., Urowitz, M. B., *et al.* (1991). Kidney biopsy in systemic lupus erythematosus: Survival analyses according to biopsy results. *Arthritis Rheum.* **34**, 1268–1273.
86. Nossent, J. C., Henzen-Logmans, S. C., Vroom, T. M., *et al.* (1990). Contribution of renal biopsy data in predicting outcome in lupus nephritis: Analysis of 116 patients. *Arthritis Rheum.* **33**, 970–977.
87. Reveille, J. D., Bartolucci, A., and Alarcon, G. S. (1990). Prognosis in systemic lupus erythematosus: Negative impact of increasing age at onset, black race and thrombocytopenia, as well as causes of death. *Arthritis Rheum.* **33**, 37–48.
88. Ward, M. M., and Studenski, S. (1992). Clinical prognostic factors in lupus nephritis: The importance of hypertension and smoking. *Arch. Intern. Med.* **152**, 2082–2088.
89. Balow, J. E. (2002). Choosing therapy for proliferative lupus nephritis. *Arthritis Rheum.* **46**, 1981–1983.
90. Sztejnbok, M., Stewart, A., Diamond, H., and Kaplan, D. (1971). Azathioprine in the treatment of systemic lupus erythematosus: A controlled study. *Arthritis Rheum.* **14**, 639–645.
91. Hahn, B. H., Kantor, O. S., and Osterland, C. K. (1975). Azathioprine plus prednisone compared with prednisone alone in the treatment of systemic lupus erythematosus. *Ann. Intern. Med.* **83**, 597–605.
92. Felson, D. T., and Anderson, J. (1984). Evidence for the superiority of immunosuppressive drugs and prednisone over prednisone alone in lupus nephritis. *N. Engl. J. Med.* **311**, 1528–1533.
93. Haubitz, M., Schellong, S., Gobel, U., *et al.* (1998). Intravenous pulse administration of cyclophosphamide versus daily oral treatment in patients with antineutrophil cytoplasmic antibody-associated vasculitis and renal involvement: A prospective, randomized study. *Arthritis Rheum.* **41**, 1835–1844.
94. Ponticelli, C., Zucchelli, P., Banfi, G., *et al.* (1982). Treatment of diffuse proliferative lupus nephritis by intravenous high-dose methylprednisolone. *Q. J. Med.* **51**, 16–24.
95. Ponticelli, C. (1997). Treatment of lupus nephritis—the advantages of a flexible approach. *Nephrol. Dial. Transplant.* **12**, 2057–2059.
96. Boumpas, D. T., Austin, H. A., Vaughan, E. M., *et al.* (1992). Severe lupus nephritis: Controlled trial of pulse methylprednisolone versus two different regimens of pulse cyclophosphamide. *Lancet* **340**, 741–745.
97. Sesso, R., Monteiro, M., Sato, E., *et al.* (1994). A controlled trial of pulse cyclophosphamide versus pulse methylprednisolone in severe lupus nephritis. *Lupus* **3**, 107–112.
98. Gourley, M. F., Austin, H. A., Scott, D., *et al.* (1996). Methylprednisolone and cyclophosphamide, alone or combination, in patients with lupus nephritis: A randomized, controlled trial. *Ann. Intern. Med.* **125**, 549–557.
99. Illei, G. G., Austin, H. A., Crane, M., *et al.* (2001). Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. *Ann. Intern. Med.* **135**, 296–298.
100. Eiser, A. R., Grishman, E., and Dreznin, S. (1993). Intravenous pulse cyclophosphamide in the treatment of type IV lupus nephritis. *Clin. Nephrol.* **40**, 155–159.
101. McCune, W. J., Golbus, J., Zelders, W., *et al.* (1988). Clinical and immunologic effects of monthly administration of intravenous cyclophosphamide in severe systemic lupus erythematosus. *N. Engl. J. Med.* **318**, 1423–1431.
102. Valeri, A., Radhakrishnan, J., Estes, D., *et al.* (1994). Intravenous pulse cyclophosphamide treatment of severe lupus nephritis: A prospective five-year study. *Clin. Nephrol.* **42**, 71–78.
103. Belmont, H. M., Storch, M., Buyon, J., and Abramson, S. (1995). New York University/Hospital for Joint Diseases experience with pulse cyclophosphamide treatment: Efficacy in steroid unresponsive lupus nephritis. *Lupus* **4**, 104–108.
104. Lehman, T. J., and Onel, K. (2000). Intermittent intravenous cyclophosphamide arrests progression of the renal chronicity index in childhood systemic lupus erythematosus. *J. Pediatr.* **136**, 243–247.
105. Mok, C. C., Ho, C. T., Siu, Y. P., *et al.* (2001). Treatment of diffuse proliferative lupus glomerulonephritis: A comparison of two cyclophosphamide-containing regimens. *Am. J. Kidney Dis.* **38**, 256–264.
106. Barbano, G., Gusmano, R., Damasio, B., *et al.* (2002). Childhood-onset lupus nephritis: A single-center experience of pulse intravenous cyclophosphamide therapy. *J. Nephrol.* **15**, 123–129.
107. Bootsma, H., Spronk, P., Derksen, R., *et al.* (1995). Prevention of relapses in systemic lupus erythematosus. *Lancet* **345**, 1595–1599.
108. Ciruelo, E., de la Cruz, J., Lopez, I., and Gomez-Reino, J. J. (1996). Cumulative rate of relapse of lupus nephritis after successful treatment with cyclophosphamide. *Arthritis Rheum.* **39**, 2028–2034.
109. Moroni, G., Quaglini, S., Maccario, M., *et al.* (1996). "Nephritic flares" are predictors of bad long-term renal outcome in lupus nephritis. *Kidney Int.* **50**, 2047–2053.
110. Ioannidis, J. P., Boki, K. A., Katsorida, M. E., *et al.* (2000). Remission, relapse, and re-remission of proliferative lupus nephritis treated with cyclophosphamide. *Kidney Int.* **57**, 258–264.

111. Illei, G. G., Takada, K., Parkin, D., *et al.* (2002). Renal flares are common in patients with severe lupus nephritis treated with pulse immunosuppressive therapy. *Arthritis Rheum.* **46**, 995–1002.
112. Ligtenberg, G., Grootscholten, C. M., Derkson, R. H., and Berden, J. H. (2002). Cyclophosphamide pulse therapy versus azathioprine and methylprednisolone pulses in proliferative lupus nephritis: First results of a randomized, prospective multicenter study (abstract). *J. Am. Soc. Nephrol.* **13**, 14A.
113. Contreras, G., Pardo, V., Leclercq, B., *et al.* (2002). Maintenance therapy for proliferative forms of lupus nephritis: A randomized clinical trial comparing quarterly intravenous cyclophosphamide (IVCY) versus oral mycophenolate mofetil (MMF) or azathioprine (AZA) (abstract). *J. Am. Soc. Nephrol.* **13**, 14A.
114. Briggs, W. A., Choi, M. J., and Scheel, P. J., Jr. (1998). Successful mycophenolate mofetil treatment of glomerular disease. *Am. J. Kidney Dis.* **31**, 213–217.
115. Choi, M. J., Eustace, J. A., Gimenez, L. F., *et al.* (2002). Mycophenolate mofetil for treatment of primary glomerular diseases. *Kidney Int.* **61**, 1098–1114.
116. Glicklich, D., and Acharya, A. (1998). Mycophenolate mofetil therapy for lupus nephritis refractory to intravenous cyclophosphamide. *Am. J. Kidney Dis.* **32**, 318–322.
117. Dooley, M. A., Cosio, F. G., Nachman, P. H., *et al.* (1999). Mycophenolate mofetil therapy in lupus nephritis: Clinical observations. *J. Am. Soc. Nephrol.* **10**, 833–839.
118. Chan, T. M., Wong, W. S., Lau, C. S., *et al.* (2001). Prolonged follow-up of patients with diffuse proliferative lupus nephritis (DPLN) treated with prednisolone and mycophenolate mofetil (MMF) (abstract). *J. Am. Soc. Nephrol.* **12**, 195A.
119. Ginzler, E. M. (2001). Clinical trials in lupus nephritis. *Curr. Rheumatol. Rep.* **3**, 199–204.
120. Wei, N., Klippel, J. H., Huston, D. P., *et al.* (1983). Randomized trial of plasma exchange in mild systemic lupus erythematosus. *Lancet* **1**, 17–22.
121. Lewis, E. J., Hunsicker, L. G., Shu-Ping, Lan, M. A., *et al.* (1992). A controlled trial of plasmapheresis therapy in severe lupus nephritis. *N. Engl. J. Med.* **326**, 1373–1378.
122. Wallace, D. J., Goldfinger, D., Pepkowitz, S. H., *et al.* (1998). Randomized controlled trial of pulse/synchronization cyclophosphamide/apheresis for proliferative lupus nephritis. *J. Clin. Apheresis* **13**, 163–166.
123. Danieli, M. G., Palmieri, C., Salvi, A., *et al.* (2002). Synchronized therapy and high-dose cyclophosphamide in proliferative lupus nephritis. *J. Clin. Apheresis* **17**, 72–77.
124. Wallace, D. J. (1999). Apheresis for lupus erythematosus. *Lupus* **8**, 174–180.
125. Boumpas, D. T., Austin, H. A., Vaughan, E. M., *et al.* (1993). Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide therapy. *Ann. Intern. Med.* **119**, 366–369.
126. McDermott, E. M., and Powell, R. J. (1996). Incidence of ovarian failure in systemic lupus after treatment with pulse cyclophosphamide. *Ann. Rheum. Dis.* **55**, 224–229.
127. Blumenfeld, Z., Shapiro, D., Shteinberg, M., *et al.* (2000). Preservation of fertility and ovarian function and minimizing gonadotoxicity in young women with systemic lupus erythematosus treated by chemotherapy. *Lupus* **9**, 401–405.
128. Abu-Shakra, M., Ehrenfeld, M., and Shoenfeld, Y. (2002). Systemic lupus erythematosus and cancer: Associated or not? *Lupus* **11**, 137–144.
129. Cibere, J., Sibley, J., and Haga, M. (2001). Systemic lupus erythematosus and the risk of malignancy. *Lupus* **10**, 394–400.
130. Nived, O., Bengtsson, A., Jonsen, A., *et al.* (2001). Malignancies during follow-up in an epidemiologically defined systemic lupus erythematosus inception cohort in southern Sweden. *Lupus* **10**, 500–504.
131. Donadio, J. V., Burgess, J. H., and Holley, K. E. (1977). Membranous lupus nephropathy: A clinicopathologic study. *Medicine* **56**, 527–536.
132. Kolanski, S. L., Chung, J. B., and Albert, D. A. (2002). What do we know about lupus membranous nephropathy? An analytic review. *Arthritis Rheum.* **47**, 450–455.
133. Appel, G. B., Williams, G. X., Meltzer, J. I., and Pirani, C. L. (1976). Renal vein thrombosis, nephrotic syndrome and systemic lupus erythematosus. *Ann. Intern. Med.* **85**, 310–317.
134. Ordonez, J. D., Hiatt, R. A., Killebrew, E. J., and Fireman, B. H. (1993). The increased risk of coronary heart disease associated with nephrotic syndrome. *Kidney Int.* **44**, 638–642.
135. Keane, W. F. (2001). Metabolic pathogenesis of cardiorenal disease. *Am. J. Kidney Dis.* **38**, 1372–1375.
136. Moroni, G., Maccario, M., Banfi, G., *et al.* (1998). Treatment of membranous lupus nephropathy. *Am. J. Kidney Dis.* **31**, 681–686.
137. Radhakrishnan, J., Kunis, C. L., D'Agati, V., and Appel, G. B. (1994). Cyclosporine treatment of lupus membranous nephropathy. *Clin. Nephrol.* **42**, 147–154.
138. Hallegua, D., Wallace, D. J., Metzger, A. L., *et al.* (2000). Cyclosporine for lupus membranous nephritis: Experience with ten patients and review of the literature. *Lupus* **9**, 241–251.
139. Austin, H. A., Vaughan, E. M., and Balow, J. E. (2000). Lupus membranous nephropathy: Randomized, controlled trial of prednisone, cyclosporine, and cyclophosphamide (abstract). *J. Am. Soc. Nephrol.* **11**, 81A.
140. Moroni, G., Trendelenburg, M., Del Papa, N., *et al.* (2001). Anti-C1q antibodies may help in diagnosing a renal flare in lupus nephritis. *Am. J. Kidney Dis.* **37**, 490–498.
141. Hill, G. S., Delahousse, M., Nochy, D., *et al.* (2002). Outcome of relapse in lupus nephritis: Roles of reversal of renal fibrosis and response of inflammation to therapy. *Kidney Int.* **61**, 2176–2186.
142. Remuzzi, G., Ruggenenti, P., and Perico, N. (2002). Chronic renal diseases: Renoprotective benefits of renin–angiotensin system inhibition. *Ann. Intern. Med.* **136**, 604–615.
143. Hill, G. S., Delahousse, M., Nochy, D., *et al.* (2001). Proteinuria and tubulointerstitial lesions in lupus nephritis. *Kidney Int.* **60**, 1893–1903.

144. The Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Arch. Intern. Med.* **157**, 2413–2446.
145. Saland, J. M., Ginsberg, H., and Fisher, E. A. (2002). Dyslipidemia in pediatric renal disease: Epidemiology, pathophysiology, and management. *Curr. Opin. Pediatr.* **14**, 197–204.
146. Petri, M., Roubenoff, R., Dallal, G. E., *et al.* (1996). Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. *Lancet* **348**, 1120–1124.
147. Welch, G. N., and Loscalzo, J. (1998). Homocysteine and atherothrombosis. *N. Engl. J. Med.* **338**, 1042–1050.
148. Svenungsson, E., Jensen-Urstad, K., Heimburger, M., *et al.* (2001). Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation* **16**, 1887–1893.
149. Bridoux, F., Vrtovsnik, F., Noel, C., *et al.* (1998). Renal thrombotic microangiopathy in systemic lupus erythematosus: Clinical correlations and long-term renal survival. *Nephrol. Dial. Transplant.* **14**, 298–304.
150. Bobrie, G., Liote, F., Houillier, P., *et al.* (1987). Pregnancy in lupus nephritis and related disorders. *Am. J. Kidney Dis.* **9**, 339–343.
151. Packham, D. K., Lam, S. S., Nicholls, K., *et al.* (1992). Lupus nephritis and pregnancy. *Q. J. Med.* **83**, 315–324.
152. Julkunen, H. (2001). Pregnancy and lupus nephritis. *Scand. J. Urol. Nephrol.* **35**, 319–327.
153. Jones, D. C., and Hayslett, J. P. (1996). Outcome of pregnancy in women with moderate or severe renal insufficiency. *N. Engl. J. Med.* **335**, 226–232.
154. Lehman, T. J., Sherry, D. D., Wagner-Weiner, L., *et al.* (1989). Intermittent intravenous cyclophosphamide for lupus nephritis. *J. Pediatr.* **114**, 1055–1060.
155. McCurdy, D. K., Lehman, T. J. A., Bernstein, B., *et al.* (1992). Lupus nephritis: Prognostic factors in children. *Pediatrics* **89**, 240–246.
156. Silverman, E. (1996). What's new in the treatment of pediatric SLE. *J. Rheumatol.* **23**, 1657–1660.
157. Niaudet, P. (2000). Treatment of lupus nephritis in children. *Pediatr. Nephrol.* **14**, 158–166.
158. Correia, P., Cameron, J. S., Ogg, C. S., *et al.* (1984). End-stage renal failure in systemic lupus erythematosus with nephritis. *Clin. Nephrol.* **22**, 293–302.
159. Jarrett, M. P., Santhanan, S., and Delgreco, F. (1983). The clinical course of end-stage renal disease in systemic lupus erythematosus. *Arch. Intern. Med.* **143**, 1353–1356.
160. Kimberly, R. P., Lockshin, M. D., Sherman, R. L., *et al.* (1981). "End-stage" lupus nephritis: Clinical course to and outcome on dialysis: experience in 39 patients. *Medicine* **60**, 277–287.
161. Nossent, J. C., Swaak, A. J. G., and Berden, J. H. M. (1990). Systemic lupus erythematosus: Analysis of disease activity in 55 patients with end stage renal failure treated with hemodialysis or continuous ambulatory peritoneal dialysis. *Am. J. Med.* **89**, 169–174.
162. Nossent, J. C., Swaak, A. J. G., and Berden, J. H. M. (1991). Systemic lupus erythematosus after renal transplantation: Patient and graft survival and disease activity. *Ann. Intern. Med.* **114**, 183–188.
163. Radhakrishnan, J., Williams, G. S., Appel, G. B., and Cohen, D. J. (1994). Renal transplantation in anticardiolipin antibody-positive lupus erythematosus patients. *Am. J. Kidney Dis.* **23**, 286–289.
164. Lochhead, K. M., Pirsch, J. D., D'Alessandro, A. M., *et al.* (1996). Risk factors for renal allograft loss in patients with systemic lupus erythematosus. *Kidney Int.* **49**, 512–517.
165. Stone, J. H., Amend, W. J., and Criswell, L. A. (1997). Outcome of renal transplantation in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **27**, 17–26.
166. Ward, M. M. (2000). Outcomes of renal transplantation among patients with end-stage renal disease caused by lupus nephritis. *Kidney Int.* **57**, 2136–2143.
167. Bartosh, S. M., Fine, R. N., and Sullivan, E. K. (2001). Outcome after renal transplantation of young patients with systemic lupus erythematosus: A report of the North American Pediatric Renal Transplant Cooperative Study. *Transplantation* **72**, 973–978.
168. Stone, J. H., Millward, C. L., Olson, J. L., *et al.* (1998). Frequency of recurrent lupus nephritis among ninety-seven renal transplant patients during the cyclosporine era. *Arthritis Rheum.* **41**, 678–686.
169. Brodsky, R. A., Petri, M., Smith, B. D., *et al.* (1998). Immunoablative high-dose cyclophosphamide without stem cell rescue for refractory, severe autoimmune disease. *Ann. Intern. Med.* **129**, 1031–1035.
170. Brodsky, R. A. (2002). High-dose cyclophosphamide for aplastic anemia and autoimmune diseases. *Curr. Opin. Oncol.* **14**, 143–146.
171. Traynor, A. E., Schroeder, J., Rosa, R. M., *et al.* (2000). Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haematopoietic stem-cell transplantation: A phase 1 study. *Lancet* **356**, 701–707.
172. Brunner, M., Greinix, H. T., Redlich, K., *et al.* (2002). Autologous blood stem cell transplantation in refractory systemic lupus erythematosus with severe pulmonary impairment. *Arthritis Rheum.* **46**, 1580–1584.
173. Furst, D. E. (2002). Stem cell transplantation for autoimmune disease: Progress and problems. *Curr. Opin. Rheumatol.* **14**, 220–224.
174. Marmont, A. M. (2001). Lupus: Tinkering with haematopoietic stem cells. *Lupus* **10**, 769–774.
175. Shaughnessy, P. J., Ririe, D. W., Ornstein, D. L., *et al.* (2001). Graft failure in a patient with systemic lupus erythematosus (SLE) treated with high-dose immunosuppression and autologous stem cell rescue. *Bone Marrow Transplantation* **27**, 221–224.

31

CARDIOVASCULAR SYSTEMIC LUPUS ERYTHEMATOSUS

Michelle Petri

The first recognition of cardiac involvement in lupus was a report by Kaposi in 1872 of cardiac irregularity and dyspnea [1]. The pathologic study of cardiac lupus dates from the report of Libman and Sacks [2] of verrucous endocarditis. Ironically, they did not recognize the association of verricous endocarditis with systemic lupus erythematosus (SLE). It is now understood that all three layers of the heart (pericardium, myocardium, and endocardium) can be involved by lupus.

Cardiac involvement in SLE is often forgotten, because it is not a common cause of flare. In our prospective study of flare, serositis was present in only 7 to 9% of flares [3]. However, the words of Brigden *et al*., written in 1960, remain true today: "Heart lesions develop in nearly all patients at some time during the course of their disease when life is prolonged by modern. . . . therapy" [4]. This chapter will review the cardiac manifestations of active lupus, but will also emphasize a growing source of morbidity and mortality in SLE patients, accelerated atherosclerosis.

CARDIAC MANIFESTATIONS OF ACTIVE SYSTEMIC LUPUS ERYTHEMATOSUS

Pericarditis

Clinical Features

Pericarditis is the most common cardiac manifestation of active lupus, although often it is not evident clinically. Although pericarditis can occur at any time during the course of SLE, it tends to be one of the earlier cardiac manifestations, and can even be the first manifestation of lupus [5]. Pericarditis was the presenting sign of lupus in 4 of 28 patients who ultimately developed it in one series [6]. Pericarditis in SLE presents in the typical way, with precordial pain, usually positional (aggravated by lying down), often with a pleuritic quality, and sometimes with dyspnea. Coexistent pleurisy and/or effusions are common, occurring in 14 of 28 cases in one series [6]. Pericarditis usually appears as an isolated attack or as recurrent episodes, with or without symptoms [4]. Patients may have fever and tachycardia. Friction rubs are rare, perhaps because they are present often for only a few hours and are missed. The "classic" pericardial friction rub has three components, occurring with ventricular contraction, atrial contraction, and at the end of rapid ventricular filling [7]. In a French series, of 28 cases of pericarditis, 23 had pain, 12 had a rub, and 4 required pericardiocentesis because of tamponade [6].

Patients with pericardial effusion (as opposed to thickening) are more likely to have pericardial pain and active lupus elsewhere [8, 9]. In one study, only the patients with moderate or severe pericardial effusion had clinical or electrocardiographic evidence of pericarditis [9]. When present, pericardial effusions are usually small and do not cause hemodynamic problems.

Pericardial tamponade has been reported, however, even in treated patients [5]. Earlier studies may have included SLE patients with uremia as the cause (or a contributing factor to) pericardial tamponade [7]. In the modern era, most pericardial effusions do not cause hemodynamic problems [8]. Pericardial hemorrhage (from vasculitis) caused fatal pericardial tamponade in one anticoagulated patient [10]. Constrictive

pericarditis is very rare [11]. Only four cases of constrictive pericarditis have been reported [12–14]. One 17-year-old male with constrictive pericarditis was admitted with ascites, without any prior history of pericarditis [11]. In two of the four cases constrictive pericarditis developed in spite of corticosteroid therapy [11, 14]. All four known cases have occurred in males [11].

Diagnosis

If a patient presents for the first time with pericarditis, it is usually impossible to invoke SLE as the cause until appropriate laboratory tests, suggesting the diagnosis, return. However, patients with idiopathic pericarditis more often give a history of recent viral infection, and are more often male. In idiopathic pericarditis there is usually a leukocytosis, whereas a finding of leukopenia would suggest SLE [7].

Pericardial friction rubs may be heard in sicker and untreated patients, but are often absent in milder cases, especially those patients already on corticosteroid and/or NSAID treatment. A significant rise in jugular venous pressure is unusual [4].

In one series, most patients showed electrocardiographic evidence of acute or chronic pericarditis [5]. The diagnosis of pericarditis can be confirmed by EKG findings of elevated ST segments and tall T waves (although slight T-wave changes or transient elevation of ST segments are most characteristic), or by cardiac echocardiogram findings of pericardial effusion or thickened pericardium. Serial electrocardiograms may show a progression of changes in pericarditis. Initially, a diffuse elevation of ST segments (without reciprocal ST segment depression) is found. This is followed by a lowering of ST segments back toward baseline and subsequent T-wave inversion. In most cases, T waves then return to normal [7]. In the days before echocardiograms, the diagnosis of pericarditis was based on the presence of a pericardial friction rub in 71%, diagnostic evolutionary EKG changes in 33%, and on evidence of pericardial effusion in 50% [12]. Effusions may be accompanied by a drop in voltage. After severe attacks T waves may not recover their original voltage [4]. In the series of Godeau *et al*., of 28 total cases, 5 had low voltage, 10 had ST changes, and 20 had repolarization changes [6].

Both effusion and thickening are frequent in echocardiogram studies [15, 16]. Most effusions are mild. In one study, of 19 patients with pericardial effusions, 5 were moderate or severe and 14 were mild [9]. The echocardiographic findings of pericardial thickening have been described [15], and diagnostic criteria exist [17]. Echocardiography (two-dimensional echocardiogram and Doppler echocardiography) is the modality of choice in evaluating pericardial disease, because it is both noninvasive and sensitive [18]. However, echocardiography may be an insensitive technique in diagnosing pericarditis when it is not accompanied by effusion or thickening. For example, in one study, only 42% of the patients with previous pericarditis had an abnormal echocardiogram [19].

Prevalence

The frequency of pericarditis depends on the modality of diagnosis. Published series of patients (Table 1) [4–6, 8, 12, 15, 16, 19–44] find pericarditis in 12–47% of living SLE patients. However, at any one time (cross-sectional analysis), only a minority of SLE patients, 3%, had a pericardial effusion [44]. In general, the echocardiogram is more sensitive than clinical diagnosis, with 19–54% of patients having pericardial effusion or thickening. The echocardiogram is an essential tool in the clinical management of sick patients with cardiac lupus, because clinical diagnosis alone may be faulty. For example, in one patient with a rapidly enlarging heart, pericardial friction rub, and paradoxic pulse, there was no pericardial effusion [35]. Pericardial abnormalities are the most common echocardiographic finding in SLE patients [8, 41]. However, significant pericardial disease is uncommon, even using echocardiograms, being found in only 7% in one study [9]. Autopsy studies find a much higher prevalence of pericardial involvement, ranging up to 61–100% (Table 1).

Pathology

Pericardial fluid in SLE is usually exudative, the amount of fluid varying from 100 to more than 1000 cc [5]. White blood counts are in the 30,000 range, primarily neutrophils [45]. Although not helpful in patient management, the fluid may contain anti-DNA and have low complement levels [46]. Hunder *et al*. found complement-fixing material in pericardial fluid in SLE, which was felt to be immune complexes [47].

At autopsy, a diffuse or focal fibrinous pericarditis, often with many hematoxylin bodies, with or without effusion, is found. In the series of Brigden *et al*., the layers of the pericardium were obliterated with occasional deposits of fresh fibrin or effusion [4]. In another autopsy study, of 11 cases, 6 had acute pericarditis and 5 had chronic obliterative pericarditis (2 of these had pericardiomediastinal adhesions) [22]. The histophathology in a case of constrictive pericarditis showed fibrosis and mild chronic inflammation, with IgG, IgM, and complement deposition on immunofluorescence [11]. Immunopathogenetic analyses of pericardium in 2 of 9 patients in an autopsy series

TABLE 1 Prevalence of Pericarditis in SLE

Study, year	No. of patients	Clinical ascertainment (%)	Echocardiography	Postmortem (%)
1940–1959				
Armas-Cruz *et al*., 1958 [20]	108	12		
Copeland *et al*., 1958 [21]	47	23.4		
	18			83
Griffith and Vural, 1951 [22]	18	17		61
Gross, 1940 [23]	23			61
Harvey *et al*., 1954 [24]	138	45.7		
Humphreys, 1948 [25]	21			42.9
Jessar *et al*., 1953 [26]	44	22.7		
	15			46.7
Shearn, 1959 [5]	83	31.3		62
	16			43.8
1960–1979				
Brigden *et al*., 1960 [4]	60	43		
	27			74
Bulkley and Roberts, 1975 [27]	36	30.6		53
Collins *et al*., 1978 [28]	17		0% effusion	
Dubois, 1976 [30]	520	31		
Dubois and Tuffanelli, 1964 [31]	520	31		
Elkayam *et al*., 1977 [32]	32	0	6.3% effusion 0% thickening	
Estes and Christian, 1971 [29]	150	19.3		
Grigor *et al*., 1978 [315]	50	20		
Hejtmancik *et al*., 1964 [12]	142	17		
Ito *et al*., 1979 [33]	48		46% effusion 0% thickening	
James *et al*., 1965 [34]	8	100		100
Kong *et al*., 1962 [35]	30	47	40	
Maniscalco *et al*., 1975 [16]	25		44% effusion 24% thickening	
Ropes, 1976 [36]	142	28.9		
	58			82.8
1980–present				
Badui *et al*., 1985 [37]	100	25	39%	
Bidani *et al*., 1980 [38]	10			60
Bomalaski *et al*., 1983 [39]	47		49% effusion	
Chia *et al*., 1981 [15]	21		24% effusion 29% thickening	
Crozier *et al*., 1990 [40]	50		54% effusion	
Doherty *et al*., 1988 [41]	50		42% effusion	
Godeau *et al*., 1981 [6]	103		27%	
Klinkhoff *et al*., 1985 [42]	47		9% effusion 13% thickening	
Leung *et al*., 1990 [8]	75		37%	
Nihoyannopoulos *et al*., 1990 [19]	96		21%	
Pistiner *et al*., 1991 [43]	464	12		
Sturfelt *et al*., 1992 [44]	75	35	19% effusion	
Rantapaa-Dahlqvist *et al*., 1997 [77]	50		40%	

demonstrated the vascular deposition of immunoglobulin and complement [38]. Pericardial lesions were present in an additional 4 patients in the autopsy series, as well [38]. On histopathology, small pericardial blood vessels were surrounded by an infiltrate of lymphocytes, plasma cells, macrophages, and rare polymorphonuclear leukocytes. On immunofluorescence, IgG was present in a predominantly granular pattern around small pericardial vessels [38]. Thus, Bidani and colleagues concluded that immune complex deposition was the cause of pericarditis [38].

Treatment

In early studies, pericarditis usually responded quickly to corticosteroids, with serial chest x-rays showing rapid and radiologic evidence of resorption of fluid [4]. Shearn commented in his review that the "often transient nature of pericarditis makes evaluation of therapy for this condition most difficult [5]. However, many studies have noted pericardial effusion developing or persisting even with corticosteroid treatment, such as the autopsy study of Kong *et al*., in which 11 of 12 patients with pericardial effusion had taken corticosteroids [35]. Occasional patients progressed to the point of tamponade [5]. Nonsteroidal anti-inflammatory drugs are helpful for mild cases of pericarditis. Patients presenting with pericardial tamponade may necessitate pericardiocentesis. Pericardiocentesis in one patient with pericardial tamponade punctured the left anterior descending coronary artery, resulting in death [35]. Refractory cases of large pericardial effusions may benefit from a pericardial window.

Myocarditis

Clinical Features

Myocarditis, as recognized clinically, is rare in SLE. The clinical detection of myocarditis ranges from 3 to 15%, although it appears to be much more common in autopsy studies, suggesting the largely subclinical nature of the myocardial pathology. Patients may present in florid congestive heart failure, or more subacutely with tachycardia and dyspnea.

Most myocarditis in SLE is subclinical. Myocardial abnormalities were found in 20% of patients using echocardiograms, but only one patient with an echocardiographic pattern of myocarditis developed myocardial dysfunction clinically [9].

Even autopsy studies have shown that myocarditis usually does not lead to cardiac dilatation [22]. Brigden *et al*. had no patient in whom congestive heart failure was attributed solely to myocarditis [4]. Shearn had only one patient with heart failure attributable to myocarditis [5].

However, other series have found myocarditis as a cause of congestive heart failure. Harvey *et al*. [24] found that myocarditis was the cause of heart failure in 8 of their 9 patients. Hejtmancik *et al*. found myocarditis to be the major cause in 6 of their 10 cases [12]. Kong *et al*. had 17 patients with cardiomegaly; at autopsy, 15 had myocarditis, but of varying degrees of severity [35].

The differential diagnosis of congestive heart failure in SLE would include myocarditis, but also anemia, renal failure, pulmonary disease, atherosclerotic heart disease, coronary arteritis, valvular disease, and hypertension.

Diagnosis

In an understatement, Shearn said, "The clinical recognition of myocarditis is difficult" [5]. In our most recent case, the patient, who had a hematologic and renal flare, was not recognized to have myocarditis as well until she presented in congestive heart failure. Myocarditis should be considered in patients with tachycardia not due to fever, in patients with a third heart sound (S3), in patients with abnormal EKGs, in those with new murmurs or conduction disturbances, and in those with congestive heart failure [5]. Brigden *et al*. suggested that prolongation of the conduction time of either P–R, QRS, or Q–T interval would have been evidence of myocarditis (in the absence of another cause of ventricular hypertrophy), but that they did not encounter these EKG changes in their series [4]. Hejtmancik *et al*. made a clinical diagnosis of myocarditis in 21% of their patients (after first excluding hypertension and coronary artery disease), based on (1) cardiac enlargement, (2) conspicuous ventricular gallop, and (3) electrocardiographic abnormalities [12]. Kong *et al*. found myocarditis at autopsy in 15 of their 16 patients with gallop rhythm [35]. The diagnosis of myocarditis can be supported by the finding of global hypokinesis on cardiac echocardiogram and confirmed by right ventricular endomyocardial biopsy [48, 49].

Prevalence

In large series of patients, the clinical diagnosis of myocarditis has been made in up to 21% (Table 2) [5, 6, 8, 9, 12, 19, 22, 24, 27, 29, 31, 34–38, 41, 44, 50, 51]. More recent series that relied on clinical detection suggest the frequency does not exceed 14% [6, 37]. Autopsy studies, mainly done in the 1950s and 1960s, frequently found myocarditis. More recent postmortem studies [27, 34,

TABLE 2 Prevalence of Myocarditis

Study, year (chronologic)	Number of patients	Clinical diagnosis (%)	Postmortem (%)	Echo (%)
Griffith and Vural, 1951 [22]	18		78	
Harvey *et al*., 1954 [24]	38		55	
Shearn, 1959 [5]	16		50	
Larson, 1961 [50]	52		15	
Kong *et al*., 1962 [35]	30		50	
Dubois and Tuffanelli, 1964 [31]	520	8		
Hejtmancik *et al*., 1964 [12]	16		81 (any) 38 (severe)	
	142	21		
James *et al*., 1965 [34]	8		12.5	
Estes and Christian, 1971 [29]	150	8		
Bulkley and Roberts, 1975 [27]	36		8	
Ropes, 1976 [36]	128	10		
Borenstein *et al*., 1978 [51]	140	3.6		
Bidani *et al*., 1980 [38]	10		0	
Godeau *et al*., 1981 [6]	103	14.5		
Badui *et al*., 1985 [37]	100	14		
Doherty *et al*., 1988 [41]	50			8
Leung *et al*., 1990 [8]	75			5
Nihoyannopoulos *et al*., 1990 [19]	93			1
Cervera *et al*., 1992 [9]	70			20
Sturfelt *et al*., 1992 [44]	75			12

38], reflecting the era of corticosteroid treatment, found much lower frequencies, from 0 to 8%.

Echocardiographic studies cannot definitively diagnose myocarditis, but global hypokinesis, in the absence of other known causes, is strongly suggestive. Large echo series have found frequencies of global hypokinesis between 5 and 20%. However, segmental areas of hyopkinesis on echocardiogram can also be indicative of myocarditis [52]. Newer imaging modalities, such as magnetic resonance, are largely unstudied [53].

Pathology

A common misperception is that myocarditis in SLE is a myositis. CPK levels are usually normal [52, 54]. In fact, only one study found any association with myositis elsewhere [51]. Myocarditis in SLE is a complicated process, with arteritis or arteriopathy, not primary disease of the myocardial fibers, playing a major role [55]. Kong *et al*. found pathologic evidence of myocarditis (fibrinoid and collagenous degeneration, interstitial edema, necrosis, and/or cellular infiltration) in 15 of 30 autopsies [35]. The cellular infiltrates of myocarditis consist of foci of interstitial plasma cells and lymphocytes [4]. Immunofluorescence studies confirm that the etiopathogenesis is vascular. In one study, most of the immune deposits were present in the walls of blood vessels of the myocardium [38]. Immunofluorescence studies of endomyocardial biopsies reveal perivascular deposits of IgG and vascular deposits of C3 [52].

Treatment

Myocarditis that comes to clinical attention is usually an urgent situation. Treatment with high-dose intravenous methylprednisolone (such as the "pulse" regimen, 1000 mg daily for 3 days), followed by high-dose IV or oral corticosteroid maintenance therapy, is indicated. The addition of intravenous "pulse" cyclophosphamide, in refractory cases, may be helpful. Supportive therapy for congestive heart failure, including diuresis, digoxin, and afterload reduction (such as with angiotensin converting enzyme (ACE) inhibitors) may be necessary. Anticoagulation should be considered in those patients who have progressed to the stage of cardiomyopathy. Efficacy of therapy can be assessed by serial echocardiographic studies or right ventricular endomyocardial biopsies [56]. In one patient, response

to corticosteroid therapy was confirmed by both endomyocardial biopsy and cardiac catheterization [52]. Nihoyannopoulos *et al*. followed one patient in whom corticosteroid treatment improved diffuse ventricular hypokinesis [19].

Left Ventricular Dysfunction

Clinical Features

Although ventricular dysfunction is commonly found in SLE patients, it is usually subclinical. For the clinician, ventricular dysfunction has a long differential diagnosis, including myocarditis, arteritis, hypertension, and atherosclerotic disease. Ventricular dysfunction is most likely to come to the attention of the clinician through a physical examination finding of gallop rhythm, new murmur, or congestive heart failure; a chest X-ray finding of cardiomegaly; EKG changes; or by an echocardiogram done for other purposes (valvular disease or pericarditis). However, the literature on left ventricular dysfunction is largely an echocardiographic one, emphasizing subclinical degrees of diastolic and/or systolic dysfunction.

Diagnosis

The diagnosis of subtle degrees of left ventricular diastolic or systolic dysfunction is made echocardiographically. Left ventricular systolic function can be evaluated by the ventricular ejection fraction. Diastolic function can be determined by the diastolic descent rate of the anterior mitral leaflet, the ratio of mean systolic velocity to mean diastolic velocity in the left ventricular posterior wall, and the ratio of peak mitral inflow velocity during the atrial filling period to that in the early filling period [57]. Routine M-mode echocardiography may miss the mild degree of dysfunction present in SLE, leading one group to use computer-assisted analysis of digitized echoes [58].

Prevalence

Echocardiographic studies (Table 3) [8, 12, 15, 16, 19, 27, 37, 40–42, 44, 59–62] show that 4 to 71% of SLE patients have some degree of left ventricular dysfunction. Some of the segmental abnormalities found may be due to past myocardial infarctions. Autopsy studies [12, 27] have also shown a large range of frequencies,

TABLE 3 Prevalence of Left Ventricular Dysfunction

Study, year	Modality	No. of patients	Finding	Frequency (%)
Autopsy				
Bulkley and Roberts, 1975 [27]	Autopsy	36	Congestive heart failure	43
Hejtmancik *et al*., 1964 [12]	Autopsy	16	Cardiomegaly	69
Echo				
Crozier *et al*., 1990 [40]	Echo	50	Systolic dysfunction	
Chia *et al*., 1981 [15]	Echo	21	Left ventricular dysfunction	71
Doherty *et al*., 1988 [41]	Echo	50	Global hypokinesis	8
			Segmental hypokinesis	16
			Left ventricular dilation	10
Giunta *et al*., 1993 [61]	Echo	75	Left ventricular diastolic dysfunction	31
Klinkhoff *et al*., 1985 [42]	Echo			
Leung *et al*., 1990 [8]	Echo	75	Left ventricular hypokinesis	5
			Segmental hypokinesis	4
Maniscalco *et al*., 1975 [16]	Echo	25	Depressed ejection fraction	28
Nihoyannopoulos *et al*., 1990 [19]	Echo	93	Global or segmental hypokinesis	5
Roldan *et al*., 1992 [62]	Echo	54	Left ventricular dysfunction	20
Sturfelt *et al*., 1992 [44]	Echo	75	Global hypokinesis	12
			Segmental hypokinesis	7
Other				
Badui *et al*., 1985 [37]	Noninvasive	100	Congestive heart failure	10
Bahl *et al*., 1992 [59]	Radionuclide ventriculography	20	Segmental hypokinesis	20
Hospenpud *et al*., 1984 [60]	Exercise thallium scintigraphy	26	Regional hypokinesis	38.5

with 11 or 43% of SLE patients having cardiomegaly or congestive heart failure.

Initially, it was thought that left ventricular systolic function was not affected or affected only mildly by SLE [44, 57, 58, 61], or, if there was a difference, that it was due to hypertension, coronary artery disease, or other comorbid processes [63]. However, SLE patients may have systolic dysfunction that only becomes apparent with exercise [59].

Diastolic dysfunction, although subclinical, is found more consistently [57, 61]. Giunta *et al*. found that disease duration was longer in patients with diastolic dysfunction [61]. Similarly, Enomoto *et al*. found that diastolic function deteriorated progressively with age [57]. In contrast, several groups have found that disease activity is a major determinant of diastolic dysfunction [8, 33, 58, 64].

Pathology

The coronary catheterization study of Strauer *et al*. [64] found multiple abnormalities in the five SLE patients studied, including: (1) increased end-diastolic pressures; (2) decreased contractility; (3) decreased left ventricular ejection fraction; (4) increased left ventricular stiffness; and (5) reduction of coronary vascular reserve. This suggests that multiple mechanisms contribute to ventricular dysfunction. There has been one case report of endomyocardial fibrosis presenting as congestive heart failure and restrictive cardiomyopathy [65].

Corticosteroid therapy could contribute to ventricular dysfunction through multiple mechanisms, including fatty infiltration [27, 51]. A second potential factor is hypertension aggravated by corticosteroids [66]. Deterioration in diastolic function over time [57, 63] could be due to these processes or to progressive atherosclerosis. Several studies [8, 40] have found abnormal left ventricular function in patients without coronary artery disease or hypertension, but in the setting of active disease, suggesting that the mechanism was lupus myocarditis.

Treatment

Because left ventricular dysfunction studies are primarily echocardiographic, treatment has not been adequately studied. However, ther is one report of ventricular function (measured echocardiographically) worsening with active lupus and improving with corticosteroid treatment [33]. However, the ventricular dysfunction that progressively worsens in inactive patients might be best addressed by aggressive risk factor (hypertension, hyperlidemia, smoking) modification and pharmacologic therapy [63].

Valvular Disease

Clinical Features

Verrucous endocarditis can affect valve leaflets, papillary muscles, and the mural endocardium, as initially described by Libman and Sacks [2]. However, Libman and Sacks [2] and Gross [23] found the tricuspid valve involved most often, whereas more recent studies have found the mitral valve (followed by aortic) to be most affected. In the corticosteroid era, valvular vegetations are found less frequently. Shearn found that none of 11 patients who received corticosteroids had verrucous endocarditis, but that 4 patients, who died before corticosteroid therapy was available, did [5]. In their landmark autopsy study, Bulkley and Roberts also commented on the rarity of vegetations in corticosteroid treated patients [27]. Occasionally, the presentation may be fulminant, with congestive heart failure due to mitral regurgitation [67], or brain emboli secondary to valvular vegetations.

Verrucous endocarditis (vegetations, "Libman-Sacks") affects the mitral valve most frequently, followed by the aortic valve. The presence of vegetations predisposes patients to bacterial endocarditis [4, 68]. Although verrucous endocarditis can produce both systolic and diastolic murmurs, these are rarely of sufficient hemodynamic importance to cause congestive heart failure.

There is virtually no correlation between the presence of verrucous endocarditis and cardiac murmurs. shearn found that systolic nurmurs occurred in 70% of SLE patients [5]. Most murmurs were low intensity, and were heard loudest (47%) at the apex. Because murmurs were also associated with fever, infection, tachycardia, and anemia, the differential diagnosis of a new murmur was complex. Systolic murmurs were heard in each of the four patients who were later found to have Libman-Sacks endocarditis, but the murmurs did not have any unique quality [5]. Griffith and Vural heard murmurs in only two of six patients with Libman-Sacks endocarditis, and, vice versa, found Libman-Sacks endocarditis in only two of seven patients with systolic murmurs [22].

Diastolic murmurs occur in only 4% of SLE patients [20, 26]. The differential diagnosis of diastolic murmurs in SLE includes rheumatic or congenital heart disease, bacterial endocarditis, Libman-Sacks endocarditis, and left ventricular dilatation. In general, even when the valvular vegetations of Libman-Sacks endocarditis are

large, they do not involve the line of closure, and therefore should not deform the valve. Even involvement of the chordae tendineae should not be sufficient to distort the valve. There are several documented cases, however, in which Libman-Sacks endocarditis appeared to be the only explanation for a diastolic murmur [2, 24, 69]. Two of the four patients with Libman-Sacks endocarditis in Shearn's series had a diastolic murmur suggestive of mitral stenosis. However, diastolic murmurs were also heard in two patients without Libman-Sacks endocarditis [5].

It is rare for valvular disease in SLE to be clinically significant. In a series of 421 patients, only 1 to 2% had significant morbidity or mortality. Of the 14 cases with available pathology, only 6 had evidence of SLE valvulopathy, either verrucous vegetations or valvulitis with necrosis and vasculitis [70].

Diagnosis

Transesophageal echocardiogram is the modality of choice in terms of sensitivity in detecting valvular disease due to either lupus or anti-phospholipid antibody syndrome [42, 62]. Most previous series used M-mode echocardiography or two-dimensional Doppler echocardiography and are not completely comparable. Patients with new murmurs or with valvular abnormalities on echocardiogram should have blood cultures to rule out bacterial endocarditis.

Prevalence

The prevalence of valvular disease in SLE is very high (Table 4) [8, 9, 12, 15, 16, 19, 27, 35, 37, 41, 44, 57, 61, 62, 71–76]. Valvular disease, for the most part, however, is mild and asymptomatic.

Pathology

Valvular disease occurs predominantly as vegetations (what was termed Libman-Sacks endocarditis in the past), or thickening (that can present as either a regurgitant or stenotic lesion). The mitral valve is affected most often, followed by the aortic valve. Mitral and aortic regurgitation are the most common findings, with stenotic lesions very rare. Aortic cup sclerosis has been identified as a common lesion [77].

The typical valvular and mural endocarditis lesions, which are verrucous, occur as a single vegetation or as mulberry-like clusters. When occurring on valves, the vegetations are often on the ventricular surface, near, but not distorting, the line of closure [78].

The original histologic description of Libman-Sacks endocarditis [23, 68] emphasized the multiplication of endothelial cells, proliferation of Anitschow myocytes, and infiltration of mononuclear cells in the valve ring and valve base, especially the valve pocket. Aggregations of hemosiderin were frequent, along with some fibrosis. Cells underwent karyolysis to form hematoxylin bodies. The mural endothelium was affected, especially near the mitral valve [4].

A more recent description of Libman-Sacks endocarditis was based on two patients who underwent aortic valve replacement. Histologic studies showed three distinct zones, an outer exudative layer of fibrin, nuclear debris, and hematoxylin-stained bodies; a middle organizing layer of proliferation of capillaries and fibroblasts, and an inner layer of neovascularization. Immunofluorescence showed immunoglobulin and complement deposition in the walls of small junctional vessels in the inner zone of neovascularization, suggesting that circulating immune complexes were critical in the development of the vegetations [78]. Bidani *et al*. found immunoglobulins and complement deposition in the valve stroma and vegetations in one patient with Libman-Sacks endocarditis [38].

It is not clear whether Libman-Sacks endocarditis evolves into the valvular thickening that is the second important form of SLE valvulopathy. In modern series, valvular thickening is found more commonly than vegetations (Table 4) [8, 44, 61]. Galve *et al*. found that patients with Libman-Sacks endocarditis were younger, had shorter disease duration, and had received less corticosteroid therapy than those with thickened valves [71]. The patients with valvular thickening were more likely to have stenotic or regurgitant lesions and to require valve replacement [71]. Some authors have expressed concern that corticosteroid treatment might increase the chance that a valve would develop thickening. Changes in valve thickening can occur over time, with valve thickening resolving or new valve thickening appearing [71, 79].

Studies are conflicting on the role that anti-phospholipid antibodies play in the development of the vegetations of Libman-Sacks endocarditis [80]. Valvulopathy is common in the primary anti-phospholipid antibody syndrome, usually found in about a third of patients in large series [62, 73, 76, 81–86]. Thrombus formation, usually on the mitral valve, can be massive and require valve replacement [87]. Mitral and/or aortic valve thrombus (or vegetations) can also be a precipitant of embolic strokes. In SLE patients, some series have shown significantly more valvulopathy in those with anti-cardiolipin antibody [9, 19, 61, 72, 82, 88, 89]. However, other equally large series have found no difference [62, 73, 75, 76, 90–94]. In patients with the secondary form of anti-phospholipid antibody syndrome and valvulopathy, there is deposition of

TABLE 4 Prevalence of Valvular Disease

Study, year	Modality	No. of patients	Finding	Frequency (%)
Kong *et al*., 1962 [35]	Autopsy	30		13
Hejtmancik *et al*., 1964 [12]	Autopsy	16		50
Bulkley and Roberts, 1975 [27]	Autopsy	36		50
Maniscalco *et al*., 1975 [16]	Echo	25	Mitral valve thickening	8
Chia *et al*., 1981 [15]	Echo	21		
Badui *et al*., 1985 [37]	Echo	100	Valvular disease	9
Doherty *et al*., 1988 [41]	Echo	50	Mitral valve thickening	12
Galve *et al*., 1988 [71]	Echo	74	Vegetations	9
			Thickening	8
Nihoyannopoulos *et al*., 1990 [19]	Echo	93	Valvular pathology	28
Khamashta *et al*., 1990 [72]	Echo	132		23
Leung *et al*., 1990 [8]	Echo	75	Thickening—gross	8
			Thickening—focal	12
			Mitral regurgitation	25
			Aortic regurgitation	8
Enomoto *et al*., 1991 [57]	Echo	43		93
Roldan *et al*., 1992 [62]	Echo	54		74
Cervera *et al*., 1992 [9]	Echo	70		44
Sturfelt *et al*., 1992 [44]	Echo	75	Valvular pathology	27
			Vegetations	4
			Thickening	4
			Mitral regurgitation	39
			Aortic regurgitation	13
			Tricuspid regurgitation	87
Giunta *et al*., 1993 [61]	Echo	75	Vegetations	4
			Thickening	12
Gleason *et al*., 1993 [73]	Echo	20		40
Metz *et al*., 1994 [74]	Echo	52		33
Meyer *et al*., 1995 [75]	Echo	92		10
Gabrielli *et al*., 1995 [76]	Echo	39		38
Roldan *et al*., 1996 [79]	Echo	69	Thickening	51
			Vegetations	43
			Regurgitation	25
			Stenosis	4

immunoglobulin and complement, but in addition there is binding of anticardiolipin antibody [95].

Fenfluramine/Phentermine

Some non-SLE patients with obesity, treated with the appetite suppressant drugs fenfluramine/phentermine in combination [96], fenfluramine alone [97], and dexfenfluramine alone [98], have developed valvulopathy. In the series of Connolly *et al*., 24 women developed valvular disease after an average 12 months of therapy, but one after 1 month of therapy [96]. Mitral, aortic, and tricuspid valves were affected. Eight of the patients also had developed pulmonary hypertension. In the patients who underwent valve replacement, a plaque-like substance of proliferating myofibroblasts in an exuberant extracellular matrix encasing the leaflets and choidal structures, resembling carcinoid or ergotamine valvular disease, was found [96]. Whether SLE patients exposed to appetite suppressant drugs have developed valvular heart disease is unknown. Fenfluramine and dexfenfluramine were withdrawn from the market by the FDA in September, 1997.

Treatment

Systemic lupus erythematosus patients with large, sterile vegetations should be anticoagulated to lessen

embolic complications. We have used high-dose corticosteroids for 4 to 6 weeks to shrink vegetations, but this approach is controversial [80]. Some studies have suggested that corticosteroid treatment may contribute to ultimate valve thickening, but this is unproven.

Arrythmias and Conduction Disturbances

Clinical Features

The strongest association of SLE with conduction disturbance is congenital heart block, usually in the setting of maternal anti-Ro and anti-La [99, 100]. Both myocarditis [101] and cardiomyopathy [102] can occur in addition to fetal congenital heart block. The most common cardiac arrhythmia in adult SLE is sinus tachycardia (Table 5) [12, 22, 25, 32, 37, 103, 104].

Diagnosis

The diagnosis of arrhythmias is likely to be biased in that patients who have active SLE and who are hospitalized are more likely to have electrocardiograms performed. Accurate ascertainment of arrhythmias would likely require continuous electrocardiographic monitoring. Arrhythmias are found more commonly in SLE patients with pericarditis and myocarditis.

Prevalence

Approximately 10% of adult SLE patients have conduction disturbances [6, 37, 103]. There are only a few cases of adult SLE complete heart block with anti-Ro and/or anti-La [105–107].

One of the most common arrhythmias is sinus tachycardia, found in 6 to 100% of patients (Table 5) [29]. One group has suggested that sinus tachycardia is a manifestation of active disease [108].

Pathology

Autopsy studies have found arteritis of the sinus node, vascular occlusion, vasculopathy [34], and fibroblastic replacement of the sinoarterial and atrioventricular nodes [107].

Treatment

Systemic lupus erythematosus patients with life-threatening conduction defects can be treated with permanent pacemakers [106].

TABLE 5 Frequency of Sinus Tachycardia

Study, year	Frequency (%)
Humphreys, 1948 [25]	100
Griffith and Vural, 1951 [22]	100
Hejtmancik *et al.*, 1964 [12]	50
Okado and Shiokawa, 1975 [103]	28
Elkayam *et al.*, 1977 [32]	6
Del Rio *et al.*, 1978 [104]	20
Badui *et al.*, 1985 [37]	11

Coronary Arteritis

Clinical Features

Coronary arteritis is extremely rare in SLE. In some cases, it has been found at autopsy, with no clinical correlate during life. The most common clinical presentation is angina and/or myocardial infarction, in a child or young adult who does not have a long history of corticosteroid therapy. There is no clear correlation with extracardiac disease activity, although it has been present in some cases [109–112]. Three of eight SLE patients who had a coronary artery aneurysm had no physical or laboratory evidence of active SLE [111]. Aortic aneurysms can also occur in SLE [113].

Diagnosis

It is often difficult to distinguish coronary arteritis from accelerated atherosclerosis. Serial coronary angiography has been proposed as the most useful diagnostic modality [109, 114]. Arteritis is suggested when coronary aneurysms are found, if there are smooth focal lesions, or if there are rapidly developing stenoses [109, 114]. However, Wilson *et al.* described a patient with rapidly progressive coronary artery occlusions in whom only advanced atherosclerosis was found at autopsy [111]. Thrombosis or spasm can further confuse the interpretation of coronary angiograms [115].

Prevalence

There are few studies that allow any estimate of the prevalence of coronary arteritis (Table 6) [4, 12, 35, 55, 109–112, 114, 116–122]. In one study in the 1960s, 6 of 16 patients were found to have arteritis at autopsy [12]. Otherwise, case series do not have more than 3 patients. The cases identified have a predilection for pediatric patients or very young adults, with rare exceptions [123]. Unfortunately, where follow-up is given, the outcome is

TABLE 6 Coronary Arteritis

Case report or series	No. of patients	Age at diagnosis of arteritis
Keat and Shore, 1958 [117]	1	14
Brigden *et al*., 1960 [4]	2	
Kong *et al*., 1962 [35]	1	
Hejtmancik *et al*., 1964 [12]	6	
Bor, 1968 [112]	1	10 boy
Bonfiglio *et al*., 1972 [110]	1	16 girl
Simon *et al*., 1973 [55]	1	25 man
Benisch and Pervez, 1974 [120]	1	
Heibel *et al*., 1976 [114]	1	45
Homcy *et al*., 1982 [109]	3	27, 34, 21
Englund and Lucas, 1983 [122]	1	14 boy
Korbet *et al*., 1984 [118]	1	26
Wallace and Dubois, 1987 [116]	1	35
Wilson *et al*., 1992 [111]	1	25
Koh *et al*., 1998 [316]	1	22 woman
Dhond *et al*., 1999 [317]	1	
Matayoshi *et al*., 1999 [318]	1	29 woman
Uchida *et al*., 2001 [123]	1	55 man

usually death. This is quite paradoxical, in that autopsy studies indicated that arteritis rarely resulted in myocardial infarction.

Pathology

Histopathology demonstrates transmural vasculitis [110] with both lymphocytic and neutrophilic infiltration of a thrombus in the case described by Homcy *et al*. [109]. Immunofluorescence studies demonstrate immunoglobulin and complement deposition in coronary arteritis [118].

Treatment

The differentiation of coronary arteritis from atherosclerosis is essential for appropriate management. Coronary artery bypass surgery, angioplasty, or stent placement would be considered in patients with severe atherosclerotic disease, but would be contraindicated in patients with coronary arteritis. Case reports suggest that corticosteroid therapy can have rapid benefit in patients with coronary arteritis. Patient 4 in the series of Homcy *et al*. had refractory angina with arteritis suggested on serial coronary angiograms [109]. Corticosteroid therapy resulted in relief of angina and angiographic improvement [109]. Not all patients with coronary arteritis do as well on corticosteroids, however. Heibel *et al*. describe a patient with coronary arteritis who was treated with prednisone and cyclophosphamide, but had new myocardial damage after starting therapy [114]. Angina did not resolve for 3 weeks [114]. A 16-year-old girl, treated with 120 mg of prednisone, died of cardiac arrest, and had fibrinoid necrosis of extramural coronary arteries [110].

Pulmonary Hypertension

Clinical Features

Pulmonary hypertension is note frequent in SLE patients, in contrast to mixed connective tissue disease and scleroderma, particularly limited scleroderma ("CREST"). It is usually asymptomatic, discovered on a screening echo Doppler. Rare SLE patients will present with chest pain, dyspnea, or even pedal edema and be found to have pulmonary hypertension.

In one series of SLE patients, those with pulmonary hypertension by Doppler (14% of the group) had a shorter duration of SLE and corticosteroid therapy and a higher prevalence of Raynaud's phenomenon [124]. A 5-year follow-up study of these patients documented a gradual worsening, with 45% having pulmonary hypertension by Doppler [125].

Diagnosis

Diagnosis is best made by Doppler echocardiography, which can estimate right ventricular systolic pressure if the patient has a small amount of tricuspid regurgitation. Doppler echocardiography has a close correlation with simultaneous right heart catheterization measurement of pulmonary artery pressures. Owing to its invasive nature, right heart catheterization is more appropriately reserved for symptomatic patients, and to guide therapy. Ventilation perfusion scans, to rule out the possibility of multiple pulmonary emboli, are important.

Prevalence

The advent of echo Doppler allowed for sensitive, noninvasive screening for pulmonary hypertension, usually using a cutoff of right ventricular systolic pressure (RVSP) of 30 mm Hg or more (Table 7) [4, 8, 12, 37, 40, 44, 124, 126, 127]. Earlier studies, that determined the prevalence clinically, found a cumulative frequency of only 2–9%. These studies would only be able to detect late clinical manifestations of pulmonary hypertension and would underestimate the true prevalence.

TABLE 7 Prevalence of Pulmonary Hypertension

Study, year	Descriptor	No. of patients	Frequency (%)
Brigden *et al*., 1960 [4]	Clinical detection	60	3
Hejtmancik *et al*., 1964 [12]	Clinical detection	142	1
Perez and Kramer, 1981 [126]	Clinical detection	43	9
Quismorio *et al*., 1984 [127]	Clinical detection	400	1
Badui *et al*., [37]	Noninvasive studies	100	9
Simonson *et al*., 1989 [124]	Echo (RVSP >30 mm Hg)	36	14
Crozier *et al*., 1990 [40]	Echocardiography	50	2
Leung *et al*., 1990 [8]	Echocardiography	75	1
Sturfelt *et al*., 1992 [44]	Echo (RVSP >30 mm Hg)	68	16

Pathology

The cardiac indices in the patients in the series of Simonson *et al*. were low, suggesting increased pulmonary vascular resistance as the cause [124]. Several lines of evidence suggest that pulmonary hypertension may be a complication of pulmonary artery vasospasm. Raynaud's phenomenon is more common in SLE patients with pulmonary hypertension [126, 128]. Primary pulmonary hypertension occurs in patients with Raynaud's phenomenon [129–132]. Cold-induced pulmonary artery spasm has been found in some scleroderma patients (with Raynaud's) [133, 134]. However, other mechanisms may operate as well, including pulmonary vasculitis [135], anti-phospholipid antibody syndrome [128], and interstitial lung disease [126].

Treatment

Effective treatment is now available for severe pulmonary hypertension with the advent of continuous intravenous prostacyclin and its analogs [136]. The endothelin-receptor antagonist bosentan is effective for pulmonary hypertension secondary to connective tissue disease [137]. Patients with severe pulmonary hypertension should also be anticoagulated [138].

Fenfluramine/Phentermine

Because morbid obesity is common in SLE patients who require chronic corticosteroid therapy, some were exposed to appetite suppressant therapy. The association of pulmonary hypertension with appetite suppressant drugs dates to the use of the drug aminorex in Europe in 1967 to 1972 [139]. A second outbreak of pulmonary hypertension occurred in association with dexfenfluramine (Redux) and fenfluramine (Pondimin) [140]. The odds ratio for pulmonary hypertension for just 3 months use of these drugs was 2.3 [141]. The combination of appetite suppressant drugs used most in the United States, fenfluramine and phentermine, has also commonly been associated with pulmonary hypertension with short-term use [142]. In animals, these drugs cause dose-related increases in pulmonary artery pressure [143], perhaps by inhibition of potassium current in vascular smooth muscle cells or the vasoconstrictor action of serotonin [143]. The extent to which morbidly obese SLE patients have been exposed to these drugs, and whether pulmonary hypertension has newly evolved or worsened in them, is not known. Dexfenfluramine and fenfluramine were removed from the U.S. market in September, 1997.

TABLE 8 Prevalence of Hypertension

Study, year	Frequency (%)
Griffith and Vural, 1951 [22]	24
Harvey *et al*., 1954 [24]	14
Shearn, 1959 [5]	32
Brigden *et al*., 1960 [4]	44
Kong *et al*., 1962 [35]	53
Hejtmancik *et al*., 1964 [12]	22
Okado and Shiokawa, 1975 [103]	44
Budman and Steinberg, 1976 [144]	45
Doherty *et al*., 1988 [41]	50
Crozier *et al*., 1990 [40]	14
Schieppati and Remuzzi, 1993 [145]	40

Hypertension

Clinical Features

Hypertension was not a major feature of early series of SLE patients (Table 8) [4, 12, 22, 24, 35, 40, 41, 103, 144, 145]. Today, however, it is a major clinical challenge.

Several studies found that hypertension was more common in those with underlying lupus nephropathy [5]. All patients with hypertension in the series of Armas-Cruz *et al*. [20] and 86% of those in the series of Estes and Christian [29] had lupus nephritis. Pollack and Kant found a correlation of mean diastolic blood pressure and increasing renal damage [146].

Budman and Steinberg, however, found that hypertension can occur in SLE in the absence of important renal disease [144]. Approximately two-thirds of their hypertensive patients had creatinine clearances greater than 60 cc/min and nonnephrotic range proteinuria [144]. Others have found an association with corticosteroids [147], and, specifically, a worsening of hypertension by corticosteroids [4, 5]. Hypertension is especially likely to develop or worsen when patients with nephropathy are given corticosteroids [148]. Kaplan and colleagues reported an inverse correlation of hypertension and rheumatoid-like arthritis [149].

We examined the relationship of prednisone and blood pressure using the Hopkins Lupus Cohort database, in which patients are seen on a quarterly basis [150]. Using regression methods appropriate for longitudinal data, we found that an increase in prednisone dose of 10 mg led to an increase in mean arterial pressure, adjusting for all other factors that affect blood pressure [150].

In the Hopkins Lupus Cohort database, hypertension is an independent predictor of coronary artery disease [151] and poor renal outcome [152]. In addition, it is one of the strongest predictors of preterm birth in lupus pregnancy [153].

Prevalence

Although earlier studies, perhaps because of shortened survival, did not find a high prevalence of hypertension, more recent studies have found a high frequency (Table 8), up to 50%.

Treatment

Consensus has been reached on the treatment of hypertension in the general population [154], emphasizing that the previous cutoff of 140/90 does not identify all patients who truly have hypertension. In the future, treatment may be offered sooner to those with lower levels of hypertension.

Because many hypertensive patients with SLE will have underlying renal disease, an important long-term benefit of ACE-inhibitors may be the lessening of renal scarring reported in diabetic nephropathy [155]. ACE-inhibitors are usually well tolerated in SLE, although an occasional patient may develop an ACE-inhibitor-induced chronic cough. Patients unable to tolerate ACE-inhibitors are candidates for angiotensin receptor antagonists. More recently, in the general population an angiotensin receptor antagonist, losartan, was found to prevent more cardiovascular morbidity and mortality than a beta-blocker for a similar reduction in blood pressure [156].

Coronary Atherosclerosis in SLE

Cliniclal Features

Coronary atherosclerosis ("premature" or "accelerated" atherosclerosis) is a clinical conundrum of the modern era of lupus management. Patients present, usually starting in their early 40's, with angina, myocardial infarction, or sudden death. However, patients have presented in their early 20's with coronary atherosclerosis [157]. In observational cohorts, the prevalence is 6 to 12%, but up to 40% are affected if more sensitive testing is done in asymptomatic patients [158, 159]. The risk of myocardial infarction in young women with SLE is increased 52-fold [160]. Young SLE women are 2.27 times more likely to be hospitalized for myocardial infarction, 3.80 times more likely for congestive heart failure, and 2.05 times more likely for stroke, than non-SLE women [161]. The differential diagnosis includes coronary arteritis, thrombosis secondary to the anti-phospholipid antibody syndrome, or coronary vasospasm.

Diagnosis

The antemortum diagnosis of coronary artery disease is an example of the potential utility of high technology [162], noninvasive testing, but the lack of wide screening of asymptomatic SLE patients is due to the high costs. Coronary angiography remains the gold standard, but would not be appropriate for screening. Exercise EKGs lack both sensitivity and specificity [163–166]. In the carotid distribution, extensive studies have been done using carotid duplex [167–169]. In these prevalence studies, the prevalence of plaque has varied from 15 to 40%. Risk factors for plaques have included, in one study, age, duration of lupus, blood pressure, LDL, and prednisone treatment [167]. In a second study, risk factors for carotid intima media thickness included increased oxLDL, anti-oxLDL, triglycerides, lipoprotein(a), decreased HDL, increased homocysteine and the lupus anticoagulant [169].

Rest and perfusion myocardial SPECT scans are currently one of the most sensitive and specific means of assessing the presence of atherosclerosis, but they are expensive [170–173]. Using dual isotope myocardial

perfusion imaging (DIMPI), one study found that 40% of all women with SLE and 35% of SLE women with no cardiovascular events had abnormal scans. The perfusion defect was reversible in 90% [174].

In a single photon emission computed tomography (SPECT) study comparing SLE patients who were symptomatic vs asymptomatic for cardiovascular disease, 27 of the 33 symptomatic SLE patients had abnormal scans, but also 12 of 28 asymptomatic patients [175]. Using TI-201 perfusion tomoscintigraphy, with exercise, 11 of 15 SLE patients had transient perfusion abnormalities, but only one out of three had changes consistent with atherosclerosis. This suggests that disorders of the microcirculation occur in addition to atherosclerosis of major coronary arteries [176]. More recently, prevalence studies have been done using electron beam or helical computerized tomography to detect coronary calcification.

The very earliest stage, vascular stiffness, has also been examined. Using pulse wave velocity in premenopausal SLE patients, elevated C3, elevated anti-DNA, leukopenia, hypertension, and carotid plaque were found to be predictors [177]. Using high-resolution brachial ultrasound, less endothelium-dependent dilation was found in SLE patients than in controls [178].

Prevalence

Myocardial infarction was not common in the early autopsy series [35], but was a major feature of the Bulkley and Roberts [27] and subsequent autopsy series [179], especially those from Toronto [180, 181]. Prospective studies, from disparate populations, have found, using the clinical detection of angina and/or myocardial infarction, frequencies on the order of 7 to 9% (Table 9) [6, 8, 12, 22, 27, 35, 37, 38, 44, 60, 151, 179–186]. Cross-sectional or retrospective studies using more sensitive screening studies (such as nuclear medicine myocardial scans), have found reversible (exercise-induced) ischemia in 11 to 23% [44, 60], suggesting that this is a more realistic estimate.

Pathogenesis

Immune Complex or Arteritis

Although coronary arteritis is rarely detected antemortum, autopsy studies have detected it frequently (Table 9) [22, 27, 35, 38, 179–181]. In fact, both arteritis and atherosclerosis have been found within a single patient, suggesting that arteritis might have predisposed to the later development of atherosclerosis [12]. Animal models also suggest that this contributes to atherosclerosis. Vascular injury, through immune complexes, followed by exposure to atherosclerotic risk factors, can lead to atherosclerosis in animal models [187–190]. Immunization of rabbits with heat-shock protein 60/65 leads to aortic intima atherosclerosis [191]. Coronary vasculopathy and myocardial infarctions are found in murine lupus models, often in association with anti-cardiolipin antibody [192–196]. Immune complexes from lupus sera accelerated uptake of cholesterol by smooth muscle cells [197]. One small study in human SLE suggested that patients treated with corticosteroids had less intimal proliferation in their coronary vessels, suggesting that suppression of arteritis initially might lead to less atherosclerosis later [198].

Anti-phospholipid Antibodies

Anti-phospholipid antibodies could contribute to coronary artery disease through thrombosis [199] or vasculopathy [200]. The association of anti-phospholipid antibodies with coronary artery disease has been shown in some [201] but not all studies (Table 10) [201–212]. We have found that the lupus anticoagulant is associated with angina/myocardial infarction [168], but not carotid plaque. Lupus anticoagulant was increased in SLE cases with cardiovascular disease vs those without [169].

Anti-phospholipid antibodies may function also as antibodies against oxidized lipoproteins [213], an additional mechanism (the "oxidative modification hypothesis") [214] by which they might contribute to atherosclerosis. In one study, anti-oxLDL was higher in SLE cases with cardiovascular disease [169]. However, several studies have failed to find an association of anti-oxLDL with arterial thrombosis [215], arterial disease [216], or atherosclerosis [217].

Finally, one of the plasma protein targets of anti-phospholipid antibodies, β_2 glycoprotein I, may be an important control against atherosclerosis that is perturbed by anti-phospholipid antibodies [218]. Anti-β_2 glycoprotein I accelerates uptake of oxLDL *in vitro* [219]. There is also interest in lysophosphatidycholine, LPC, a high-affinity ligand for G2A, a lymphocyte-expressed protein-coupled receptor. Genetic deletion of the receptor results in autoimmunity [220]. LPC is reduced in SLE [221] and anti-LPC has been detected [222].

Chronic Infection

In the non-SLE patient, there is great interest in chronic infections, including *Chlamydia pneumoniae*, as potential causes of atherosclerosis [223–233]. Simple antibiotic regimens could conceivably eliminate these infections and reduce later coronary artery disease. Whether these chronic infections lead to accelerated atherosclerosis in SLE is currently unknown.

TABLE 9 Coronary Artery Disease in SLE

Study, year	Total patients	Design	Modality	Frequency (%)
Griffith and Vural, 195 [22]	11	Autopsy		4.5
Kong *et al*., 1962 [35]	30	Autopsy	Myocardial infarction	7
Hejtmancik *et al.*, 1964 [12]	142	Retrospective	Angina in 6 patients, EKG changes in 4	4.9
Bulkely and Roberts, 1975 [27]	36	Autopsy	>50% Narrowing	22
	36		Myocardial infarction	11
Urowitz *et al.*, 1976 [184]	81	Prospective	Angina, myocardial infarction	7.4
Bidani *et al*., 1980 [38]	10	Autopsy		10
Godeau *et al*., 1981 [6]	103	Prospective		
Haider and Roberts, 1981 [179]	22	Autopsy	>75% Narrowing	45
Hosenpud *et al*., 1984 [60]	26	Cross-sectional	Thallium myocardial scans	
			Persistent defects	19
			Reversible defects	23
Badui *et al*., 1985 [37]	100	Cross-sectional		16
Rubuin *et al*., 1985 [181]	51	Autopsy		41
Gladman and Urowitz, 1987 [185]	507	Prospective	Angina, myocardial infarction	8.9
Jonssen *et al*., 1989 [186]	86	Prospective	Angina, myocardial infarction	19.8
Shome *et al*., 1989 [182]	65	Retrospective		6.1
Leung *et al*., 1990 [8]	75	Cross-sectional	Echocardiogram, segmental wall motion	4
Petri *et al*., 1992 [151]	229	Prospective	Angina, myocardial infarction	8.3
Sturfelt *et al*., 1992 [44]	75	Retrospective	Myocardial infarction	9
	75		Exercise-induced ischemia	11
Abu-Shakra *et al*., 1995 [180]	40	Autopsy		54
Hearth-Holmes *et al*., 1995 [183]	89	Retrospective	Angina or myocardial infarction	6

Coronary Artery Disease Risk Factors

Multiple studies have now proven that the risk of coronary artery disease (CAD) in SLE cannot be solely explained by traditional cardiovascular risk factors. After controlling for common risk factors at baseline, SLE patients have a relative risk of 10.1 for nonfatal MI [234]. In a study with control subjects matched for traditional cardiovascular risk factors, SLE patients had more carotid atherosclerosis (41 vs 9%) and left ventricular hypertrophy (32 vs 5%) [235]. On average, SLE patients with coronary artery disease have one less traditional cardiovascular risk factor than a control patient [236].

Although traditional risk factors cannot explain all atherosclerosis in SLE, they contribute significantly to the process. Our work has found that routine coronary artery disease risk factors are very frequent in SLE patients (Table 11). In fact, the average SLE patient in our cohort study has 3 or more of these routine CAD risk factors [237].

Some of these risk factors could be due to SLE. Hypertension, for example, is more prevalent in SLE patients with renal disease [20, 29]. Hypertension is associated with coronary artery disease in SLE [150], including some [150], but not all, multivariate analyses [238].

Hyperlipidemia in SLE has two major patterns. One pattern occurs in active disease, especially in pediatric patients. These patients have low HDL cholesterol and apoprotein A1 with elevated VLDL cholesterol and triglyceride levels [239]. Lahita and colleagues have found a similar dyslipoproteinemia in SLE patients with anti-cardiolipin antibody [240]. However, one group has found that disease activity, rather than anti-cardiolipin, explains the reduction in HDL [241]. There are likely defects in early cholesterol transport [242] and VLDL metabolism [241] associated with active SLE. The second pattern occurs in SLE patients on corticosteroids, with higher levels of triglyceride, cholesterol, and LDL cholesterol [243]. Sustained hypercholesterolemia, rather than baseline, or intermittent elevation, is the most important predictor [244]. A third problem identified is decreased lipolysis and chylomicron remnant removal [245].

TABLE 10 Frequency of Anti-phospholipid Antibodies in Atherosclerotic Coronary Artery Disease[a]

Study, year	Patient group	aCL patients (%)	Controls	Natural history
Hamsten *et al*., 1986 [201]	62 MI <45 years	21		Increase in thrombotic events
Morton *et al*., 1986 [202]	83 CABG, late graft occlusion	20		
Gavaghan *et al*., 1987 [210] (correction of Morton)	83	19		Increase in late occlusions
Klemp *et al*., 1988 [212]	86 Coronary artery disease	80		
De Caterina *et al*., 1990 [203]	104 Coronary artery disease	42	51	
Cortelaro *et al*., 1992 [204]	62 MI <45 years	3.2 6.5		
Sletens *et al*., 1992 [205]	597	6.2		
Bick, 1993 [211]	43 <50 years 40 CABG failure	9 15		
Edwards *et al*., 1993 [206]	159 MI 90 Angina 38 <45 years, 21 MI, 17 angina	3.1 4.4 0	2.7	
Phadke *et al*., 1993 [207]	299 MI	6.8	4.2	
Raghavan *et al*., 1993 [209]	111 MI	18		No increase in complications
Yilmaz *et al*., 1994 [208]	76	47	0	

[a] This table is modified from: Petri, M. (1996). Epidemiology of the antiphospholipid antibody syndrome. In "The Antiphospholipid Syndrome" (R. A. Asherson, R. Cervera, S. E. Pietto, V. Shoenfeld, Eds.), p. 20. CRC Press, Boca Raton, Florida.

TABLE 11 Prevalence of CAD Risk Factors

Risk factor	Frequency (%)
Family history	41
Hypertension	48
Hypercholesterolemia	56
Obesity (major)	38
Smoking—ever	56
Smoking—current	35
Sedentary lifestyle	70
Diabetes	7
Homocysteine	15

Lipoprotein(a) has been identified as a risk factor for atherosclerosis in SLE [168, 169]. SLE patients can make anti-lp(a) [246]. Lp(a) may rise with disease flares and be reduced by corticosteroids [247]. SLE patients with higher lp(a) levels also have more immune complexes containing β_2 glycoprotein I [248].

The major cause of death in lupus nephritis patients is cardiovascular disease [249, 250]. Traditional cardiovascular risk factors, especially hypertension and hyperlipidemia, are increased [251]. Tubulointerstitial lipid deposits can be found [252]. In juvenile-onset SLE, nephrotic range proteinuria is the strongest risk factor for atherosclerosis [253].

In our longitudinal study, we found that three CAD risk factors, weight, cholesterol, and mean arterial pressure, were worsened by prednisone therapy. In the regression model, a 10-mg increase in prednisone led to a 5.5-lb increase in weight and a 8.9-mg% increase in total cholesterol, adjusting for all other factors known to affect these risk factors. Thus, even if the development of these CAD risk factors is directly due to SLE, prednisone treatment increases their levels.

Our more recent work has focused on a newly identified risk factor for cardiovascular disease, homocysteine. Homocysteine is an amino acid that has a direct toxic effect on endothelium [254] and indirect effects, including induction of a vascular endothelial cell activator [255], promotion of vascular smooth muscle proliferation, and an inhibitory effect on endothelial cell growth [256].

Hyperhomocysteinemia has been shown to increase the risk of coronary artery disease, stroke, and carotid artery stenosis in the Physicians' Health Study [257] and the Framingham Heart Study [258]. In the Hopkins Lupus Cohort Study, 15% of the 337 SLE patients had elevated homocysteine. Raised homocysteine levels were significantly associated with stroke and arterial thrombosis [259]. A retrospective study has comfirmed this association [260].

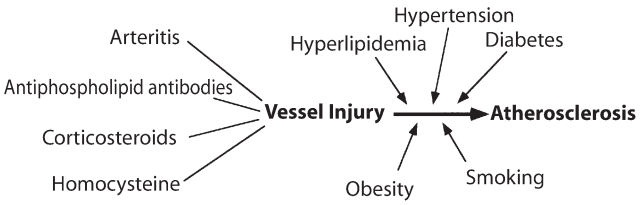


FIGURE 1 Legend—Atherosclerosis precipitation via vascular injury.

Inflammation plays a major role in the pathogenesis of atherosclerosis and myocardial infarction in the general population. The benefit of statins, for example, is mediated not just by their lipid-lowering effect, but by an anti-inflammatory effect (demonstrated by reduction in C-reactive protein) that likely stabilizes plaques. In the general population, C-reactive protein, IL-6, fibrinogen, and other markers of inflammation are predictive of atherosclerosis [261]. In SLE, C-reactive protein was not predictive in a multivariate model [167] nor in univariate analyses in our lupus cohort. However, in one case-control study, C-reactive protein was increased in SLE cases vs SLE controls [169]. We did not find fibrinogen to be predictive of atherosclerosis. In one study, fibrinogen increased with duration of disease regardless of disease activity, and was increased in SLE patients with thrombosis [262].

Prolonged corticosteroid therapy could precipitate atherosclerosis indirectly, by increasing the levels of CAD risk factors (hypertension, hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, obesity, and homocysteinemia) [150, 179, 259] or directly, via vascular injury (Fig. 1). Accelerated atherosclerosis has occurred in SLE patients without corticosteroid use, but such cases are very rare [263]. In a 1986 review, Nashel made a strong case that the latter occurs [264]. In animals, corticosteroids and ACTH produce vascular injury [265, 266], alter vascular connective tissue [267], and worsen experimentally induced atherosclerosis [268]. Patients with Cushing's syndrome, before effective treatment was available, commonly developed accelerated atherosclerosis [269].

Treatment

Primary prevention of CAD is the goal, although there are no long-term studies proving that reduction of CAD risk factors reduces CAD in SLE. Most of the CAD risk factors increased by prednisone are amenable to lifestyle or pharmacologic therapy. However, a more recent study found that, other than hypertension, management of traditional cardiovascular risk factors was suboptimal [270].

Obestiy

Obesity is the most common and costly nutritional problem in the United States [271]. Over 38% of SLE patients have morbid obesity. There has been a 30% increase in obesity in the United States over the past decade, some of which is explained by a preference to calorically dense foods and a sedentary lifestyle. Corticosteroid therapy, by increasing appetite, leads to increased caloric intake. Coupled with the sedentary lifestyle so common in SLE patients, because of fatigue, arthritis, and orthopedic complications such as osteonecrosis, it is not surprising that obesity is such a problem among SLE patients.

Treatment of obesity has been discouraging, because two-thirds of people who lose weight will regain it within 1 year, and nearly all within 5 years [272]. Lifestyle modification emphasizes the role of dietary composition and exercise. Low-fat diets enhance short-term weight loss [273] and maintenance of the lowered body weight [274]. Low-fat diets will, in addition, reduce the hyperlipidemia so common in SLE. Exercise increases caloric expenditure and promotes dietary compliance. It reduces the desire for foods that are high in fat [275]. Exercise is not contraindicated in SLE and does not cause SLE flares [276]. Appetite-suppressant drugs should be avoided because of their risk of pulmonary hypertension and valvular heart disease [141]. Potentially useful drugs include a lipase inhibitor, tetrahydrolipstatin, that prevents the digestion and absorption of ingested fats [271]. Surgical therapy is limited for those with severe obesity (body-mass index >40) or to those with body-mass index between 35 to 40 who have life-threatening morbidity. Gastroplasty with or without intestinal bypass can be considered.

Diabetes Mellitus

Although only 7% of our cohort patients have diabetes mellitus requiring insulin or oral drug therapy, oral glucose tolerance testing shows the prevalence of diabetes is much higher [277]. Our work has found that hydroxychloroquine use reduces glucose levels significantly in SLE patients, thereby contributing to the protective effect of hydroxychloroquine [277]. In addition, we have found that diabetes mellitus is a risk factor for the accelerated atherosclerosis of SLE (Table 12). Lifestyle modification to reduce the risk of diabetes mellitus in SLE patients with hyperglycemia is essential, and should include dietary modification, weight loss, and exercise. The modern treatment of diabetes mellitus should include combinations of insulin and oral agents that improve glycemic control and improve insulin sensitivity [278]. The five classes of drugs to be considered are insulin, sulfonylurea, metformen,

TABLE 12 Predictors of Coronary Artery Disease in the Hopkins Lupus Cohort

Variable	OR (95% CI)	P-value
Age	1.08 (1.05–1.09)	0.001
Female sex	0.31 (0.19–0.55)	0.04
Homocysteine	1.05 (1.01–1.10)	0.02
Obesity	2.78 (1.61–4.76)	0.06
Hypertension	5.69 (3.35–9.18)	0.001
Diabetes mellitus	4.63 (2.75–7.82)	0.004
Renal insufficiency	2.77 (1.79–4.27)	0.02
Lupus anticoagulant	3.79 (2.51–5.72)	0.002

acarbose, and troglitazone. Troglitazone, for example, can reduce insulin resistance and improve glucose tolerance [279].

Hyperlipidemia

Hypercholesterolemia and elevated levels of LDL-cholesterol are very common in SLE patients (Table 11) and are risk factors for the development of accelerated atherosclerosis [151].

Lifestyle modification, with low-fat, low-cholesterol diet [183, 280], exercise, and weight loss would be the ideal primary prevention program. Many SLE patients, however, are unsuccessful in lifestyle modification and should be candidates for pharmacologic therapy. We have found that SLE patients are at increased risk for elevated liver function tests and CPK elevation with the use of lovastatin and gemfibrozil [281] and recommend that these be monitored in treated patients. SLE patients who do not respond adequately can be considered for the use of more potent statins [282, 283]. Atorvastatin has also been associated with hepatitis in an SLE patient [284].

Antimalarial therapy has a protective effect against hyperlipidemia in SLE. We showed the effectiveness of hydroxychloroquine in a longitudinal model that adjusted for other factors affecting cholesterol [150, 277]. Hydroxychloroquine exerts a beneficial effect on lipids even in corticosteroid-treated patients [285]. Antimalarial therapy reduces total cholesterol, VLDL, and LDL [286]. Chloroquine reverses the increased hepatic synthesis of lipoproteins [287]. There are, however, rare cases of chloroquine cardiomyopathy [288].

Hypertension

Hypertension is very prevalent in SLE patients (Table 8) and is aggravated by corticosteroid therapy [150]. Many SLE patients with nephropathy may have hypertension that requires three or four drugs for control. Hypertension is an independent risk factor for the development of coronary artery disease (Table 12) and renal insufficiency/failure [152].

Treatment of hypertension requires lifestyle modification with dietary factors, including lower sodium, exercise, and weight loss. Pharmacologic treatment should include the use of ACE-inhibitors because of their favorable effect in reducing renal scarring [155] and left ventricular hypertrophy [289, 290], and lack of adverse effect on the lipid profile [155]. Beta-blockers and thiazides increase insulin resistance [291] and increase the risk of diabetes [292]. In addition, beta-blockers might worsen Raynaud's phenomenon, felt to be a risk factor for pulmonary hypertension in SLE.

Estrogen Replacement Therapy

In the postmenopausal woman with SLE, estrogen replacement therapy has the potential benefit of reducing hyperlipidemia and possibly atherosclerotic risk [293, 294], osteoporosis, senility [295], and tooth loss, in addition to quality-of-life concerns, In studies in the general female population, hormone replacement therapy has increased, rather than decreased, the early risk of myocardial infarction [296]. Estrogen replacement therapy has not been given routinely in SLE because of concerns of increasing hypercoagulability [297] (especially in those patients with anti-phospholipid antibodies) or causing SLE flares. In one cohort study, postmenopausal nurses who took hormonal replacement therapy were more likely to develop new lupus [298]. These issues will not be definitively addressed until the current SELENA trial, a randomized double-blind, placebo-controlled clinical trial of hormonal replacement therapy, is completed. Many women continue to have fears of the increased risk of breast carcinoma with estrogen replacement therapy [299]. Raloxifene, a designer estrogen, may exert its estrogen-like effects on bone without stimulating the endometrium or increasing breast cancer risk, but whether it will be protective against cardiovascular disease is unknown [300].

Management of Myocardial Infarction

The acute management of an SLE patient with myocardial infarction is similar to that of a non-SLE patient. SLE patients are candidates for thrombolytic therapy (especially if they have anti-phospholipid antibodies), angioplasty, and coronary artery bypass graft surgery. The difficulty is in determining acutely whether the myocardial infarction is due to atherosclerosis, arteritis, or thrombosis secondary to anti-phospholipid antibody syndrome. Early coronary angiography can be helpful. Serial coronary angiograms may provide evi-

dence of rapid changes in vessels that is consistent with arteritis rather than atherosclerosis. High-dose corticosteroid therapy should only be given at the time of myocardial infarction if there is proof of arteritis by angiogram or a very high suspicion based on extracardiac active lupus, because of the possible adverse effect it may have in causing marked scar thinning [301].

Anti-phospholipid Antibodies

Systemic lupus erythematosus patients who have anti-phospholipid antibody syndrome will be treated with long-term high-intensity warfarin [302]. A more recent retrospective study strongly suggests that low-dose aspirin may be a useful prophylactic therapy [303]. Antimalarial therapy may have an antithrombotic benefit [277]. In an *in vitro* study, statins blocked the binding of anti-phospholipid antibodies to endothelial cells [304].

Other Treatments

The presence of macrophages and activated T cells in atherosclerotic plaques suggest that immunity plays a role in atherosclerotic progression. CD40–CD40 ligand interactions may be important in the development of atherosclerosis. In endarterectomy specimens, CD40 is expressed by macrophages and possibly other cell types. In early atherosclerosis, monocytes adhere to the endothelium and then enter the intima. Macrophages accumulate cholesterol, become foam cells, and smooth muscle proliferates. T cells, two-thirds of which are $CD4^+$, are found in these atherosclerotic lesions. CD40–CD40 ligand interactions mediate the signaling that leads to the activation of both antigen-presenting cells and T cells. Thus, it is hypothesized that, in atherosclerotic plaques, triggering of CD40L on T cells leads to regulation of T-cell expansion and cytokine production [305]. Anti-CD40 ligand therapy was examined in two multicenter studies in SLE; one trial was halted because of unexpected thrombosis and the other showed no benefit [306]. Other immune-based therapies may be considered in the future.

Cardiovascular Mortality

Urowitz and colleagues first drew attention to the bimodal pattern of mortality in SLE, with early deaths due to active disease and infection and later deaths due to cardiovascular disease [184]. With better survival from SLE, both the morbidity and mortality from accelerated atherosclerosis can be expected to increase (Table 13) [29, 43, 151, 180, 181, 184, 186, 307–312].

TABLE 13 Cardiovascular Mortality

Study	Percentage of deaths due to cardiovascular disease
Urowitz *et al*., 1976 [184]	45
Karsh *et al*., 1979 [308]	25
Wallace *et al*., 1981 [311]	20
Rosner *et al*., 1982 [307]	3
Helve, 1985 [312]	6
Rubin *et al*., 1985 [181]	16
Jonsson *et al*., 1989 [186]	44
Shome *et al*., 1989 [182]	3
Reveille *et al*., 1990 [310]	9
Pistiner *et al*., 1991 [43]	15
Petri *et al*., 1992 [151]	8
Abu-Shakra *et al*., 1995 [180]	10.5 (MI); 15.4 (vascular)
Ward *et al*., 1995 [309]	16

In the study of causes of death in 144 SLE patients in an inception cohort, cardiovascular disease was the third leading cause of death (after SLE and infection) with 13 deaths from myocardial infarction, 7 from congestive heart failure, 2 from valvular heart disease, and 1 sudden death. Five cardiovascular deaths occurred in women under the age of 50. Surprisingly, African-Americans were less likely to die of cardiovascular disease [309].

Cardiovascular Surgery

Systemic lupus erythematosus patients are at greater risk of complications from cardiovascular surgery, [313, 314]. In one study, acute renal failure and wound complications were identified [313]. Reinforcement of suture lines has been recommended [314].

SUMMARY

Pericarditis is the most common form of cardiovascular lupus clinically detected. Other forms of cardiac lupus are rare, or are subclinical. The major challenge to clinicians is accelerated atherosclerosis, one of the major causes of mortality.

Acknowledgments

The Hopkins Lupus Cohort is supported by NIH RO1 AR 43727. The Hopkins Lupus Cohort has received support from the General Clinical Research Center NIH/NCRR M01 RR 00052.

References

1. Kaposi, M. (1872). Neue beiträge zur kenntnis des lupus erythematosus. *Arch. Dermat. U. Syph.* **4**, 36.
2. Libman, E., and Sacks, B. (1924). A hitherto undescribed form of valvular and mural endocarditis. *Arch. Intern. Med.* **33**, 701–737.
3. Petri, M., Genovese, M., Engle, E., and Hochberg, M. (1991). Definition, incidence and clinical description of flare in systemic lupus erythematosus: A prospective cohort study. *Arthritis Rheum.* **34**, 937–944.
4. Brigden, W., Bywaters, E. G. L., Lessof, M. H., and Ross, I. P. (1960). The heart in systemic lupus erythematosus. *Br. Heart J.* **22**, 1–16.
5. Shearn, M. (1959). The heart in systemic lupus erythematosus. *Am. Heart J.* **58**, 452–466.
6. Godeau, P., Guilleven, L., Fechner, J., Herreman, G., and Wechsler, B. (1981). Manifestations cardiques du lupus erythemateaux aigu dissemine. *Nouv. Presse Med.* **10**, 2175–2178.
7. Marks, A. D. (1972). The cardiovascular manifestations of systemic lupus erythematosus. *Am. J. Med. Sci.* **264**, 254–265.
8. Leung, W.-H., Wong, K.-L., Lau, C.-P., Wong, C.-K., and Cheng, C.-H. (1990). Cardiac abnormalities in systemic lupus erythematosus: A prospective M-mode, cross-sectional and Doppler echocardiographic study. *Int. J. Cardiol.* **27**, 367–375.
9. Cervera, R., Font, J., Paré, C., Azqueta, M., Pérez-Villa, F., López-Soto, A., *et al.* (1992). Cardiac disease in systemic lupus erythematosus: Prospective study of 70 patients. *Ann. Rheum. Dis.* **51**, 156–159.
10. Leung, W. H., Lau, C. P., Wong, C. K., and Leung, C. Y. (1990). Fatal cardiac tamponade in systemic lupus erythematosus: A hazard of anti-coagulation. *Am. Heart J.* **119**, 422–423.
11. Jacobson, E. J., and Reza, M. J. (1978). Constrictive pericarditis in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 972–974.
12. Hejtmancik, M. R., Wright, J. C., and Quint, R. (1964). The cardiovascular manifestations of systemic lupus erythematosus. *Am. Heart J.* **68**, 119–130.
13. Yurchak, P. M., Levine, S. A., and Gorlin, R. (1965). Contrictive pericarditis complicating disseminated lupus erythematosus. *Circulation* **31**, 113–118.
14. Starkey, R. H., and Hahn, B. H. (1973). Rapid development of constrictive pericarditis in a patient with systemic lupus erythematosus. *Chest* **63**, 448–450.
15. Chia, B. L., Mah, E. P., and Feng, P. H. (1981). Cardiovascular abnormalities in systemic lupus erythematosus. *J. Clin. Ultrasound* **9**, 237–243.
16. Maniscalco, B. S., Felner, J. M., McCans, J. L., and Chiapella, J. A. (1975). Echocardiographic abnormalities in systemic lupus erythematosus [abstract]. *Circulation* **52**(Suppl. II), II-211.
17. Schnittger, I., Bowden, R., Abrams, J., and Popp, R. L. (1978). Echocardiography: Pericardial thickening and constrictive pericarditis. *Am. J. Cardiol.* **42**, 388–395.
18. Feigenbaum, H. (1972). "Echocardiography." Lea & Febiger, Philadelphia.
19. Nihoyannopoulos, P., Gomez, P. M., Joshi, J., Loizou, S., Walport, M. J., and Oakley, C. M. (1990). Cardiac abnormalities in systemic lupus erythematosus: Association with raised anticardiolipin antibodies. *Circulation* **82**, 369–375.
20. Armas-Cruz, R., Harnecker, J., Ducach, G., Jalil, J., and Gonzales, F. (1958). Clinical diagnosis of systemic lupus erythematosus. *Am. J. Med.* **25**, 409–419.
21. Copeland, G. D., von Capeller, D., and Stern, T. N. (1958). Systemic lupus erythematosus: A clinical report of forty-seven cases with pathologic findings in eighteen. *Am. J. Med. Sci.* **236**, 318–326.
22. Griffith, G. C., and Vural, I. L. (1951). Acute and subacute disseminated lupus erythematosus: A correlation of clinical and postmortem findings in eighteen cases. *Circulation* **3**, 492.
23. Gross, L. (1940). The cardial lesions in Libman-Sacks disease with a consideration of its relationship to acute diffuse lupus erythematosus. *Am. J. Pathol.* **16**, 375–407.
24. Harvey, A. M., Shulman, L. E., Tumulty, P. A., Conley, C. L., and Schoenrich, E. H. (1954). Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine* **33**, 291–437.
25. Humphreys, E. (1948). The cardiac lesions of acute disseminated lupus erythematosus. *Ann. Intern. Med.* **28**, 12–14.
26. Jessar, R. A., Lamont-Havers, W., and Ragan, C. (1953). Natural history of lupus erythematosus disseminatus. *Ann. Intern. Med.* **38**, 717–731.
27. Bulkley, B. H., and Roberts, W. C. (1975). The heart in systemic lupus erythematosus and the changes induced in it by corticosteroid therapy: A study of 36 necropsy patients. *Am. J. Med.* **58**, 243–264.
28. Collins, R., Turner, R. A., Nomeir, M., *et al.* (1978). Cardiopulmonary manifestations of systemic lupus erythematosus. *J. Rheumatol.* **5**, 299–306.
29. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85–95.
30. Dubois, E. L. (1976). "Lupus Erythematosus: A Review of the Current Status of Discoid and Systemic Lupus Erythematosus and Their Varients," 2nd ed. Univ. of Southern California Press, Los Angeles.
31. Dubois, E. L., and Tuffanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus. *J. Am. Med. Assoc.* **190**, 104–111.
32. Elkayam, U., Weiss, S., and Laniado, S. (1977). Pericardial effusion and mitral valve involvement in systemic lupus erythematosus: Echocardiographic study. *Ann. Rheum. Dis.* **36**, 349–353.
33. Ito, M., Kagiyama, Y., Omura, I., Hiramatsu, Y., Kurata, E., Kanaya, S., *et al.* (1979). Cardiovascular manifestations in systemic lupus erythematosus. *Jpn. Circ. J.* **43**, 985–994.
34. James, T. N., Rupe, C. E., and Monto, R. W. (1965). Pathology of the cardiac conduction system in systemic lupus erythematosus. *Ann. Intern. Med.* **63**, 402–410.

35. Kong, T. Q., Kellum, R. E., and Haserick, J. R. (1962). Clinical diagnosis of cardiac involvement in systemic lupus erythematosus: A correlation of clinical and autopsy findings in thirty patients. *Circulation* **26**, 7–11.
36. Ropes, M. W. (1976). "Systemic Lupus Erythematosus." Harvard Univ. Press, Cambridge, Massachusetts.
37. Badui, E., Garcia-Rubi, D., Robles, E., Jimenez, J., Juan, L., Deleze, M., *et al*. (1985). Cardiovascular manifestations in systemic lupus erythematosus. Prospective study of 100 patients. *Angiology* **36**, 431–441.
38. Bidani, A. K., Roberts, J. L., Schwartz, M. M., and Lewis, E. J. (1980). Immunopathology of cardiac lesions in fatal systemic lupus erythematosus. *Am. J. Med.* **69**, 849–858.
39. Bomalaski, J. S., Talano, J. V., and Perlman, S. (1983). The value of echocardiography in patients with systemic lupus erythematosus [abstract]. *Clin. Res.* **31**, 68.
40. Crozier, I. G., Li, E., Milne, M. J., and Nicholls, M. G. (1990). Cardiac involvement in systemic lupus erythematosus detected by echocardiography. *Am. J. Cardiol.* **65**, 1145–1148.
41. Doherty III, N. E., Feldman, G., Maurer, G., and Siegel, R. J. (1988). Echocardiographic findings in systemic lupus erythematosus. *Am. J. Cardiol.* **61**, 1144.
42. Klinkhoff, A. V., Thompson, C. R., Reid, G. D., and Tomlinson, C. W. (1985). M-mode and two dimensional echocardiographic abnormalities in systemic lupus erythematosus. *J. Am. Med. Assoc.* **253**, 3273–3277.
43. Pistiner, M., Wallace, D. J., Nessim, S., Metzger, A. L., and Klinenberg, J. R. (1991). Lupus erythematosus in the 1980s: A survey of 570 patients. *Semin. Arthritis Rheum.* **21**, 55–64.
44. Sturfelt, G., Eskilsson, J., Nived, O., Truedsson, L., and Valind, S. (1992). Cardiovascular disease in systemic lupus erythematosus: A study of 75 patients from a defined population. *Medicine* **71**, 216–223.
45. Mandell, B. F. (1987). Cardiovascular involvement in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **17**, 126–141.
46. Doherty, N. E., and Siegel, R. J. (1985). Cardiovascular manifestations of systemic lupus erythematosus. *Am. Heart J.* **110**, 1257–1265.
47. Hunder, G. G., Mullen, B. J., and McDuffie, F. C. (1974). Complement in pericardial fluid of lupus erythematosus. *Ann. Intern. Med.* **80**, 453–458.
48. Tamburino, C., Fiore, C. E., Foti, R., Salomone, E., Di Paola, R., and Grimaldi, D. R. (1989). Endomyocardial biopsy in diagnosis and management of cardiovascular manifestations of systemic lupus erythematosus (SLE). *Clin. Rheumatol.* **8**, 108–112.
49. Herskowitz, A., Campbell, S., Deckers, J., Kasper, E. K., Boehmer, J., Hadian, D., *et al*. (1993). Demographic features and prevalence of idiopathic myocarditis in patients undergoing endomyocardial biopsy. *Am. J. Cardiol.* **71**, 982–986.
50. Larson, D. L. (1961). "Systemic Lupus Erythematosus." Little, Brown & Co., Boston.
51. Borenstein, D. G., Fye, W. B., Arnett, F. C., and Stevens, M. B. (1978). The myocarditis of systemic lupus erythematosus: Association with myositis. *Ann. Intern. Med.* **89**, 619–624.
52. Berg, G., Bodet, J., Webb, K., Williams, G., Palmer, D., Ruoff, B., *et al*. (1985). Systemic lupus erythematosus presenting as isolated congestive heart failure. *J. Rheumatol.* **12**, 1182–1185.
53. Been, M., Thomson, B. J., Smith, M. A., Ridgway, J. P., Douglas, R. H. B., Been, M., *et al*. (1988). Myocardial involvement in systemic lupus erythematosus detected by magnetic resonance imaging. *Eur. Heart J.* **9**, 1250–2156.
54. Takahashi, T., Suzuki, T., Suzuki, A., Yamada, T., Koido, N., Akizuki, S., *et al*. (1999). [A case of systemic lupus erythematosus with interstitial myocarditis leading to sudden death]. *Ryumachi* **39**, 573–579.
55. Simon, N., Cohen, H., Glick, G., Kanter, A., Levin, B., and Pirani, C. L. (1973). Clinical pathologic conference. *Am. Heart J.* **86**, 539–552.
56. Fairfax, M. J., Osborn, T. G., Williams, G. A., Tsai, C. C., and Moore, T. L. (1988). Endomyocardial biopsy in patients with systemic lupus erythematosus. *J. Rheumatol.* **15**, 593–596.
57. Enomoto, K., Kaji, Y., Mayumi, T., Tsuda, Y., Kanaya, S., Nagasawa, K., *et al*. (1991). Left ventricular function in patients with stable systemic lupus erythematosus. *Jpn. Heat J.* **32**, 445–453.
58. Murai, K., Oku, H., Takeuchi, K., Kanayama, Y., Inoue, T., and Takeda, T. (1987). Alterations in myocardial systolic and diastolic function in patients with active systemic lupus erythematosus. *Am. Heart J.* **113**, 966–971.
59. Bahl, V. K., Aradhye, S., Vasan, R. S., Malhotra, A., Reddy, K. S., and Malaviya, A. N. (1992). Myocardial systolic function in systemic lupus erythematosus: A study based on radionuclide ventriculography. *Clin. Cardiol.* **15**, 433–435.
60. Hospenpud, J. D., Montanaro, A., Hart, M. V., Haines, J. E., Specht, H. D., Bennett, R. M., *et al*. (1984). Myocardial perfusion abnormalities in asymptomatic patients with systemic lupus erythematosus. *Am. J. Med.* **77**, 286–292.
61. Giunta, A., Picillo, U., Maione, S., Migliaresi, S., Valentini, G., Arnese, M., *et al*. (1993). Spectrum of cardiac involvement in systemic lupus erythematosus: Echocardiographic, echo-doppler observations and immunological investigation. *Acta Cardiol.* **48**, 183–197.
62. Roldan, C. A., Shively, B. K., Lau, C. C., Gurule, F. T., Smith, E. A., and Crawford, M. H. (1992). Systemic lupus erythematosus valve disease by transesophageal echocardiography and the role of antiphospholipid antibodies. *J. Am. Coll. Cardiol.* **20**, 1127–1134.
63. Winslow, T. M., Ossipov, M. A., Fazio, G. P., Foster, E., Simonson, J. S., and Schiller, N. B. (1993). The left ventricle in systemic lupus erythematosus: Initial observations and a five-year follow-up in a university medical center population. *Am. Heart J.* **125**, 1117–1122.
64. Strauer, B. E., Brune, I., Schenk, H., Knoll, D., and Perings, E. (1976). Lupus cardiomyopathy: Cardiac mechanics, hemodynamics, and coronary blood flow in

uncomplicated systemic lupus erythematosus. *Am. Heart J.* **92**, 715–722.
65. Rangel, A., Basave, M., Lavalle, C., Hernandez, L., Ochoa, J., Chavez, E., *et al*. (2000). Endomyocardial fibrosis (Davies disease) coincidental with systemic lupus erythematosus. *Arch. Inst. Cardiol. Mex.* **70**, 66–71.
66. Hartford, M., Wikstrand, J., Wallentin, I., Ljungman, S., Wilhelmsen, L., and Berglund, G. (1984). Diastolic function of the heart in untreated primary hypertension. *Hypertension* **6**, 329.
67. Ueno, K., Fujimoto, S., Fujimoto, T., Nakano, H., Nakajima, T., Yamano, S., *et al*. (1999). [A case of systemic lupus erythematosus discovered from left heart failure due to lupus induced mitral regurgitation]. *Ryumachi* **39**, 778–783.
68. Klemperer, P. (1950). The concept of collagen diseases. *Am. J. Pathol.* **26**, 505–519.
69. Daugherty, G. W., and Baggenstoss, A. H. (1950). Syndrome characterized by glomerulonephritis and arthritis. Libman-Sacks disease with predominantly renal involvement. *Arch. Intern. Med.* **85**, 900–923.
70. Straaton, K. V., Chatham, W. W., Reveille, J. D., Koopman, W. J., and Smith, S. H. (1988). Clinically significant valvular heart disease in systemic lupus erythematosus. *Am. J. Med.* **85**, 645–650.
71. Galve, E., Candell-Riera, J., Pigrau, C., Permanyer-Miralda, G., Garcia-Del-Castillo, H., and Soler-Soler, J. (1988). Prevalence, morphologic types, and evolution of cardiac valvular disease in systemic lupus erythematosus. *N. Engl. J. Med.* **319**, 817–823.
72. Khamashta, M. A., Cervera, R., Asherson, R. A., Font, J., Gil, A., Coltart, D. J., *et al*. (1990). Association of antibodies against phospholipids with heart valve disease in systemic lupus erythematosus. *Lancet* **335**, 1541–1544.
73. Gleason, C. B., Stoddard, M. E., Wagner, S. G., Longaker, R. A., Pierageli, S., and Harris, E. N. (1993). A comparison of cardiac valvular involvement in the primary antiphospholipid syndrome versus anticardiolipin-negative systemic lupus erythematosus. *Am. Heart J.* **125**, 1123–1129.
74. Metz, D., Jolly, D., Graciet-Richard, J., Pennaforte, J. L., Nazeyrollas, P., Chabert, J. P., *et al*. (1994). Prevalence of valvular involvement in systemic lupus erythematosus and association with antiphospholipid syndrome: A matched echocardiographic study. *Cardiology* **85**, 129–136.
75. Meyer, O., Golstein, M., Nicaise, P., Labarre, C., and Kahn, M. F. (1995). Heart valve disease in systemic lupus erythematosus: Role of antiphospholipid antibodies. *Clin. Rev. Allergy Immunol.* **13**, 49–56.
76. Gabrielli, F., Alcini, E., Di Prima, M. A., Mazzacurati, G., and Masala, C. (1995). Cardiac valve involvement in systemic lupus erythematosus and primary antiphospholipid syndrome: Lack of correlation with antiphospholipid antibodies. *Int. J. Cardiol.* **51**, 117–126.
77. Rantapaa-Dahlqvist, S., Neumann-Andersen, G., Backman, C., Dahlen, G., and Stegmayr, B. (1997). Echocardiographic findings, lipids and lipoprotein(a) in patients with systemic lupus erythematosus. *Clin. Rheumatol.* **16**, 140–148.
78. Shapiro, R. F., Gamble, C. N., Wiesner, K. B., Castles, J. J., Wolf, A. W., Hurley, E. J., *et al*. (1977). Immunopathogenesis of Libman-Sacks endocarditis: Assessment by light and immunofluorescent microscopy in two patients. *Ann. Rheum. Dis.* **36**, 508–516.
79. Roldan, C. A., Shively, B. K., and Crawford, M. H. (1996). An echocardiographic study of valvular heart disease associated with systemic lupus erythematosus. *N. Engl. J. Med.* **335**, 1424–1430.
80. Nesher, G., Ilany, J., Rosenmann, D., and Abraham, A. S. (1997). Valvular dysfunction in antiphospholipid syndrome: Prevalence, clinical features, and treatment. *Semin. Arthritis Rheum.* **27**, 27–35.
81. Vianna, J. L., Khamashta, M. A., Ordi-Ros, J., Font, F., Cervera, R., Lopez-Soto, A., *et al*. (1994). Comparison of the primary and secondary antiphospholipid syndrome: A European multicenter study of 114 patients. *Am. J. Med.* **96**, 3–9.
82. Galve, E., Ordi, J., Barquinero, J., Evangelista, A., Vilardell, M., and Soler-Soler, J. (1992). Valvular heart disease in the primary antiphospholipid syndrome. *Ann. Intern. Med.* **116**, 293–298.
83. Brenner, B., Blumenfeld, Z., Markiewicz, W., and Reisner, S. A. (1991). Cardiac involvement in patients with primary anti-phospholipid syndrome. *J. Am. Coll. Cardiol.* **18**, 931–936.
84. Garcia-Torres, R., Amigo, M. C., de la Rossa, A., Moron, A., and Reyes, P. A. (1996). Valvular heart disease in primary antiphospholipid syndrome: Clinical and morphological findings. *Lupus* **5**, 56–61.
85. Badui, E., Solorio, S., Martinez, E., Bravo, G., Enciso, R., Barile, L., *et al*. (1995). The heart in the primary antiphospholipid syndrome. *Arch. Med. Res.* **26**, 115–120.
86. Cervera, R., Khamashta, M. A., Font, J., Reyes, P. A., Vianna, J. L., Lopez-Soto, A., *et al*. (1991). High prevalence of significant heart valve lesions in patients with the primary antiphospholipid syndrome. *Lupus* **1**, 43–47.
87. Ford, P. H., Ford, S. E., and Lillicrap, D. P. (1988). Association of lupus anticoagulant with severe valvular heart disease in SLE. *J. Rheumatol.* **15**, 597–600.
88. Chartash, E. K., Lans, D. M., Paget, S. A., Qamar, T., and Lockshin, M. D. (1989). Aortic insufficiency and mitral regurgitation in patients with systemic lupus erythematosus and the antiphospholipid syndrome. *Am. J. Med.* **86**, 407–412.
89. Leung, W.-H., Wong, K.-L., Lau, C.-P., Wong, C.-K., and Liu, H.-W. (1990). Association between antiphospholipid antibodies and cardiac abnormalities in patients with systemic lupus erythematosus. *Am. J. Med.* **89**, 411–419.
90. Li, E. K., Crozier, I. G., Milne, M. J., Nicholls, M. G., and Cohen, M. G. (1990). Lack of association between anticardiolipin antibodies and heart valve disease in Chinese patients with systemic lupus erythematosus [letter]. *Lancet* **336**, 504–505.
91. Ong, M. L., Veerapen, K., Chambers, J. B., Lim, M. N., Manivasagar, M., and Wang, F. (1992). Cardiac abnor-

malities in systemic lupus erythematosus: Prevalence and relationship to disease activity. *Int. J. Cardiol.* **34**, 69–74.
92. Ramonda, R., Doria, A., Villanova, C., Vaccaro, E., Punzi, L., Fasoli, G., *et al*. (1992). Evaluation de l'atteinte cardiaque au cours du lupus erythemateux disseminé: Étude clinique et échocardiographque. *Rev. Rhum. Mal Osteoartic.* **59**, 790–796.
93. Lolli, C., Foscoli, M., Giofré, R., Tarquini, S. P., and Toschi, G. P. (1993). Anomalie cardiache nel Lupus Eritematoso Sistemico: Prevalenza e relazione con durata, attivitá della malattia e presenza di anticorpi antifosfolipidi. *G. Ital. Cardiol.* **23**, 1125–1134.
94. Laganá, B. (1993). Anormalitá cardiache in corso di lupus eritematoso sistemico e loro associazione con gli anticropi antifosfolipidi. *Rec. Progr. Med.* **84**, 662–672.
95. Ziporen, L., Goldberg, I., Arad, M., Hojnik, M., Ordi-Ros, J., Afek, A., *et al*. (1996). Libman-Sacks endocarditis in the antiphospholipid syndrome: Immunopathologic findings in deformed heart valves. *Lupus* **5**, 196–205.
96. Connolly, H. M., Crary, J. L., McGoon, M. D., Hensrud, D. D., Edwards, B. S., Edwards, W. D., *et al*. (1997). Valvular heart disease associated with fenfluramine–phentermine. *N. Engl. J. Med.* **337**, 581–588.
97. Graham, D. J., and Green, L. (1997). Further cases of valvular heart disease associated with flenfluramine–phentermine. *N. Engl. J. Med.* **337**, 635.
98. Cannistra, L. (1997). Valvular heart disease associated with dexfenfluramine. *N. Engl. J. Med.* **337**, 636.
99. Buyon, J. P., Winchester, R. J., Slade, S. G., Arnett, F., Copel, J., Friedman, D., *et al*. (1993). Identification of mothers at risk for congenital heart block and other neonatal lupus syndromes in their children. Comparison of enzyme-linked immunosorbent assay and immunoblot for measurement of anti-SS-A/Ro and anti-SS-B/La. *Arthritis Rheum.* **36**, 1263–1273.
100. Petri, M., Watson, R., and Hochberg, M. C. (1989). Anti-Ro antibodies and neonatal lupus. *Rheum. Dis. Clin. North Am.* **15**, 335–360.
101. Ferrazzini, G., Fasnacht, M., Arbenz, U., Seger, R., Biedermann, R., Simma, B., *et al*. (1996). Neonatal lupus erythematosus with congenital heart block and severe heart failure due to myocarditis and endocardititis of the mitral valve. *Intensive Care Med.* **22**, 464–466.
102. Taylor-Albert, E., Reichlin, M., Toews, W. H., Overholt, E. D., and Lee, L. A. (1997). Delayed dilated cardiomyopathy as a manifestation of neonatal lupus: Case reports, autoantibody analysis, and management. *Pediatrics* **99**, 733–735.
103. Okada, T., and Shiokawa, Y. (1975). Cardiac lesions in collagen disease. *Jpn. Circ. J.* **39**, 479–484.
104. del Rio, A., Vázquez, J. J., Sobrino, J. A., Gil, A., Barbado, J., Maté, I., *et al*. (1978). Myocardial involvement in systemic lupus erythematosus: A noninvasive study of left ventricular function. *Chest* **74**, 414–417.
105. Mevorach, D., Raz, E., Shalev, O., Steiner, I., and Ben-Chetrit, E. (1993). Complete heart block and seizures in an adult with systemic lupus erythematosus: A possible pathophysiologic role for anti-SS-A/Ro and Anti-SS-B/La autoantibodies. *Arthritis Rheum.* **36**, 259–262.
106. Martinez-Costa, X., Ordi, J., Barberá, J., Selva, A., Bosch, J., and Vilardell, M. (1991). High grade atrioventricular heart block in 2 adults with systemic lupus erythematosus. *J. Rheumatol.* **18**, 1926–1928.
107. Bharati, S., de la Fuente, D. J., Kallen, R. J., Freij, Y., and Lev, M. (1975). Conduction system in systemic lupus erythematosus with atrioventricular block. *Am. J. Cardiol.* **35**, 299–304.
108. Guzman, J., Cardiel, M. H., Arce-Salinas, A., and Alarcon-Segovia, D. (1994). The contribution of resting heart rate and routine blood tests to the clinical assessment of disease activity in systemic lupus erythematosus. *J. Rheumatol.* **21**, 1845–1848.
109. Homcy, C. J., Liberthson, R. R., Fallon, J. T., Gross, S., and Miller, L. M. (1982). Ischemic heart disease in systemic lupus erythematosus in the young patient: Report of 6 cases. *Am. J. Cardiol.* **49**, 478–484.
110. Bonfiglio, T. A., Botti, R. E., and Hagstrom, J. W. C. (1972). Coronary arteritis, occlusion and myocardial infarction due to lupus erythematosus. *Am. Heart J.* **83**, 153–158.
111. Wilson, V. E., Eck, S. L., and Bates, E. R. (1992). Evaluation and treatment of acute myocardial infarction complicating systemic lupus erythematosus. *Chest* **101**, 420–424.
112. Bor, I. (1968). Cardiac infarction in the Libman-Sacks endocarditis [letter]. *N. Engl. J. Med.* **279**, 164.
113. Ohara, N., Miyata, T., Kurata, A., Oshiro, H., Sato, O., and Shigematsu, H. (2000). Ten years' experience of aortic aneurysm associated with systemic lupus erythematosus. *Eur. J. Vasc. Endovasc. Surg.* **19**, 288–293.
114. Heibel, R. H., O'Toole, J. D., Curtiss, E. I., Medsger, T. A., Jr., Reddy, S. P., and Shaver, J. A. (1976). Coronary arteritis in systemic lupus erythematosus. *Chest* **69**, 200–203.
115. Takatsu, Y., Hattori, R., Sakaguchi, K., Yui, Y., and Kawai, C. (1985). Acute myocardial infarction associated with systemic lupus erythematosus documented by coronary arteriograms. *Chest* **88**, 147–149.
116. Wallace, D. J., and Dubois, E. L. (1987). "Dubois' Lupus Erythematosus," 3rd ed. Lea & Febiger, Philadelphia.
117. Keat, E. C., and Shore, J. H. (1958). Gangrene of the legs in disseminated lupus erythematosus. *Br. Med. J.* **5061**, 2527–2531.
118. Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1984). Immune complex deposition and coronary vasculitis in systemic lupus erythematosus. *Am. J. Med.* **77**, 141–146.
119. Kurokawa, H., Kondo, T., Shiga, Y., Nomura, M., Mizuno, Y., Ashiwara, M., *et al*. (1986). [Myocardial infarction due to thrombi in coronary aneurysms in a young woman with systemic lupus erythematosus]. *J. Cardiogr.* **16**, 249–258.
120. Benisch, B. M., and Pervez, N. (1974). Coronary artery vasculitis and myocardial infarction with systemic lupus erythematosus. *N. Y. State J. Med.* **74**, 873–874.
121. Castleman, B., and Dole, V. P. (1962). Case records of the Massachusetts General Hospital: Case 1-1962. *N. Engl. J. Med.* **266**, 42–49.

122. Englund, J. A., and Lucas, R. U. (1983). Cardiac complications in children with systemic lupus erythematosus. *Pediatrics* **72**, 724–730.
123. Uchida, T., Inoue, T., Kamishirado, H., Nakata, T., Sakai, Y., Takayanagi, K., *et al*. (2001). Unusual coronary artery aneurysm and acute myocardial infarction in a middle-aged man with systemic lupus erythematosus. *Am. J. Med. Sci.* **322**, 163–165.
124. Simonson, J. S., Schiller, N. B., Petri, M., and Hellmann, D. B. (1989). Pulmonary hypertension in systemic lupus erythematosus. *J. Rheumatol.* **16**, 918–925.
125. Winslow, T. M., Ossipov, M. A., Fazio, G. P., Simonson, J. S., Redberg, R. F., and Schiller, N. B. (1995). Five-year follow-up study of the prevalence and progression of pulmonary hypertension in systemic lupus erythematosus. *Am. Heart J.* **129**, 510–515.
126. Perez, H. D., and Kramer, N. (1981). Pulmonary hypertension in systemic lupus erythematosus: Report of four cases and review of the literature. *Semin. Arthritis Rheum.* **11**, 177–181.
127. Quismorio, F. P., Jr., Sharma, O., Koss, M., Boylen, T., Edmiston, A. W., Thornton, P. J., *et al*. (1984). Immunopathologic and clinical studies in pulmonary hypertension associated with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **13**, 349–359.
128. Asherson, R. A., Hackett, D., Gharavi, A. E., Harris, E. N., Kennedy, H. G., and Hughes, G. R. V. (1986). Pulmonary hypertension in systemic lupus erythematosus: A report of three cases. *J. Rheumatol.* **13**, 416–420.
129. Wakaki, K., Koizumi, F., and Fukase, M. (1984). Vascular lesions in systemic lupus erythematosus (SLE) with pulmonary hypertension. *Acta Pathol. Jpn.* **34**, 593–604.
130. Wagenvoort, C. A., and Wagenvoort, N. (1970). Primary pulmonary hypertension: A pathologic study of the vessels in 156 clinically diagnosed cases. *Circulation* **42**, 1163–1184.
131. Walcott, G., Burchell, H. B., and Brown, A. L. (1970). Primary pulmonary hypertension. *Am. J. Med.* **49**, 70–79.
132. Kuramochi, S., Tashiro, Y., Torikata, C., and Watanabe, Y. (1982). Systemic lupus erythematosus associated with multiple nodular hyperplasia of the liver. *Acta Pathol. Jpn.* **32**, 547–560.
133. Furst, D. E., Davis, J. A., Clements, P. J., Chopra, S. K., Theofilopoulos, A. N., and Chia, D. (1981). Abnormalities of pulmonary vascular dynamics and inflammation in early progressive systemic sclerosis. *Arthritis Rheum.* **24**, 1403–1408.
134. Rozkovec, A., Bernstein, R., Asherson, R. A., and Oakley, C. M. (1983). Vascular reactivity and pulmonary hypertension in systemic sclerosis. *Arthritis Rheum.* **26**, 1037–1040.
135. Asherson, R. A., and Oakley, C. M. (1986). Pulmonary hypertension and systemic lupus erythematosus [editorial]. *J. Rheumatol.* **13**, 1–5.
136. de la Mata, J., Gomez-Sanchez, M. A., Aranzana, M., and Gomez-Reino, J. J. (1994). Long-term iloprost infusion therapy for severe pulmonary hypertension in patients with connective tissue diseases. *Arthritis Rheum.* **37**, 1528–1533.
137. Rubin, L. J., Badesch, D. B., Barst, R. J., Galie, N., Black, C. M., Keogh, A., *et al*. (2000). Bosentan therapy for pulmonary arterial hypertension. *N. Engl. J. Med.* **346**, 896–903.
138. Rubin, L. J. (1997). Primary pulmonary hypertension. *N. Engl. J. Med.* **336**, 111–117.
139. Gurtner, H. P. (1985). Aminorex and pulmonary hypertension. *Cor Vasa* **27**, 160–171.
140. Abenhaim, L., Moride, Y., Brenot, F., *et al*. (1996). Appetite-suppressant drugs and the risk of primary pulmonary hypertension. *N. Engl. J. Med.* **335**, 609–616.
141. Curfman, G. D. (1997). Diet pills redux [editorial]. *N. Engl. J. Med.* **337**, 629–630.
142. Mark, E. J., Patalas, E. D., Chang, H. T., Evans, R. J., and Kessler, S. C. (1997). Fatal pulmonary hypertension associated with short-term use of fenfluramine and phentermine. *N. Engl. J. Med.* **337**, 602–606.
143. Weir, E. K., Reeve, H. L., Huang, J. M., *et al*. (1996). Anorexic drugs aminorex, fenfluramine, and dexfenfluramine inhibit potassium current in rat pulmonary vascular smooth muscle and cause pulmonary vasoconstriction. *Circulation* **94**, 2216–2220.
144. Budman, D. R., and Steinberg, A. D. (1976). Hypertension and renal disease in systemic lupus erythematosus. *Arch. Intern. Med.* **136**, 1003–1007.
145. Schieppati, A., and Remuzzi, G. (1993). Hypertension in renal disease: Pathophysiological, functional, and clinical implications. *Am. J. Kidney Dis.* **21**, 58–60.
146. Pollack, V. E., and Kant, K. S. (1991). Diffuse and focal proliferative lupus nephritis: Treatment approaches and results. *Nephron* **59**, 177–183.
147. Sofer, L. J., and Bader, R. (1952). Corticotropin and cortisone in acute disseminated lupus erythematosus. *J. Am. Med. Assoc.* **149**, 1002–1008.
148. Dubois, E. L., Commons, R. R., Starr, P., Stein, C. S., Jr., and Morrison, R. (1952). Corticotropin and cortisone treatment for systemic lupus erythematosus. *J. Am. Med. Assoc.* **149**, 995.
149. Kaplan, D., Ginzler, E. M., and Feldman, J. (1992). Arthritis and hypertension in patients with systemic lupus erythematosus. *Arthritis Rheum.* **35**, 423–428.
150. Petri, M., Lakatta, C., Magder, L., and Goldman, D. W. (1994). Effect of prednisone and hydroxychloroquine on coronary artery disease risk factors in systemic lupus erythematosus: A longitudinal data analysis. *Am. J. Med.* **96**, 254–259.
151. Petri, M., Perez-Gutthann, S., Spence, D., and Hochberg, M. C. (1992). Risk factors for coronary artery disease in patients with systemic lupus erythematosus. *Am. J. Med.* **93**, 513–519.
152. Petri, M., Perez-Gutthann, S., Longenecker, J. C., and Hochberg, M. C. (1991). Morbidity of systemic lupus erythematosus: Role of race and socioeconomic status. *Am. J. Med.* **91**, 345–353.
153. Petri, M. (1997). Hopkins Lupus Pregnancy Center: 1987 to 1996. *Rheum. Dis. Clin. North Am.* **23**, 1–14.
154. The fifth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood

Pressure (JNC V) [see comments]. (1993). *Arch. Intern. Med.* **153**, 154–183.
155. Lewis, E. J., Hunsicker, L. G., Bain, R. P., and Rohde, R. D. (1993). The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. *N. Engl. J. Med.* **329**, 1456–1462.
156. Dahlof, B., Devereux, R. B., Kjeldsen, S. E., Julius, S., Beevers, G., de Faire U., *et al*. (2002). Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): A randomised trial against atenolol. *Lancet* **359**, 995–1003.
157. Meller, J., Conde, C. A., Deppisch, L. M., Donoso, E., and Dack, S. (1975). Myocardial infarction due to coronary atherosclerosis in three young adults with systemic lupus erythematosus. *Am. J. Cardiol.* **35**, 309–314.
158. Aranow, C., and Ginzler, E. M. (2000). Epidemiology of cardiovascular disease in systemic lupus erythematosus. *Lupus* **9**, 166–169.
159. Urowitz, M. B., and Gladman, D. D. (1999). Evolving spectrum of mortality and morbidity in SLE [editorial]. *Lupus* **8**, 253–255.
160. Manzi, S., Meilahn, E. N., Rairie, J. E., Conte, C. G., Medsger, T. A., Jr., Jansen-McWilliams, L., *et al*. (1997). Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: Comparison with the Framingham Study. *Am. J. Epidemiol.* **145**, 408–415.
161. Ward, M. M. (1999). Premature morbidity from cardiovascular and cerbrovascular disease in women with systemic lupus erythematosus. *Arthritis Rheum.* **42**, 338–346.
162. Patterson, R. E., Horowitz, S. F., and Eisner, R. L. (1994). Comparison of modalities to diagnose coronary artery disease. *Semin. Nucl. Med.* **24**, 286–310.
163. Grégoire, J., and Théroux, P. (1990). Detection and assessment of unstable angina using myocardial perfusion imaging: Comparison between technetium-99m sestamibi SPECT and 12-lead electrocardiogram. *Am. J. Cardiol.* **66**, 44E–46E.
164. Manca, C., Dei Cas, L., Albertini, D., Baldi, G., and Visioli, O. (1978). Different prognostic value of exercise electrocardiogram in men and women. *Cardiology* **63**, 312–319.
165. Bartel, A. G. (1979). Exercise stress testing—current status. *Cardiology* **64**, 170–189.
166. Borer, J. S., Brensike, J. F., Redwood, D. R., Itscoitz, S. B., Passamani, E. R., Stone, N. J., *et al*. (1975). Limitations of the electrocardiographic response to exercise in predicting coronary-artery disease. *N. Engl. J. Med.* **293**, 367–371.
167. Manzi, S., Selzer, F., Sutton-Tyrrell, K., Fitzgerald, S. G., Rairie, J. E., Tracy, R. P., *et al*. (1999). Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum.* **42**, 51–60.
168. Petri, M. (2000). Detection of coronary artery disease and the role of traditional risk factors in the Hopkins Lupus Cohort. *Lupus* **9**, 170–175.
169. Svenungsson, E., Jensen-Urstad, K., Heimburger, M., Silveira, A., Hamsten, A., de Faire, U., *et al*. (2001). Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation* **104**, 1887–1893.
170. Iskandrian, A. S., and Heo, J. (1992). Thallium-201 myocardial imaging. In: "Cardiovascular Nuclear Medicine and MRI" (J. H. C. Reiber and E. E. van der Wall, Eds.), pp. 223–238. Kluwer, Amsterdam.
171. Berman, D. S., Kiat, H., van Train, K. F., Friedman, J., Garcia, E. V., and Maddahi, J. (1990). Comparison of SPECT using Technetium-99m agents and thallium-201 and PET for the assessment of myocardial perfusion and viability. *Am. J. Cardiol.* **66**, 72E–79E.
172. Maddahi, J., Kiat, H., van Train, K. F., Prigent, F., Friedman, J., Garcia, E. V., *et al*. (1990). Myocardial perfusion imaging with technetium-99m sestamibi SPECT in the evaluation of coronary artery disease. *Am. J. Cardiol.* **66**, 55E–62E.
173. Taillefer, R. (1990). Technetium-99m sestamibi myocardial imaging: Same-day rest–stress studies and dipyridamole. *Am. J. Cardiol.* **66**, 80E–84E.
174. Bruce, I. N., Burns, R. J., Gladman, D. D., and Urowitz, M. B. (2000). Single photon emission computed tomography dual isotope myocardial perfusion imaging in women with systemic lupus erythematosus. I. Prevalence and distribution of abnormalities. *J. Rheumatol.* **27**, 2372–2377.
175. Sun, S. S., Shiau, Y. C., Tsai, S. C., Lin, C. C., Kao, A., and Lee, C. C. (2001). The role of technetium-99m sestamibi myocardial perfusion single-photon emission computed tomography (SPECT) in the detection of cardiovascular involvement in systemic lupus erythematosus patients with nonspecific chest complaints. *Rheumatology (Oxford)* **40**, 1106–1111.
176. Samoilenko, L. E., Fomicheva, O. A., Nasonov, E. L., Samko, A. N., Karpov Iu, A., and Sergienko, V. B. (2001). [Myocardial microcirculation in patients with systemic lupus erythematosus]. *Ter. Arkh.* **73**, 29–33.
177. Selzer, F., Sutton-Tyrrell, K., Fitzgerald, S. G., Tracy, R., Kuller, L. H., and Manzi, S. (2001). Vascular stiffness in women with systemic lupus erythematosus. *Hypertension* **37**, 1075–1082.
178. Lima, D. S., Sato, E. I., Lima, V. C., Miranda, F., Jr., and Hatta, F. H. (2002). Brachial endothelial function is impaired in patients with systemic lupus erythematosus. *J. Rheumatol.* **29**, 292–297.
179. Haider, Y. S., and Roberts, W. C. (1981). Coronary arterial disease in systemic lupus erythematosus: Quantification of degree of narrowing in 22 necropsy patients (21 women) aged 16 to 37 years. *Am. J. Med.* **70**, 775–781.
180. Abu-Shakra, M., Urowitz, M. B., Gladman, D. D., and Gough, J. (1995). Mortality studies in systemic lupus erythematosus. Results from a single center. I. Causes of death. *J. Rheumatol.* **22**, 1259–1264.
181. Rubin, L. A., Urowitz, M. B., and Gladman, D. D. (1985). Mortality in systemic lupus erythematosus: The bimodal pattern revisited. *Q. J. Med.* **55**, 87–98.
182. Shome, G. P., Sakauchi, M., Yamane, K., Takemura, H., and Kashiwagi, H. (1989). Ischemic heart disease in systemic lupus erythematosus. A retrospective study of 65

patients treated with prednisone. *Jpn. J. Med.* **28**, 599–603.
183. Hearth-Holmes, M., Baethge, B. A., Broadwell, L., and Wolf, R. E. (1995). Dietary treatment of hyperlipidemia in patients with systemic lupus erythematosus. *J. Rheumatol.* **22**, 450–454.
184. Urowitz, M. B., Bookman, A. A. M., Koehler, B. E., Gordon, D. A., Smythe, H. A., and Ogryzlo, M. A. (1976). The bimodal mortality pattern of systemic lupus erythematosus. *Am. J. Med.* **60**, 221–225.
185. Gladman, D. D., and Urowitz, M. B. (1987). Morbidity in systemic lupus erythematosus. *J. Rheumatol.* **14**(Suppl. 13), 223–226.
186. Jonsson, H., Nived, O., and Sturfelt, G. (1989). Outcome in systemic lupus erythematosus: A prospective study of patients from a defined population. *Medicine* **68**, 141–150.
187. Minick, C. R., and Murphy, G. E. (1966). Experimental induction of atherosclerosis by the synergy of allergic injury to arteries and lipid rich diet. I Effect of repeated injections of mouse serum in rabbits fed a dietary cholesterol supplement. *J. Exp. Med.* **124**, 635–652.
188. Minick, C. R., and Murphy, G. E. (1973). Experimental induction of atherosclerosis by the synergy of allergic injury to arteries and lipid rich diet. II Effect of repeatedly injecting foreign protein in rabbits fed a lipid-rich cholesterol-poor diet. *Am. J. Pathol.* **73**, 265–300.
189. Hardin, M. J., Minick, C. R., and Murphy, G. E. (1973). Experimental induction of atherosclerosis by the synergy of allergic injury to arteries and lipid rich diet. III The role of earlier acquired fibromuscular intimal thickening in the pathogenesis of later developing arteriosclerosis. *Am. J. Pathol.* **73**, 301–327.
190. Minick, C. R., Fabricant, C. G., Fabricant, J., and Litrenta, M. M. (1979). Athero/arteriosclerosis induced by infection with a Herpes virus. *Am. J. Pathol.* **96**, 673–706.
191. Kliendienst, R., Schett, G., Amberger, A., *et al*. (1995). Atherosclerosis as an autoimmune condition. *Isr. J. Med. Sci.* **31**, 596–599.
192. Hang, L. M., Izui, S., and Dixon, F. J. (1981). (NZW × BXSB) F1 hybrid: A model of acute lupus and coronary vascular disease with myocardial infarction. *J. Exp. Med.* **154**, 216–221.
193. Berden, J. H. M., Hang L. M., McConahey, P. J., and Dixon, F. J. (1983). Analysis of vascular lesions in murine SLE. I. Association with serological abnormalities. *J. Immunol.* **130**, 1699–1705.
194. Hang, L. M., Stephan-Larson, P. M., Henry, J. P., and Dixon, F. J. (1983). The role of hypertension in the vascular disease and myocardial infarcts associated with murine systemic lupus erythematosus. *Arthritis Rheum.* **26**, 1340–1345.
195. Hudgins, C. C., Steinberg, R. T., Klinman, D. M., Reeves, M. J. P., and Steinberg, A. D. (1985). Studies of consomic mice bearing the y chromosome of the BXSB mouse. *J. Immunol.* **134**, 3849–3854.
196. Yoshida, H., Fujiwara, H., Fujiwara, T., Ikehara, S., and Hamashema, Y. (1987). Quantitative analysis of myocardial infarction in NZW × BXSB F1 hybrid mice with systemic lupus erythematosus and small coronary artery disease. *Am. J. Pathol.* **129**, 477–485.
197. Kabakov, A. E., Tertov, V. V., Saenko, V. A., Poverenny, A. M., and Orekhov, A. N. (1992). The atherogenic effect of lupus sera: Systemic lupus erythematosus-derived immune complexes stimulate the accumulation of cholesterol in cultured smooth muscle cells from human aorta. *Clin. Immunol. Immunopathol.* **63**, 214–220.
198. Fukumoto, S., Tsumagari, T., Kinjo, M., and Tanaka, K. (1987). Coronary atherosclerosis in patients with systemic lupus erythematosus at autopsy. *Acta Pathol. Jpn*. **37**, 1–9.
199. Asherson, R. A., Khamashta, M. A., Baguley, E., Oakley, C. M., Rowell, N. R., and Hughes, G. R. V. (1989). Myocardial infarction and antiphospholipid antibodies in SLE and related disorders. *Q. J. Med.* **73**, 1103–1115.
200. Kattwinkel, N., Villanueva, A. G., Labib, S. B., Aretz, H. T., Walek, J. W., Burns, D. L., *et al*. (1992). Myocardial infarction caused by cardiac microvasculopathy in a patient with the primary antiphospholipid syndrome. *Ann. Intern. Med.* **116**, 974–976.
201. Hamsten, A., Norberg, R., Björkholm, M., de Faire, U., and Holm, G. (1986). Antibodies to cardiolipin in young survivors of myocardial infarction: An association with recurrent cardiovascular events. *Lancet* **1**, 113–116.
202. Morton, K. E., Gavaghan, T. P., Krilis, S. A., Daggard, G. E., Baron, D. W., Hickie, J. B., *et al*. (1986). Coronary artery bypass graft failure—an autoimmune phenomenon? *Lancet* **2**, 1353–1357.
203. De Caterina, R., D'Arcanio, A., Mazzone, A., Gazzetti, P., Bernini, W., Neri, R., *et al*. (1990). Prevalence of anticardiolipin antibodies in coronary artery disease. *Am. J. Cardiol.* **65**, 922–924.
204. Cortellaro, M., Boschetti, C., Cardillo, M., and Barbui, T. (1992). Antiphospholipid antibodies in patients with previous myocardial infarction [letter]. *Lancet* **339**, 929–930.
205. Sletnes, K. E., Smith, P., Abdelnoor, M., Arnesen, H., and Wisloff, F. (1992). Antiphospholipid antibodies after myocardial infarction and their relation to mortality, reinfarction, and non-haemorrhagic stroke. *Lancet* **339**, 451–453.
206. Edwards, T., Thomas, R. D., and McHugh, N. J., (1993). Anticardiolipin antibodies in ischaemic heart disease [letter]. *Lancet* **342**, 989.
207. Phadke, K. V., Phillips, R. A., Clarke, D. T., Jones, M., Naish, P., and Carson, P. (1993). Anticardiolipin antibodies in ischaemic heart disease: Marker or myth? *Br. Heart J.* **69**, 391–394.
208. Yilmaz, E., Adalet, K., Yilmaz, G., Badur, S., Erzengin, F., Koylan, N., *et al*. (1994). Importance of serum anticardiolipin antibody levels in coronary heart disease. *Clin. Cardiol.* **17**, 117–121.
209. Raghavan, C., Ditchfield, J., Taylor, R. J., Haeney, M. R., and Barnes, P. C. (1993). Influence of anticardiolipin antibodies on immediate patient outcome after myocardial infarction. *J. Clin. Pathol.* **46**, 1113–1115.
210. Gavaghan, T. P., Krilis, S. A., Daggard, G. E., Baron, D. E., Hickie, J. B., and Chesterman, C. N. (1987). Anti-

cardiolipin antibodies and occlusion of coronary artery bypass grafts [letter]. *Lancet* **2**, 977–978.
211. Bick, R. L. (1993). The antiphospholipid-thrombosis syndromes. Fact, fiction, confusion, and controversy [editorial]. *Am. J. Clin. Pathol.* **100**, 477–480.
212. Klemp, P., Cooper, R. C., Strauss, F. J., Jordaan, E. R., Przybojewski, J. Z., and Nel, N. (1988). Anti-cardiolipin antibodies in ischaemic heart disease. *Clin. Exp. Immunol.* **74**, 254–257.
213. Vaarala, O., Alfthan, G., Jauhiainen, M., Leirisalo-Repo, M., Aho, K., and Palosua, T. (1993). Cross reaction between antibodies to oxidized low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. *Lancet* **341**, 923–925.
214. Vaarala, O. (1998). Antiphospholipid antibodies and myocardial infarction. *Lupus* **7**(Suppl. 2), S132–S134.
215. Aho, K., Vaarala, O., Tenkanen, L., Julkunen, H., Jouhikainen, T., Alfthan, G., *et al*. (1996). Antibodies binding to anionic phospholipids but not to oxidized low-density lipoprotein are associated with thrombosis in patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **14**, 499–506.
216. Romero, F. I., Amengual, O., Atsumi, T., Khamashta, M. A., Tinahones, F. J., and Hughes, G. R. (1998). Arterial disease in lupus and secondary antiphospholipid syndrome: Association with anti-β_2-glycoprotein I antibodies but not with antibodies against oxidized low-density lipoprotein. *Br. J. Rheumatol.* **37**, 883–888.
217. Hayem, G., Nicaise-Roland, P., Palazzo, E., de Bandt, M., Tubach, F., Weber, M., *et al*. (2001). Anti-oxidized low-density-lipoprotein (OxLDL) antibodies in systemic lupus erythematosus with and without antiphospholipid syndrome. *Lupus* **10**, 346–351.
218. Khamashta, M., and Petri, M. (1996). Antiphospholipid antibodies hasten atheroma [editorial]. *Lancet* **348**, 1088.
219. Matsuura, E., Kobayashi, K., Yasuda, T., and Koike, T. (1998). Antiphospholipid antibodies and atherosclerosis. *Lupus* **7**(Suppl. 2), S135–S139.
220. Kabarowski, J. H., Zhu, K., Le, L. Q., Witte, O. N., and Xu, Y. (2001). Lysophosphatidylcholine as a ligand for the immunoregulatory receptor G2A. *Science* **293**, 702–705.
221. George, J., Harats D., Gilburd, B., Levy, Y., Langevitz, P., and Shoenfeld, Y. (1999). Atherosclerosis-related markers in systemic lupus erythematosus patients: The role of humoral immunity in enhanced atherogenesis. *Lupus* **8**, 220–226.
222. Wu, R., Svenungsson, E., Gunnarsson, I., Andersson, B., Lundberg, I., Schafer Elinder, L., *et al*. (1999). Antibodies against lysophosphatidylcholine and oxidized LDL in patients with SLE. *Lupus* **8**, 142–150.
223. Saikku, P. (1997). *Chlamydia pneumoniae* and atherosclerosis—an update. *Scand. J. Infect. Dis. Suppl.* **104**, 53–56.
224. Jackson, L. A., Campbell, L. A., Schmidt, R. A., Kuo, C. C., Cappuccio, A. L., Lee, M. J., *et al*. (1997). Specificity of detection of *Chlamydia pneumoniae* in cardiovascular atheroma: Evaluation of the innocent bystander hypothesis. *Am. J. Pathol.* **150**, 1785–1790.
225. Moazed, T. C., Kuo, C., Grayston, J. T., and Campbell, L. A. (1997). Murine models of *Chlamydia pneumoniae* infection and atherosclerosis. *J. Infect. Dis.* **175**, 883–890.
226. Blasi, F., Denti, F., Erba, M., Cosentini, R., Raccanelli, R., Rinaldi, A., *et al*. (1996). Detection of *Chlamydia pneumoniae* but not *Helicobacter pylori* in atherosclerotic plaques of aortic aneurysms. *J. Clin. Microbiol.* **34**, 2766–2769.
227. Gupta, S., and Leatham, E. W. (1997). The relation between *Chlamydia pneumoniae* and atherosclerosis [editorial]. *Heart* **77**, 7–8.
228. Juvonen, J., Juvonen, T., Laurila, A., Alakarppa, H., Lounatmaa, K., Surcel, H. M., *et al*. (1996). Immunohistochemical detection of *Chlamydia pneumoniae* in abdominal aortic aneurysms. *Ann. N. Y. Acad. Sci.* **800**, 236–238.
229. Wimmer, M. L., Sandmann-Strupp, R., Saikku, P., and Haberl, R. L. (1996). Association of chlamydial infection with cerebrovascular disease. *Stroke* **27**, 2207–2210.
230. Cook, P. J., and Lip, G. Y. (1996). Infectious agents and atherosclerotic vascular disease. *Q. J. Med.* **89**, 727–735.
231. Capron, L. (1996). Chlamydia in coronary plaques—hidden culprit or harmless hobo? *Nat. Med.* **2**, 856–857.
232. Ong, G., Thomas, B. J., Mansfield, A. O., Davidson, B. R., and Taylor-Robinson, D. (1996). Detection and widespread distribution of *Chlamydia pneumoniae* in the vascular system and its possible implications. *J. Clin. Pathol.* **49**, 102–106.
233. Kuo, C. C., Grayston, J. T., Campbell, L. A., Goo, Y. A., Wissler, R. W., and Benditt, E. P. (1995). *Chlamydia pneumoniae* (TWAR) in coronary arteries of young adults (15–34 years old). *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6911–6914.
234. Esdaile, J. M., Abrahamowicz, M., Grodzicky, T., Li, Y., Panaritis, C., du Berger, R., *et al*. (2001). Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum.* **44**, 2331–2337.
235. Roman, M. J., Salmon, J. E, Sobel, R., Lockshin, M. D., Sammaritano, L., Schwartz, J. E., *et al*. (2001). Prevalence and relation to risk factors of carotid atherosclerosis and left ventricular hypertrophy in systemic lupus erythematosus and antiphospholipid antibody syndrome. *Am. J. Cardiol.* **87**, 663–666, A11.
236. Rahman, P., Bruce, I. N., Hallett, D., *et al*. (1998). Contribution of traditional risk factors to coronary artery disease in patients with SLE. *J. Rheumatol.* **25**(Suppl. 52), S73.
237. Petri, M., Spence, D., Bone, L. R., and Hochberg, M. C. (1992). Coronary artery disease risk factors in the Hopkins Lupus Cohort: Prevalence, patient recognition, and preventive practices. *Medicine* **71**, 291–302.
238. Rahman, P., Aguero, S., Gladman, D. D., Hallett, D., and Urowitz, M. B. (2000). Vascular events in hypertensive patients with systemic lupus erythematosus. *Lupus* **9**, 672–675.
239. Ilowite, N. T., Samuel, P., Ginzler, E., and Jacobson, M. S. (1988). Dyslipoproteinemia in pediatric systemic lupus erythematosus. *Arthritis Rheum.* **31**, 859–863.

240. Lahita, R. G., Rivkin, E., Cavanagh, I., and Romano, P. (1993). Low levels of total cholesterol, high-density lipoprotein, and apolipoprotein A1 in association in patients with systemic lupus erythematosus. *Arthritis Rheum.* **36**, 1566–1574.
241. Borba, E. F., and Bonfa, E. (1997). Dyslipoproteinemias in systemic lupus erythematosus: Influence of disease activity and anticardiolipin antibodies. *Lupus* **6**, 533–539.
242. Alekberova, Z. S., Popkova, T. V., Nasonov, E. L., Reshetniak, T. M., Ozerova, I. N., and Perova, N. V. (1999). [The lipid-protein indices of the blood cholesterol transport system in systemic lupus erythematosus]. *Ter. Arkh.* **71**, 34–38.
243. Ettinger, W. H., Goldberg, A. P., Applebaum-Bowden, D., and Hazzard, W. R. (1987). Dyslipoproteinemia in systemic lupus erythematosus. Effect of corticosteroids. *Am. J. Med.* **83**, 503–508.
244. Bruce, I. N., Urowitz, M. B., and Gladman, D. D. (1999). The natural history of hypercholesterolaemia in systemic lupus erythematosus. *J. Rheumatol.* **26**, 2137–2143.
245. Borba, E. F., Bonfa, E., Vinagre, C. G., Ramires, J. A., and Maranhao, R. C. (2000). Chylomicron metabolism is markedly altered in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 1033–1040.
246. Romero, F. I., Khamashta, M. A., and Hughes, G. R. (2000). Lipoprotein(a) oxidation and autoantibodies: A new path in atherothrombosis. *Lupus* **9**, 206–209.
247. Okawa-Takatsuji, M., Aotsuka, S., Sumiya, M., Ohta, H., Kawakami, M., and Sakurabayashi, I. (1996). Clinical significance of the serum lipoprotein(a) level in patients with systemic lupus erythematosus: Its elevation during disease flare. *Clin. Exp. Rheumatol.* **14**, 531–536.
248. George, J., Gilburd, B., Langevitz, P., Levy, Y., Nezlin, R., Harats, D., *et al*. (1999). Beta2 glycoprotein I containing immune-complexes in lupus patients: Association with thrombocytopenia and lipoprotein (a) levels. *Lupus* **8**, 116–120.
249. Font, J., Ramos-Casals, M., Cervera, R., Garcia-Carrasco, M., Torras, A., Siso, A., *et al*. (2001). Cardiovascular risk factors and the long-term outcome of lupus nephritis. *Q. J. Med.* **94**, 19–26.
250. Bono, L., Cameron, J. S., and Hicks, J. A. (1999). The very long-term prognosis and complications of lupus nephritis and its treatment. *Q. J. Med.* **92**, 211–218.
251. Clark, W. F., and Moist, L. M. (1998). Management of chronic renal insufficiency in lupus nephritis: Role of proteinuria, hypertension and dyslipidemia in the progression of renal disease. *Lupus* **7**, 649–653.
252. Luzar, B., and Ferluga, D. (2000). Role of lipids in the progression of renal disease in systemic lupus erythematosus patients. *Wien. Klin. Wochenschr.* **112**, 716–721.
253. Falaschi, F., Ravelli, A., Martignoni, A., Migliavacca, D., Sartori, M., Pistorio, A., *et al*. (2000). Nephrotic-range proteinuria, the major risk factor for early atherosclerosis in juvenile-onset systemic lupus erythematosus. *Arthritis Rheum.* **43**, 1405–1409.
254. Harpper, A. E., Benevenga, N. J., and Wohlheuter, R. M. (1976). Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev.* **50**, 428–558.
255. Rodgers, G. M., and Kane, W. H. (1986). Activation of endogenous Factor V by a homocysteine induced vascular endothelial cell activator. *J. Clin. Invest.* **77**, 1909–1916.
256. Tsai, J.-C., Perrella, M. A., Yoshizumi, M., Hsieh, C.-M., Haber, E., Schlegel, R., *et al*. (1994). Promotion of vascular smooth muscle cell growth by homocysteine: A link to atherosclerosis. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6369–6373.
257. Stampfer, M. J., Malinow, M. R., Willett, W. C., *et al*. (1992). A prospective study of plasma homocysteine and risk of myocardial infarction in U.S. physicians. *JAMA* **268**, 877–881.
258. Selhub, J., Jacques, P. F., Bostom, A. G., D'Agostino, R. B., Wilson, P. W. F., Belanger, A. J., *et al*. (1995). Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N. Engl. J. Med.* **332**, 286–291.
259. Petri, M., Roubenoff, R., Dallal, G. E., Nadeau, M. R., Selhub, J. and Rosenberg, I. H. (1996). Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. *Lancet* **348**, 1120–1124.
260. Fijnheer, R., Roest, M., Haas, F. J., De Groot, P. G., and Derksen, R. H. (1998). Homocysteine, methylenetetrahydrofolate reductase polymorphism, antiphospholipid antibodies, and thromboembolic events in systemic lupus erythematosus: A retrospective cohort study. *J. Rheumatol.* **25**, 1737–1742.
261. Ridker, P. M., Hennekens, C. H., Buring, J. E., and Rifai, N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med.* **342**, 836–843.
262. Ames, P. R., Alves, J., Pap, A. F., Ramos, P., Khamashta, M. A., and Hughes, G. R. (2000). Fibrinogen in systemic lupus erythematosus: More than an acute phase reactant? *J. Rheumatol.* **27**, 1190–1195.
263. Rahman, P., Gladman, D. D., and Urowitz, M. B. (2000). Premature coronary artery disease in systemic lupus erythematosus in the absence of corticosteroid use. *J. Rheumatol.* **27**, 1323–1325.
264. Nashel, D. J. (1986). Is atherosclerosis a complication of long-term corticosteroid treatment? *Am. J. Med.* **80**, 925–929.
265. Stamler, J., Pick, R., and Katz, L. N. (1954). Effects of cortisone, hydrocortisone and corticotropin on lipemia, glycemia and atherogenesis in cholesterol-fed chicks. *Circulation* **10**, 237–246.
266. Bjorkerud, S. (1974). Effect of adrenocortical hormones on the integrity of rat aortic endothelium. In "Atherosclerosis III; Proceedings of the Third International Symposium" (G. Schettler and A. Weizel, Eds.), pp. 245–249. Springer-Verlag, Berlin.
267. Lorenzen, I., and Hansen, L. K. (1967). Effect of glucocorticoids on human vascular connective tissue. *Vasc. Dis.* **4**, 335–341.
268. Rosenfeld, S., Marmorston, J., Sobel, H., and White, A. E. (1960). Enhancement of experimental atherosclerosis by ACTH in the dog. *Proc. Soc. Exp. Biol. Med.* **103**, 83–86.

269. Christy, N. P. (1971). Cushing's syndrone: The natural history. In "The Human Adrenal Cortex" (N. P. Christy, Ed.), pp. 359–394. Harper & Row, New York.
270. Bruce, I. N., Gladman, D. D., and Urowitz, M. B. (1998). Detection and modification of risk factors for coronary artery disease in patients with systemic lupus erythematosus: A quality improvement study. *Clin. Exp. Rheumatol.* **16**, 435–440.
271. Rosenbaum, M., Leibel, R. I., and Hirsch, J. (1997). Obesity. *N. Engl. J. Med.* **337**, 396–406.
272. Methods for voluntary weight loss and control: NIH Technology Assessment Conference Panel: Consensus Development Conference, 30 March to 1 April (1992). *Ann. Intern. Med.* **119**, 764–770.
273. Schaefer, E. J., Lichtenstein, A. H., Lamon-Fava, S., *et al*. (1995). Body weight and low-density lipoprotein cholesterol changes after cosumption of a low-fat libitum diet. *JAMA* **274**, 1450–1455.
274. Sheppard, L., Kristal, A. R., and Kushi, L. H. (1991). Weight loss in women participating in a randomized trial of low-fat diets. *Am. J. Clin. Nutr.* **54**, 821–888.
275. Tremblay, A., and Buemann, B. (1995). Exercise-training, macronutrient balance and body weight control. *Int. J. Obes. Relat. Metab. Disord.* **19**, 79–86.
276. Robb-Nicholson, L. C., Daltroy, L., Eaton, H., Gall, V., Wright, E., Hartley, L. H., *et al*. (1989). Effects of aerobic conditioning in lupus fatigue: A pilot study. *Br. J. Rheum.* **28**, 500–505.
277. Petri, M. (1996). Hydroxychloroquine use in the Baltimore Lupus Cohort: Effects on lipids, glucose and thrombosis. *Lupus* **5**(Suppl. 1), S16–S22.
278. Haffner, S. M., and Miettinen, H. (1997). Insulin resistance implications for Type II diabetes mellitus and coronary heart disease. *Am. J. Med.* **103**, 152–162.
279. Nolan, J. J., Ludvik, B., Beerdsen, P., Joyce, M., and Olefsky, J. (1994). Improvement in glucose tolerance and insulin resistance in obese subjects treated with Troglitazone. *N. Engl. J. Med.* **331**, 1188–1193.
280. Katan, M. B., Grundy, S. M., and Willett, W. C. (1997). Should a low-fat, high-carbohydrate diet be recommended for everyone? *N. Engl. J. Med.* **337**, 561–567.
281. Petri, M., Turner, M., Wagner, J., Wilder, L., and Goldman, D. (1993). Dietary intervention for hypercholesterolemia in systemic lupus erythematosus [abstract]. *Arthritis Rheum.* **36**(Suppl.), S186.
282. Bertolini, S., Bon, G. B., Campbell, L. M., Farnier, M., Langan, J., Mahla, G., *et al*. (1997). Efficacy and safety of atorvastatin compared to pravastatin in patients with hypercholesterolemia. *Atherosclerosis* **130**, 191–197.
283. Dart, A., Jerums, G., Nicholson, G., d'Emden, M., Hamilton-Craig, I., Tallis, G., *et al*. (1997). A multicenter, double-blind, one-year study comparing safety and efficacy of atorvastatin versus simvastatin in patients with hypercholesterolemia. *Am. J. Cardiol.* **80**, 39–44.
284. Jimenez-Alonso, J., Osorio, J. M., Gutierrez-Cabello, F., Lopez de la Osa, A., Leon, L., and Mediavilla Garcia, J. D. (1999). Atorvastatin-induced cholestatic hepatitis in a young woman with systemic lupus erythematosus. Grupo Lupus Virgen de las Nieves. *Arch. Intern. Med.* **159**, 1811–1821.
285. Rahman, P., Gladman, D. D., Urowitz, M. B., Yuen, K., Hallett, D., and Bruce, IN. (1999). The cholesterol lowering effect of antimalarial drugs is enhanced in patients with lupus taking corticosteroid drugs. *J. Rheumatol.* **26**, 325–330.
286. Tam, L. S., Gladman, D. D., Hallett, D. C., Rahman, P., and Urowitz, M. B. (2000). Effect of antimalarial agents on the fasting lipid profile in systemic lupus erythematosus. *J. Rheumatol.* **27**, 2142–2145.
287. Borba, E. F., and Bonfa, E. (2001). Long-term beneficial effect of chloroquine diphosphate on lipoprotein profile in lupus patients with and without steroid therapy. *J. Rheumatol.* **28**, 780–785.
288. Baguet, J. P., Tremel, F., and Fabre, M. (1999). Chloroquine cardiomyopathy with conduction disorders. *Heart* **81**, 221–223.
289. The SOLVD Investigators. (1992). Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. *N. Engl. J. Med.* **327**, 685–691.
290. Swedberg, K., Held, P., Kjekshus, J., Rasmussen, K., Ryden, L., and Wedel, H. (1992). Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction. Results of the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II). *N. Engl. J. Med.* **327**, 678–684.
291. Lithell, H. (1991). Effect of antihypertensive drugs on insulin, glucose, and lipid metabolism. *Diabetes Care* **14**, 203–209.
292. Mykkänen, L., Kuusisto, J., Pyörälä, K., Laakso, M., and Haffner, S. M. (1994). Increased risk of non-insulin dependent diabetes mellitus in elderly hypertensive subjects. *J. Hypertens.* **12**, 1425–1432.
293. Grodstein, F., Stampfer, M. J., Manson, J. E., *et al*. (1996). Postmenopausal estrogen and progestin use and the risk of cardiovascular disease. *N. Engl. J. Med.* **335**, 453–461.
294. Darling, G. M., Johns, J. A., McCloud, P. I., and Davis, S. R. (1997). Estrogen and progestin compared with simvastation for hypercholesterolemia in postmenopausal women. *N. Engl. J. Med.* **337**, 595–600.
295. Kawas, C., Resnick, S., Morrison, A., *et al*. (1997). A prospective study of estrogen replacement therapy and the risk of developing Alzhemier's disease: The Baltimore longitudinal study of aging. *Neurology* **48**, 1517–1521.
296. Wells, G., and Herrington, D. M. (1999). The Heart and Estrogen/Progestin Replacement Study: What have we learned and what questions remain? *Drugs Aging* **15**, 419–422.
297. Daly, E., Vessey, M. P., Hawkins, M. M., Carson, J. L., Gough, P., and Marsh, S. (1996). Risk of venous thromboembolism in users of hormone replacement therapy. *Lancet* **348**, 977–980.
298. Sanchez-Guerrero, J., Liang, M. H., Karlson, E. W., Hunter, D. J., and Colditz, G. A. (1995). Postmenopausal estrogen therapy and the risk for developing systemic lupus erythematosus. *Ann. Intern. Med.* **122**, 430–433.

299. Colditz, G. A., Hankinson, S. E., Hunter, D. J., *et al*. (1995). The use of estrogens and progestins and the risk of breast cancer in postmenopausal women. *N. Engl. J. Med.* **332**, 1589–1593.
300. Compston, J. E. (1997). Designer oestrogens: Fact or fantasy? *Lancet* **350**, 676–677.
301. Hammerman, H., Kloner, R. A., Hale, S., Schoen, F. J., and Braunwald, E. (1983). Dose-dependent effects of short-term methylprednisolone on myocardial infarct extent, scar formation, and ventricular function. *Circulation* **68**, 446–452.
302. Khamashta, M. A., Cuadrado, M. J., Mujic, F., Taub, N. A., Hunt, B. J., and Hughes, G. R. V. (1995). The management of thrombosis in the antiphospholipid antibody syndrome. *N. Engl. J. Med.* **332**, 993–997.
303. Erkan, D., Merrill, J. T., Yazici, Y., Sammaritano, L., Buyon, J. P., and Lockshin, M. D. (2001). High thrombosis rate after fetal loss in antiphospholipid syndrome: Effective prophylaxis with aspirin. *Arthritis Rheum.* **44**, 1466–1467.
304. Meroni, P. L., Raschi, E., Camera, M., Testoni, C., Khamashta, M. A., and Tremoli, E. (2000). Fluvastatin inhibits the activation of endothelial cells induced by antiphospholipid antibodies *in vitro* [abstract]. *Arthritis Rheum.* **43**(Suppl. 9), S115.
305. Laman, J. D., de Smet, B. J. G. L., Schoneveld, A., and van Meurs, M. (1997). CD40–CD40L interactions in atherosclerosis. *Immunol. Today* **18**, 272–277.
306. Kalunian, K. C., Davis, J., Merrill, J. T., Petri, M., Buyon, J., Ginzler, E., *et al*. (2000). Treatment of systemic lupus erythematosus by inhibition of T cell costimulation [abstract]. *Arthritis Rheum.* **43**(Suppl. 9), S271.
307. Rosner, S., Ginzler, E. M., Diamond, H. S., Weiner, M., Schlesinger, M., Fries, J. E., *et al*. (1982). A multicenter study of outcome in systemic lupus erythematosus. II. Causes of death. *Arthritis Rheum.* **25**, 612–617.
308. Karsh, J., Klippel, J. H., Balow, J. E., and Decker, J. L. (1979). Mortality in lupus nephritis. *Arthritis Rheum.* **22**, 764–769.
309. Ward, M. M., Pyun, E., and Studenski, S. (1995). Causes of death in systemic lupus erythematosus. *Arthritis Rheum.* **38**, 1492–1499.
310. Reveille, J. S., Bartolucci, A., and Alarcón, G. S. (1990). Prognosis in systemic lupus erythematosus: Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. *Arthritis Rheum.* **33**, 37–48.
311. Wallace, D. J., Podell, T., Weiner, J., Klinenberg, J. R., Forouzesh, S., and Dubois, E. L. (1981). Systemic lupus erythematosus—survival patterns: Experience with 609 patients. *J. Am. Med. Assoc.* **245**, 934–938.
312. Helve, T. (1985). Prevalence and mortality rates of systemic lupus erythematosus and causes of death in SLE patients in Finland. *Scand. J. Rheumatol.* **14**, 43–46.
313. Moro, H., Hayashi, J., Okazaki, H., Takahashi, Y., Eguchi, S., Yazawa, M., *et al*. (1996). [Open heart surgery in patients with systemic diseases requiring steroid treatment]. *Nippon Kyobu Geka Gakkai Zasshi* **44**, 493–498.
314. Moro, H., Hayashi, J., Ohzeki, H., Sogawa, M., Nakayama, T., and Namura, O. (1999). Surgical management of cardiovascular lesions caused by systemic inflammatory diseases. *Thorac. Cardiovasc. Surg.* **47**, 106–110.
315. Grigor, R., Edmonds, J., Lewkonia, R., Bresnihan, B., and Hughes, G. R. (1978). Systemic lupus erythematosus. A prospective analysis. *Ann. Rheum Dis.* **37**, 121–128.
316. Koh, H. K., Yoo, D. H., Yoo, T. S., Jun, J. B., Jung, S. S., Lee, J. U., *et al*. (1998). Coexistence of coronary aneurysms and total occlusion of coronary arteries in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **16**, 739–742.
317. Dhond, M. R., Matayoshi, A., and Laslett, L. (1999). Coronary artery aneurysms associated with systemic lupus. *Clin. Cardiol.* **22**, 373.
318. Matayoshi, A. H., Dhond, M. R., and Laslett, L. J. (1999). Multiple coronary aneurysms in a case of systemic lupus erythematosus. *Chest* **116**, 1116–1118.

32

SYSTEMIC LUPUS ERYTHEMATOSUS AND THE CARDIOVASCULAR SYSTEM: VASCULITIS

Barri J. Fessler
Karen E. Rendt
Gary S. Hoffman

Clinical manifestations of vascular injury in systemic lupus erythematosus (SLE) may develop due to vasculitis and/or noninflammatory vasocclusive disease [1]. The expression of vasculitis may take several forms: isolated cutaneous lesions, single visceral organ involvement, or a multiorgan system disease [2]. Our current understanding of vasculitis in SLE patients is based on case reports or small case series. Determination of the true incidence of vasculitis in SLE is complicated by the fact that the diagnosis of vasculitis is often a clinical diagnosis, lacking histologic or arteriographic confirmation. Many of the more extensive SLE series [3–6] fail to document the incidence of vasculitis, and those that do provide proof of vasculitis indicate that it is an infrequent complication of SLE (Table 1). In view of its uncommon occurrence, it is difficult to accumulate significant numbers of SLE patients who have vasculitis, for either pathophysiologic studies or prospective trials of therapeutic agents. In addition, noninflammatory vasculopathies are confounding variables as they may mimic or rarely coexist with vasculitis [7–10].

A variety of noninflammatory vasculopathies may be observed in SLE [1]. Endothelial cell injury and/or activation resulting in intimal proliferation may lead to thickening of the walls of small blood vessels. Such changes are often widely distributed and may be associated with "micro-" or small visible infarcts that may or may not contribute to organ dysfunction [11, 12]. Activation of neutrophils and upregulation of endothelial cell adhesion molecules following exposure to complement split products and other chemoattractants may lead to leukoaggregation within the vasculature, causing transient or permanent end organ damage [13–15]. Occlusion of vessels by platelet-fibrin thrombi associated with clinical features of thrombotic thrombocytopenic purpura has also been observed in a small number of SLE patients [16–18]. Lastly, the most common and clinically significant vasculopathy in SLE is associated with the presence of anti-phospholipid antibodies (See Chapters 39–43). These different forms of vasculopathies are generally not inflammatory and are not regarded as true vasculitis. However, vasculitis may rarely occur concomitant with the anti-phospholipid antibody syndrome [8–10, 19].

PATHOGENESIS OF VASCULITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Many immunologic mechanisms are involved in the pathogenesis of vascular injury in SLE. As a consequence of complex interactions between endothelium,

TABLE 1 Incidence of Vasculitis by Organ System in SLE

Organ	Estimated frequency based on clinical diagnosis	Reported frequency based on histologic proof of vasculitis[a]
Skin [5,7,46,48,49,99,166,167]	15–50%	NA[b]
Lung [94,95,168]	1–2%	18-20%
		8/44 autopsy cases [94]
		4/20 autopsy cases [95]
Gastrointestinal tract [47,99,100,129,168–172]	1–3%	24%
		6/25 autopsy cases [172]
Central nervous system [69,70,75]	Uncommon	7–12%
		3/24 autopsy cases [69]
		4/57 autopsy cases [70]
Heart [132,133]	Rare	3–37%
		1/30 autopsy cases [132]
		6/16 autopsy cases [133]
Genitourinary tract [143,147,148–151]	Case reports	NA
Large vessels [7,152-155,158,159]	Case reports small series	NA

[a] May overestimate frequency due to sampling bias, that is, autopsy studies represent patients with fulminant/treatment resistant vasculitis.
[b] NA, not available.

inflammatory cells, autoantibodies, and immune complexes, necrotizing inflammation of blood vessel walls ensues. The trigger that initiates the cascade of interactions between cells and factors that determine the location of vascular inflammation is unknown. Upregulation of adhesion molecules on vascular endothelium and leukocytes is an important determinant of localized inflammation and has been implicated in the development of vasculitis [20]. In one *in vitro* model it has been shown that antigenic fragments of DNA (which can be found in the circulation of some lupus patients) induce human umbilical vein endothelial cells (HUVEC) to express intercellular adhesion molecule-1 (ICAM-1) on their surfaces [21]. Activation of endothelial adhesion molecules would allow adherence to the endothelium by polymorphonuclear cells (PMNs) and lymphocytes and facilitate their entry through the vessel wall into the tissue. Blocking the interaction between adhesion molecules, ICAM-1 and CD18/CD11b, located on the endothelial cell and neutrophil, respectively, prevents the development of vasculitis in an experimental model [15]. In addition, the demonstration of upregulated expression of adhesion molecules CD11a/CD18 and VLA-4 on lymphocytes from SLE patients with vasculitis further supports a role for adhesion molecules in the pathogenesis of vasculitis [22]. However, upregulation of adhesion molecules is also observed in many types of tissue inflammation, including those in which the vessel is not injured.

Immune complex deposition is thought to play a pivotal role in the development of SLE vasculitis. It is not the mere presence of circulating immune complexes that cause disease, but the inflammatory events that occur after their deposition in tissue. Factors that enhance immune complex deposition include (1) moderate antibody production with antigen excess resulting in intermediate-sized immune complexes that are not efficiently cleared; (2) antibody production against a limited number of antigenic determinants or a complex antigen, producing smaller immune complexes that are slowly eliminated; (3) the type of antibody produced, for example, immunoglobulin M (IgM) complexes are larger at all ratios of antigen, unless there is extreme antigen excess, and are more readily eliminated; (4) blockade or overload of the reticuloendothelial system, which may occur from large amounts of immune complexes [23–25]; (5) alteration in vascular permeability (the release of vasoactive amines from platelets and mast cells may cause endothelial cell retraction, allowing immune complexes to enter the vessel wall) [26]; and (6) regional differences in hydrostatic pressures, such as occurs in the glomeruli [27].

Deposition of immune complexes in the vessel wall is followed by activation of the complement cascade. Complement fragments, such as C5a desarg, promote chemotaxis of polymorphonuclear cells and expression of endothelial adhesion molecules [28, 29]. The release of polymorphonuclear enzymes and oxygen radicals

produces tissue injury. One animal study utilizing male albino New Zealand rabbits has indicated the essential role of polymorphonuclear cells in the production of vasculitis in that model system. Serum sickness was produced with bovine serum albumin (BSA) and the immune response being enhanced with either pooled rabbit anti-BSA or *Escherichia coli* endotoxin with the BSA. One group was neutrophil-depleted by pretreatment with anti-polymorphonuclear globulin at a dose sufficient to cause circulating polymorphonuclear counts of <300/mm^3. In this group necrotizing vasculitis was absent [30].

The role, if any, that autoantibodies play in the pathogenesis of vasculitis is unclear. Some have speculated that anti-endothelial antibodies may play a role in triggering the development of vasculitis. Anti-endothelial antibodies obtained from patients with lupus have been studied *in vitro* and found to upregulate endothelial cell expression of VCAM-1, ICAM-1, and E-selectin, as well as enhance leukocyte adhesion to endothelial cells [31, 31a] (Fig. 1). In addition, experimental evidence suggests the existence of cross-reactive epitopes in endothelial cells, lysophosphatidylcholine (LPC) and oxidized LDL. LPC is formed during oxida-

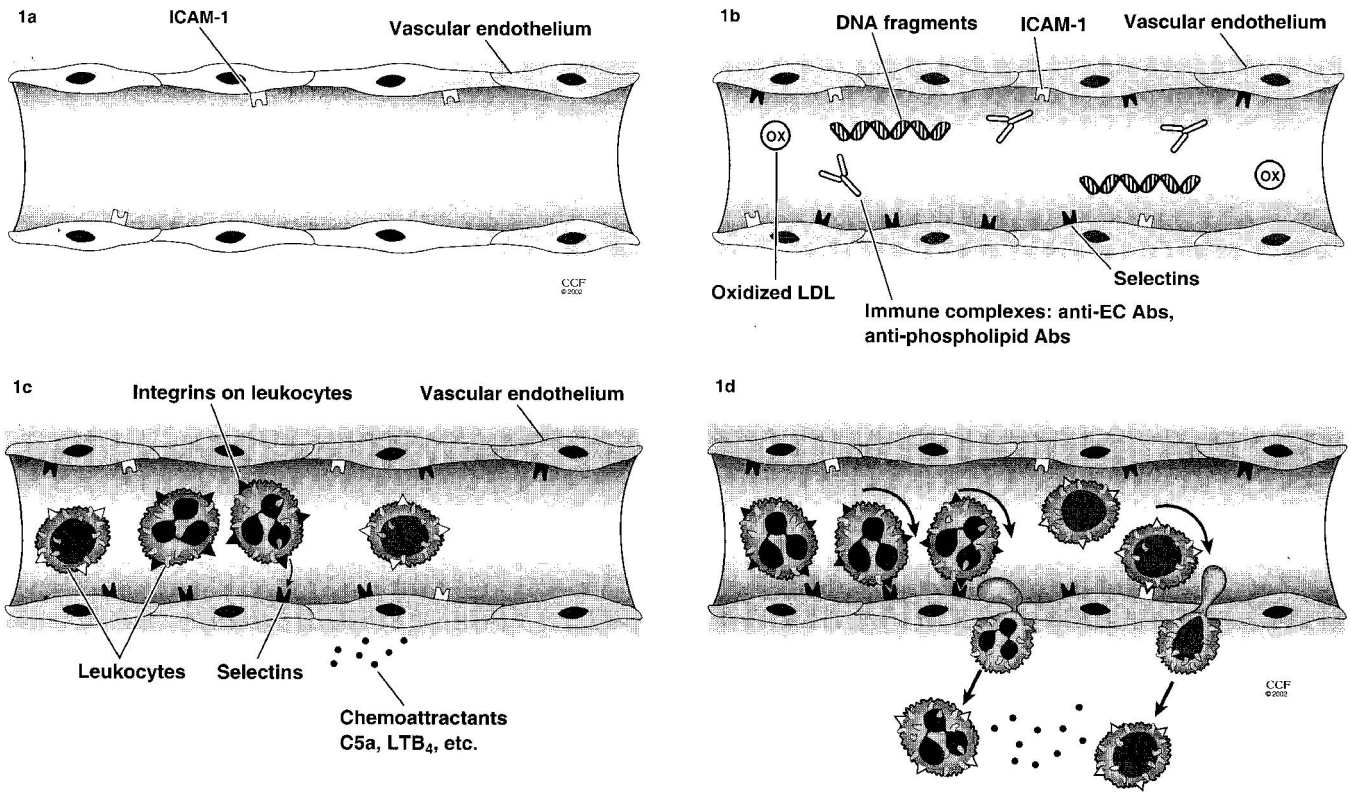


FIGURE 1 Possible mechanisms of vascular injury in SLE. In addition to soluble factors (e.g., complement, leukotrienes, immune complexes) and inflammatory cells (PMNs, lymphocytes), the endothelium itself plays an active role in vascular injury. (a) In the resting state, endothelial cells (EC) exhibit a low level of expression of surface ligands (ICAM-1, and others). (b) Appearance of adhesion melecules (selectins) on the surface of endothelial cells induced by circulating factors (fragments of DNA, anti-endothelial cell antibodies, IL-1, TNF α, lipopolysaccharide, anti-phospholipid antibodies, oxidized LDL, and others). (c) Interaction of activated lymphocytes and PMNs from the circulation with endothelial cells: binding of selectins on EC with their carbohydrate ligands on leukocytes mediates initial tethering and rolling. Chemoattractants promote directed migration of the rolling leukocytes toward the source of the chemoattractant. Binding of the chemoattractant (e.g., C5a, LTB4, PAF, IL-8, and others) to the chemoattractant receptor on the leukocyte triggers integrin expression. (d) The interaction of integrins (LFA-1, VLA-4, and others) on leukocytes with EC surface molecules from the immunoglobulin gene superfamily (ICAM-1, ICAM-2, ICAM-3, VCAM-1, and others) results in tight adhesion of the leukocytes to the ECs, thus permitting diapedesis (migration through the wall and into the tissue beyond). Activation of complement by immune complexes, and release of oxygen radicals by PMNs can contribute to local vascular injury.

tion of LDL or as a result of the action of phospholipase A2 on phosphatidylcholine; it is an important antigen in oxidized LDL. An immune reaction to LPC leading to endothelial attack culminating in vasculitis, thrombosis, or atherosclerosis, is suggested by significantly larger amounts of antibodies to LPC in patients with SLE compared with normal controls [32]. Other proposed mechanisms by which anti-endothelial antibodies may induce vascular injury include: complement fixation [33], recruitment of neutrophils, and antibody-dependent cellular cytotoxicity [34]. Anti-neutrophil cytoplasmic antibodies (ANCA), a heterogeneous group of autoantibodies directed against a variety of antigens within the neutrophil and monocyte, have been associated with certain systemic vasculitides, such as Wegener's granulomatosis [35]. The role that ANCAs play in the pathogenesis of vasculitis is unproven [36]. ANCAs have been isolated in the sera from SLE patients with varying frequency (0–93%) [37–44]. However, the presence of ANCAs in SLE does not correlate with major organ involvement, disease activity, or the presence of vasculitis [38, 40, 42]. False-positive ANCAs in lupus patients may be due to the binding of myeloperoxidase (one of the substrate antigens in ANCA immunoassays) to DNA contained within the antigen binding site of anti-DNA antibodies [45].

As there is heterogeneity in the clinical manifestations of SLE vasculitis, as will be discussed in the next section, it is reasonable to hypothesize that different mechanisms of vascular injury may be operant in different patients, or even in the same patient in different vascular beds. Thus, for example, different factors may be responsible for the development of leukocytoclastic vasculitis in the skin as compared to inflammatory aortic aneurysms. Much has yet to be learned about the determinants of vascular attack by the immune system, both from the standpoint of understanding what activates the circulating leukocyte population, as well as identifying the unique features of specific vascular beds that lead to targeting and attack by the immune system.

THE SPECTRUM OF VASCULITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Cutaneous Vasculitis

Cutaneous vasculitis is the most common form of vasculitis in SLE, occurring in 19–50% of patients [4, 5, 46–49]. Its recognition, in some instances, has correlated significantly with severity of disease and organ involvement [48–52]. Cutaneous vasculitis typically occurs as an early manifestation of SLE, developing within 5 years of disease onset [49]. Non-Caucasian patients have a higher incidence of cutaneous vasculitis than Caucasian patients [49, 53–55]. Clinical expression of vasculitis includes petechiae, palpable purpura (Fig. 2), urticaria, nodules, bullae, nail-fold (Fig. 3), and splinter hemorrhages, ulcerations, gangrene, and livedo reticularis [56]. In the early stages of evolution, cutaneous vasculitis

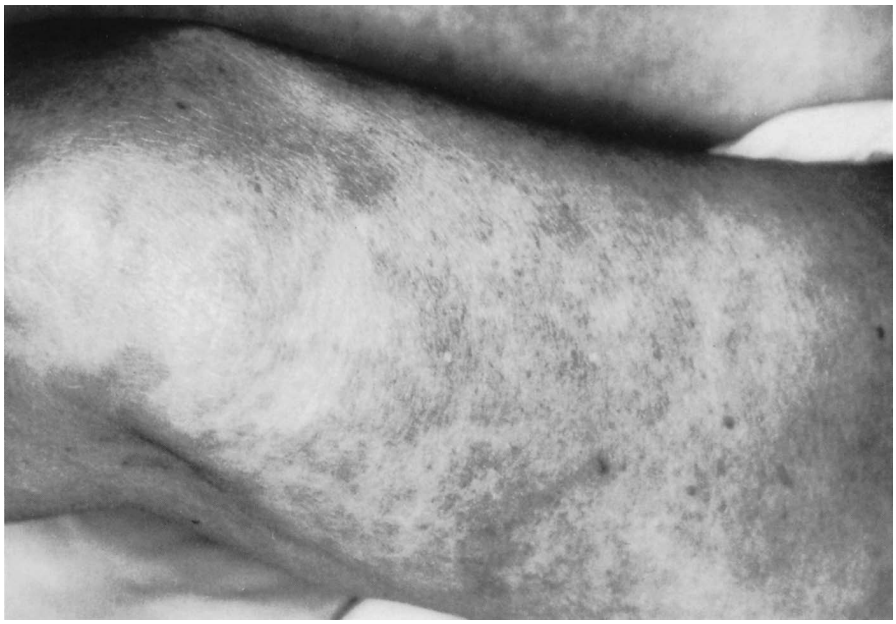


FIGURE 2 Palpable purpura of the lower extremity of a patient with active lupus who also had arthritis and pericarditis.

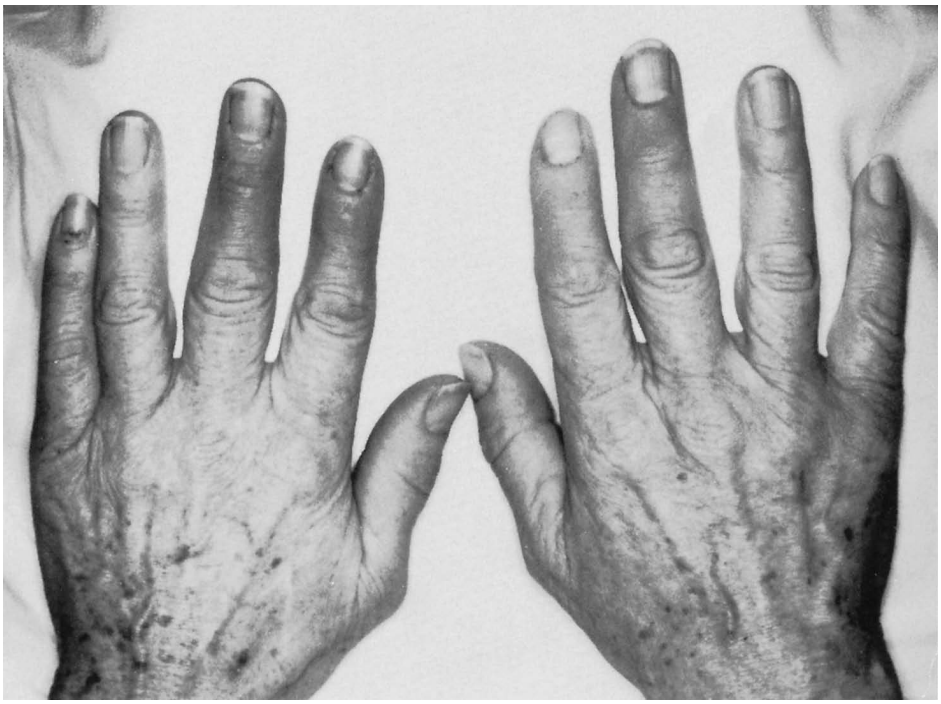


FIGURE 3 Nail-fold infarcts. There are pinpoint lesions in the nail beds, most prominent in the second, third, and fifth fingers of the left hand. There is also Raynaud's vasospasm involving the second and third fingers of the same hand.

may be flat and only later become palpable. Severe vasculitic involvement may lead to bullae formation, ulceration, or skin necrosis (Fig. 4). Purpuric lesions, palpable or nonpalpable, are the most frequently observed manifestation of inflammatory vascular disease in SLE, occurring more often in patients with anti-SSA antibodies and Sjögren's syndrome/SLE overlap [57, 58]. In addition, patients with cryoglobulins are more than twice as likely to develop cutaneous vasculitis than those without cryoglobulins [59]. Severe small-vessel vasculitis may cause small microinfarcts of the tips of the digits, which heal with "ice pick" scars (Fig. 5). Infrequently, vasculitis may manifest as urticaria (5–10%) [60–62] and requires differentiation from allergic urticaria. The lesions may be hyperpathic to light touch, persist for more than 24 h, and may display stippled petechiae that heal with hyperpigmentation.

Vasospasm may be a primary event or may occur secondary to vasculitis, embolic phenomena, vasoactive drugs, vasculopathy, or toxins. Arteritis of medium-sized vessels of the skin may cause vasospasm of the ascending dermal arterioles with pooling of blood in the superficial horizontal venous plexus and give rise to mottling (livedo reticularis). Alternatively, livedo reticularis may be associated with the vasculopathy of the antiphospholipid antibody syndrome. This finding may be generalized and/or accentuated about the outer arms, thighs, and over large joints. Livedo reticularis occurs in approximately 20% of SLE patients [51]. Punch biopsies of the skin are usually unrewarding because the deep dermal vessels are not accessible by this technique [56]. Anti-cardiolipin antibodies are found in 80% of patients with SLE and livedo reticularis [51]. Severe vasculitis of medium-sized vessels may also cause digital gangrene [4, 63], but fortunately this is rare (1–5%) [4, 5]. Cutaneous ulceration and gangrene may also develop as part of the vasculopathy associated with the anti-phospholipid antibody syndrome [64–66].

Histologically, most lupus vasculitic skin lesions demonstrate leukocytoclastic vasculitis with preferential involvement of arterioles, capillaries, and venules (Fig. 6). This is the most common form of vasculitis in SLE. In addition, small arterioles and postcapillary venules of other organs may be simultaneously affected. Infiltration and destruction of the vessel walls by polymorphonuclear cells is accompanied by polymorphonuclear fragmentation. The nuclear "debris" denotes leukocytoclasis [67]. Some cases primarily demonstrate vascular and/or perivascular infiltrate with mononuclear cells. Whether this merely represents a later stage of leukocytoclastic vasculitis is uncertain. Direct immunofluorescence of the skin lesion typically demonstrates granular IgG, complement, and fibrinogen in and/or around blood vessels [68].

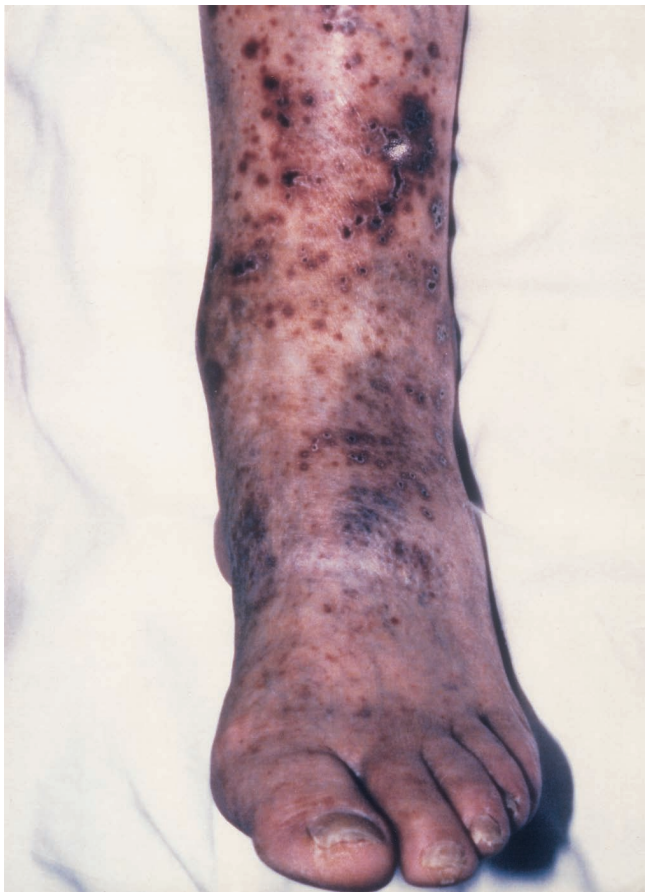


FIGURE 4 Severe palpable purpura with ulcerations affecting the lower extremity of a patient with active lupus manifested by glomerulonephritis, arthritis, serositis, and central nervous system disease.

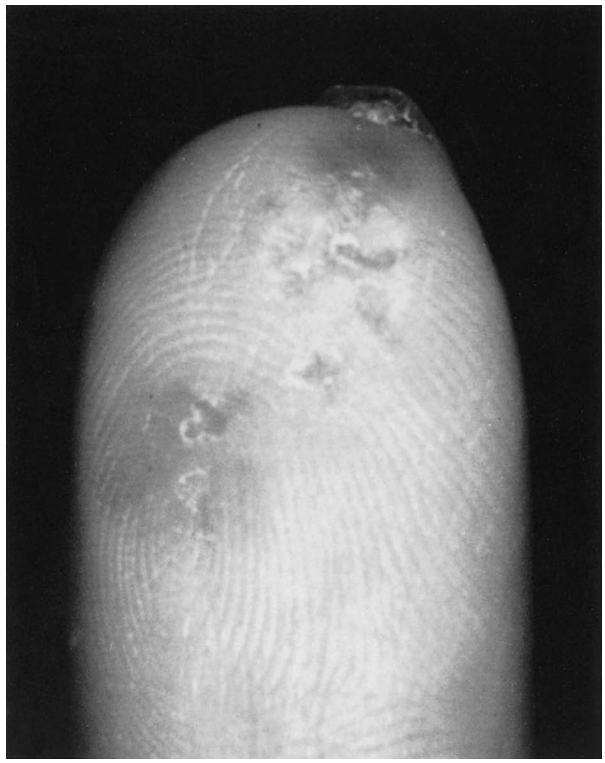


FIGURE 5 Healed microinfarcts of the fingertips. Often referred to as "ice pick" scars, they appear as ischemic ulcers surrounded by areas of hyperemia.

Central Nervous Systems and Peripheral Nervous System Vasculitis

Neuropsychiatric manifestations may be seen in up to 75% of SLE patients [3–5, 69, 70]. Central nervous system (CNS) abnormalities are more common than peripheral neuropathy (3–18%). CNS vasculitis may rarely be the sole presenting symptom of lupus [71]. Several studies have noted a correlation of cutaneous vasculitis with CNS disease [49–52, 72]. Feinglass *et al.* [50] observed that of a total of 140 cases, 52 had neuropsychiatric manifestations, among whom a clinical diagnosis of cutaneous vasculitis was made in 46%. In contrast, of the 88 without neuropsychiatric disease, cutaneous vasculitis was clinically recognized in only 17%.

Although CNS symptoms are usually attributed to vasculitis, demonstration of CNS vasculitis on postmortem examination of SLE patients is infrequent [69, 70, 73, 74]. Rather, an occlusive vasculopathy

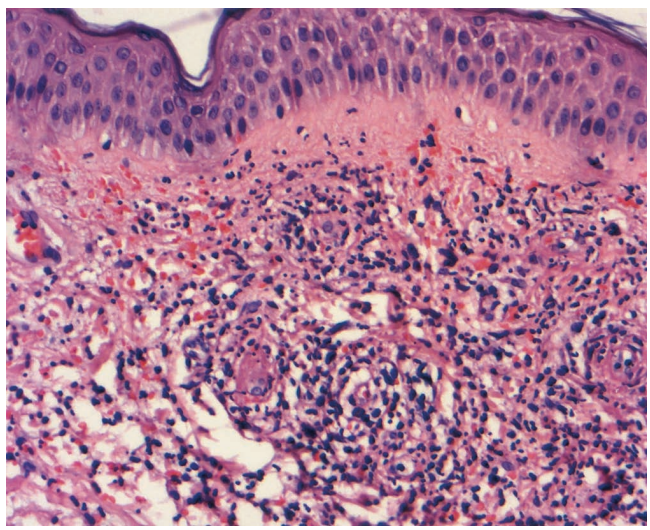


FIGURE 6 Skin biopsy demonstrating leukocytoclastic vasculitis involving postcapillary venules with fibrinoid necrosis within vessels and a perivascular mixed inflammatory infiltrate with nuclear dust. (Photo courtesy of Kathleen J. Smith, M.D.)

characterized by intimal proliferation of arterioles and capillaries and vascular hyalinization, associated with infarcts and hemorrhage, is observed. Johnson and Richardson [69] found vasculitic lesions in only 3 of 24 (12%) autopsy cases and Ellis and Verity [70] recorded arteritis in 7% of 57 cases.

Central nervous system vascular disease usually affects small vessels (diameter ≤2 mm). The result is multiple microinfarcts that may vary in age from region to region. This feature allows distinction from hypertensive encephalopathy and the CNS lesion of thrombotic thrombocytopenic purpura. The former has the additional feature of hypertrophy and hyalinization of the media of larger vessels, and thrombocytopenic purpura is distinguished by its microangiopathic peripheral blood smear, as well as the paucity of CNS parenchymal disease. When parenchymal vascular lesions are present in thrombocytopenic purpura, they are usually of the same age. The CNS lesions of SLE are most common in the cerebral cortex and brain stem and correlate with generalized seizures and cranial nerve deficits, respectively. Large-vessel vasculitis of the CNS is distinctly rare, with less than a dozen cases described in the literature [75]. Subarachnoid hemorrhage is an uncommon complication of SLE and may result from a ruptured intracerebral aneurysm [76–80]. Pathology specimens have revealed medium-sized vessel vasculitis.

If vasculitis affects the retina, it is usually bilateral. One eye is usually more severely affected than the other. Lesions tend to be focal and characterized by intraretinal hemorrhage and local retinal infarction with variable adjacent retinal exudation (Figs. 7 and 8). Fluorescein angiography may be useful in identifying the extent of disease and response to treatment (Fig. 9). Papillitis may result from vascular disease of nutrient vessels to the optic nerve [81, 82]. Only a few postmortem ocular specimens have been studied. Findings have included vasculitis in the choroidal circulation [83, 84]. Retinopathy may be a marker of disease activity and CNS involvement [85, 86].

Spinal cord vascular lesions may manifest as transverse myelopathy [87]. In one series, 2 of 12 cases had histopathologic evidence of vasculitis. Perivascular round cell infiltrates, collagen proliferation, thrombotic occlusion of small arteries and arterioles, and micro-

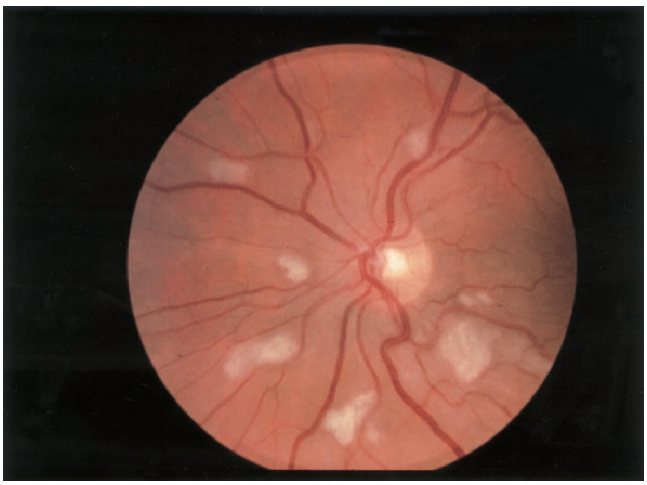


FIGURE 7 Fundus photograph reveals cotton wool spots or nerve fiber layer infarcts in a patient with SLE. (Photo courtesy of Careen Lowder, M.D.)

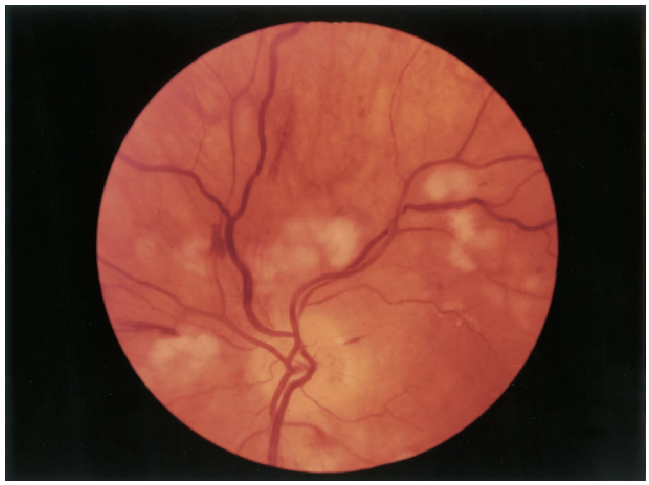


FIGURE 8 Fundus photograph of the right eye reveals flame shaped hemorrhages and cotton wool spots, the two most frequently reported ocular findings in SLE. (Photo courtesy of Careen Lowder, M.D.)

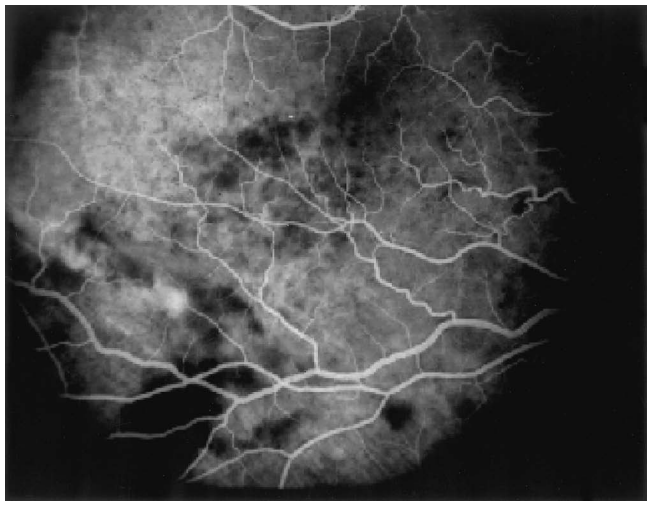


FIGURE 9 Fluorescein angiogram reveals focal areas of choroidal nonperfusion as seen in patients with choroidal ischemia. (Photo courtesy of Careen Lowder, M.D.)

scopic hemorrhage in the cord parenchyma were noted in all cases. Whether more extensive sampling of tissue would have demonstrated frank vasculitis in all cases is a matter of speculation.

Peripheral neuropathy in SLE may be due to vasculitis of the vasa nervorum that may result in multifocal sensorimotor neuropathy with prominent sensory symptoms [88, 89]. Mononeuritis multiplex has also been reported [88, 90, 91]. Nerve biopsy may show evidence of necrotizing vasculitis with perivascular inflammatory infiltrates and intimal thickening [92, 93].

Pulmonary Vasculitis

The contribution of vasculitis to pulmonary pathology in SLE has been reported to range from 0 to 40% [94–98]. Fayemi [94] reported 20 autopsy cases, of which 6 had pulmonary symptoms. Four had vasculitis involving the muscular and elastic arteries, which contrasts with more commonly recognized predilection for small vessels noted in other organs. Gross *et al.* [95] reported abnormalities of the pulmonary vasculature in 8 of 43 cases, including involvement of small arteries and arterioles. Radiographically, lesions may appear as areas of atelectasis, infiltrates, or, rarely, nodules or cavities caused by ischemic necrosis. Despite the extensive vascular changes that can occur, cor pulmonale or right ventricular hypertrophy have been uncommon. This may reflect rapid evolution of vascular pathology.

Gastrointestinal Vasculitis

Vasculitis within the abdomen is uncommon [7, 47, 99] and associated with a high mortality rate (Table 2) [100]. Vasculitis may affect the vessels within the mesentery [101], (Fig. 10), pancreas [102–104], peritoneum (Fig. 11), and, rarely, liver [105–108] and gall bladder [109, 110].

Mesenteric vasculitis is estimated to occur in approximately 1–2% of SLE patients [47, 99, 101] and may affect the vessels supplying the stomach, small, intestine, or large intestine [111–114]. Clinically, gastrointestinal symptoms may range from nausea, bloating, vomiting, diarrhea, postprandial pain and/or fullness, to frank hemorrhage and acute abdominal pain. The less acute gastrointestinal symptoms might erroneously be attributed to side effects of commonly prescribed medications such as corticosteroids, NSAIDs, hydroxychloroquine, and azathioprine. Physical exam findings of guarding and rebound may be absent or appear only late in the disease course in the patient taking corticosteroids or other immunosuppressive agents. Unexplained acidosis, a falling hematocrit, and hypotension are worrisome clues to a possible intra-abdominal crisis [115]. X rays may be normal or reveal thumbprinting, ileus, or pseudo-obstruction [110]. Computed tomography (CT) scan may reveal bowel-wall thickening [116] and prominence of mesenteric vessels [117]. Vasculitis involving the intestinal tract is typically a small-vessel vasculitis affecting arterioles and venules [118] that are beyond the resolution of arteriography. Rarely, periarteritis nodosa-like aneurysmal lesions in medium-sized vessels may occur. When present, aneurysms may thrombose or rupture, causing hemorrhage and death [118, 119]. Intestinal vasculitis may lead to mucosal ischemia, resulting in ulceration, hemorrhage, infarction, or perforation [118, 120, 121]. Exploratory laparoscopy or laparotomy should be considered early, as a delay in diagnosis of ruptured or infarcted bowel has a very high mortality rate. Medina *et al.* [122] found that among 44 SLE patients with acute abdomen requiring surgery, none of 33 died who were operated on within 48 h of presentation, whereas 10 of 11 died who were operated on >48 h after presentation. In most but not all [123] series, patients with mesenteric vasculitis have evidence of lupus activity affecting other organs. Pneumatosis cystoides intestinalis, although more frequently associated with other connective tissue diseases such as scleroderma or rheumatoid arthritis, may be a benign finding [124] or may be associated

TABLE 2 Gastrointestinal Involvement and Morbidity

Series	No. of patients	Gastrointestinal involvement (%)	Histologic proof (%)[a]	Death (%)
Matolo and Albo [171]	51	27.5	Unclear	43
Estes and Christian [5]	150	16	1.3	8
Harvey *et al.* [3]	106	14	58	NR[b]
Zizic *et al.* [100]	140[c]	11	82	53

[a] Of all patients in whom a clinical diagnosis of gastrointestinal vasculitis was made, the percentage in whom actual histologic proof of vasculitis was documented.

[b] NR, not reported.

[c] Patient selection emphasized acute surgical presentations.

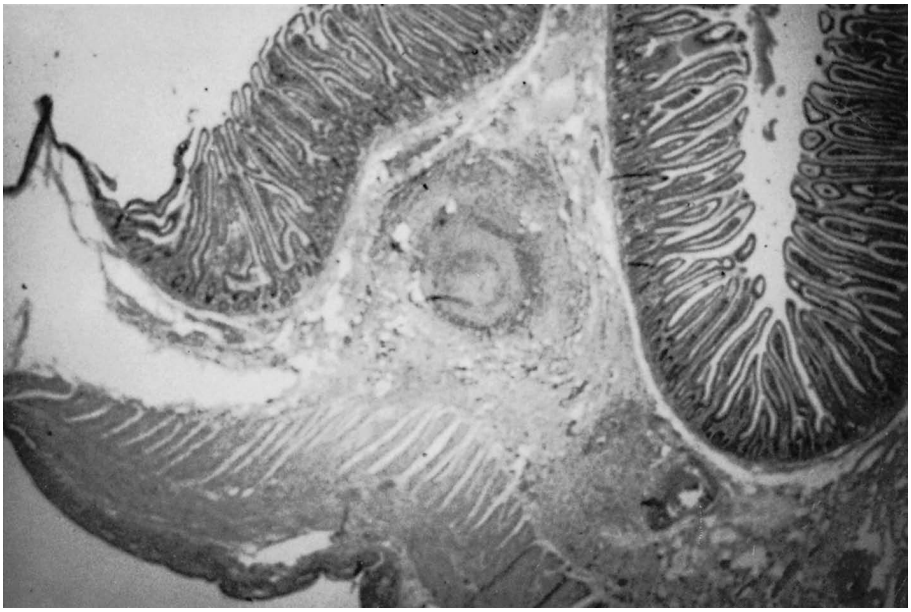


FIGURE 10 Gastrointestinal vasculitis. Transmural intestinal segment demonstrating a medium-sized vessel with thrombosis and whose wall is infiltrated with inflammatory cells.

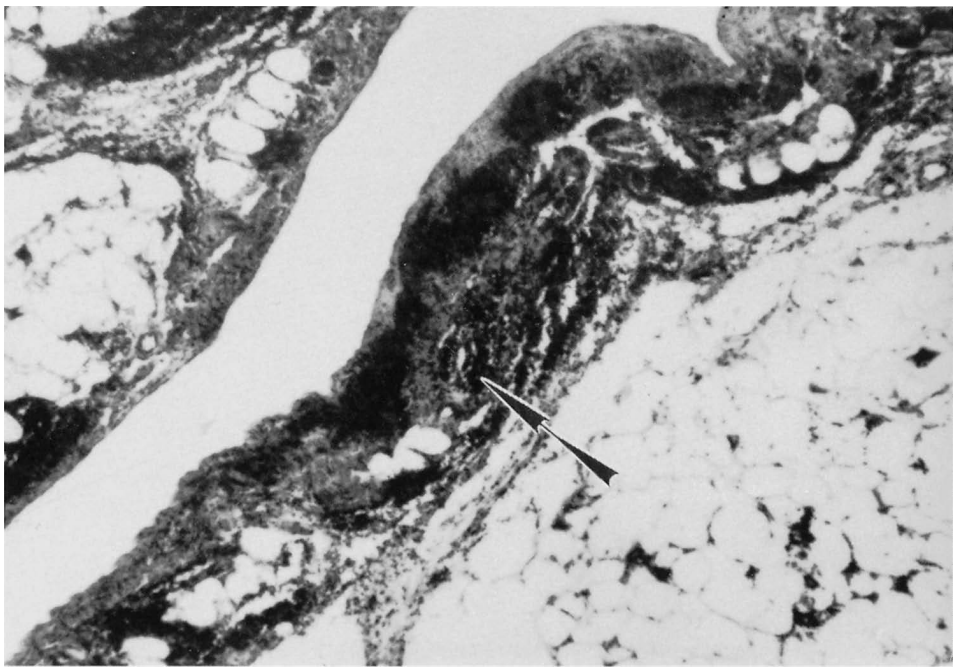


FIGURE 11 Vasculitis of the peritoneum. This patient presented with massive ascites. At laparotomy, no abnormal viscus was found but a peritoneal biopsy demonstrated extensive small-vessel vasculitis.

with intestinal vasculitis in SLE patients [101, 125, 126]. Inflammatory vascular disease affecting venules supplying the intestines has also been implicated in the etiology of protein losing enteropathy in SLE [127, 128].

Vasculitis may also affect other organs within the abdominal cavity. Histologic evidence of necrotizing arteritis of the liver has been reported in 18–20% of postmortem cases [107, 129]. Spontaneous hepatic rupture caused by small- and medium-sized vessel vasculitis is a

rare complication [105, 130]. Acute acalculous cholecystitis due to vasculitis may occur in isolation or may be associated with widespread disease activity [109, 110]. Vasculitis has been implicated as a cause of pancreatitis in a subset of SLE patients [102, 103].

Because of the rarity of the condition, there is no standardized approach to the treatment of gastrointestinal vasculitis. Controlled trials of treatment regimens do not exist. High-dose corticosteroids (1–2 mg/kg IV methylprednisolone) and bowel rest are generally effective in treating those patients whose vasculitic manifestations include abdominal pain, ileus, even ulceration and hemorrhage, but obviously one must carefully rule out bowel infarction, perforation, or obstruction before settling on nonsurgical management strategies. Case reports exist describing concomitant use of cytotoxic agents, most commonly cyclophosphamide, in management of lupus gastrointestinal vasculitis [115, 131].

Vasculitis of the Heart

Coronary artery vasculitis is exceedingly rare in SLE and has been documented primarily in case reports [132–140]. Vasculitis affecting the coronary arteries may occur in the absence of extracardiac lupus activity, thereby making the diagnosis especially challenging. Diagnosis is usually made by serial coronary angiographic studies. However, the sensitivity and specificity of angiography is unknown. Angiographic findings suggestive of vasculitis include arterial aneurysms without concomitant distal stenoses, smooth focal tapering stenoses, and/or rapidly developing arterial occlusions on repeated studies. Both the mainstem and its branches including the sinus node artery have been involved [137]. Vessels that are no longer the site of active vasculitis may have persistent aneurysms or stenoses that may thrombose and cause myocardial infarction. In some cases, vasculitic changes have been accompanied by fibrosis of the conduction system [140]. Rare examples of cardiac valve dysfunction caused by small-vessel vasculitis have also been documented [137].

Vasculitis of the Genitourinary System

True vasculitis of the kidney is the least frequent manifestation of vascular disease within the kidney, occurring in less than 5% of SLE nephritis patients [141, 142] (Fig. 12). Vascular immune deposits, noninflammatory necrotizing vasculopathy (Fig. 13), and thrombotic microangiopathy are other forms of vascular disease implicated in the pathogenesis of SLE-associated renal disease [12, 143, 144]. In renal vasculitis, the vessels affected are usually small- and medium-sized arteries, most commonly intralobular arteries. It is most frequently associated with diffuse proliferative glomerulonephritis. The presence of vasculitis on renal biopsy is believed to be an independent indicator of adverse renal prognosis [145, 146].

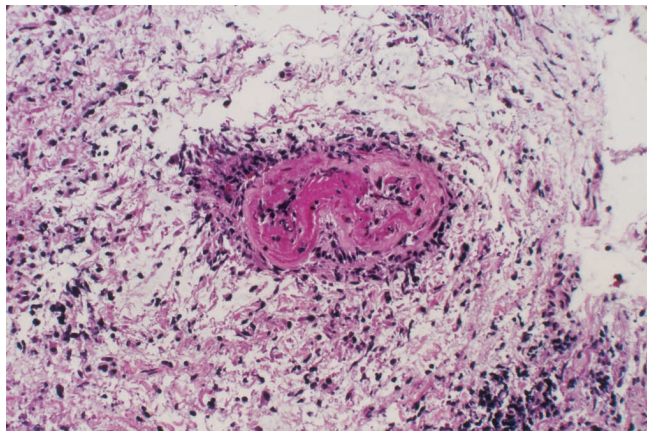


FIGURE 12 Acute vasculitis: necrotizing vasculitis with transmural infiltration by neutrophils and lymphocytes. (Photo courtesy of William J. Cook, M.D.)

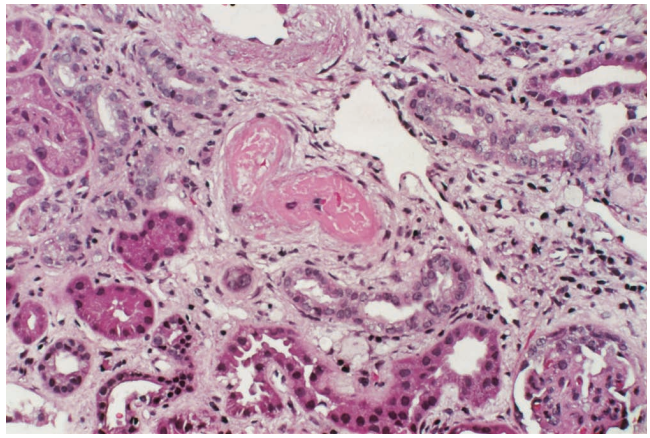


FIGURE 13 Lupus vasculopathy: The arteriole on this renal biopsy is narrowed by intimal deposits of eosinophilic material which likely represents a mixture of fibrin and immune deposits. Although the endothelium is necrotic, there is no inflammation. (Photo courtesy of William J. Cook, M.D.)

Case reports of lower genitourinary tract involvement include examples of hydronephrosis, hydroureter, and acute renal failure in the absence of cytotoxic therapy [147, 148]. Histologic examination of the bladder has demonstrated interstitial cystitis. In rare instances, ureteral vessels have been affected, and displayed necrotizing vasculitis with extensive fibrinoid changes. Such lower genitourinary tract disease should be considered in an SLE patient with deteriorating

TABLE 3 Vasculitis of the Placenta

Series	No. of patients	No. of placentas	Fetal outcome (live births)	Types of vascular lesions studied			
				Vasculitis	Thrombosis	Intimal proliferation	Tortuosity
Abramowsky *et al*. [162]	10	10	4	5	2	Reported[a]	Reported
Hanly *et al*. [163]	11	11	7	0	2	NR[b]	NR
Benirschke and Driscoll [165]	5	5	Unknown	1	Unknown	Unknown	Unknown
Total	26	26	11	6			

[a] Not quantitated.
[b] NR, not reported.

renal function in the absence of red blood cell casts and in whom calculi and extrinsic causes of compression have been excluded. In the cases reported, obstruction was relieved by corticosteroid therapy [147, 148]. In addition, rare cases of necrotizing vasculitis of the penis [149] ovaries [150], and uterus [151] have been reported in lupus patients.

Large-Vessel Disease

Large-vessel vasculitis of the aorta and its branches is exceedingly rare in SLE. Cases of Takayasu's arteritis associated with SLE have been described [152–155]. However, the coexistence of these two diseases may merely be an issue of semantics, that is reconciled when one recognizes that in fact aortitis or disease of aortic branch vessels may rarely be a part of SLE [156, 157].

Large-vessel involvement has been reported to affect the abdominal aorta, subclavian, carotid, coronary, and lower-limb arteries [138, 158–160]. Large-vessel vasculitis of the extremities resulting in gangrene is very uncommon in SLE patients. In a literature review of all reported English-language cases of limb-threatening vascular occlusion, 12 cases were identified, of which 4 were due to vasculitis [161]. Pathologic specimens demonstrated full-thickness necrotizing arteritis with polymorphonuclear cell infiltration [159, 161]. Immune complexes were not identified. Sympathectomy and streptokinase have not proven to be useful in these cases. Corticosteroids have allegedly provided relief of intermittent claudication and limited the damage caused by large-vessel vasculitis [158, 160].

Vasculitis of the Placenta

The contribution of placental vasculitis to fetal mortality has been subject to preliminary evaluation that has led to conflicting observations (Table 3). Abramowsky *et al.* [162] have studied the placentas of 10 women with SLE. Five of 10 specimens demonstrated vascular fibrinoid necrosis and mononuclear and/or polymorphonuclear inflammatory infiltrates. Some vessels were tortuous and others aneurysmal. These changes were seen in the 5 multiparous patients who suffered fetal loss after the first trimester, but were noticeably absent in the primagravids. Hanly *et al.* in a prospective study of 11 cases could not document any distinct form of placental pathology. They found no evidence of vasculitis [163]. Demonstration of immune complexes in diseased as well as in normal placentas raises questions as to what role immune complexes play in placental insufficiency and abortion [164]. The correlation of fetal outcome with placental thrombosis, phospholipid antibodies, vasculopathy, or vasculitis is not yet clear. In addition, many of the histologic features described in placentas of SLE patients are very similar to those in preeclampsia and may be present to a lesser degree in the placentas of normal nonlupus patients [165].

Differential Diagnosis of SLE Vasculitis

Immune responses in SLE are inherently abnormal and further compromised by immunosuppressive therapy. Deterioration in clinical status must take into account the possibilities of infection, drug toxicity, disease progression, thrombosis from anti-phospholipid antibodies, and less commonly, disseminated intravascular coagulation or thrombotic thrombocytopenic purpura. Any of these multisystem processes can mimic vasculitis or, conversely, be complicated by vasculitis.

Management

Because (1) the diagnosis of lupus vasculitis is often uncertain, (2) large numbers of similarly affected patients are not available for prospective studies, and (3) vasculitis usually does not occur in the absence of a

variety of other pathologic events, it is difficult to perform controlled therapeutic trials for lupus vasculitis. For life-threatening major organ involvement with vasculitis, aggressive therapy with corticosteroids, cyclophosphamide, intravenous immunoglobulin, and/or plasmapheresis has proven useful in selected patients. Treatment must remain a matter of clinical judgement; one must consider the patient's global illness, of which vasculitis may be only one factor and not necessarily the dominant consideration.

References

1. Belmont, H. M., Abramson, S. B., and Lie, J. T. (1996). Pathology and pathogenesis of vascular injury in systemic lupus erythematosus. *Arthritis Rheum* **39**, 9–22.
2. Fauci, A. S., Haynes, B. F., and Katz, P. (1978). The spectrum of vasculitis. Clinical, pathologic, immunologic and therapeutic considerations. *Ann. Intern. Med.* **89**, 660–676.
3. Harvey, A. M., Shulman, L. E., Tumulty, P. A., *et al.* (1954). Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine* **33**, 291.
4. Dubois, E. L., and Tufanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus: Computer analysis of 520 cases. *JAMA* **190**, 104.
5. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85–95.
6. Cervera, R., Khamashta, M. A., Font, J., *et al.* (1993). Systemic lupus erythematosus: Clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. *Medicine* **72**, 113–124.
7. Drenkard, C., Villa, A. R., Reyes, E., Abello, M., and Alarcón-Segovia, D. (1997). Vasculitis in systemic lupus erythematosus. *Lupus* **6**, 235–242.
8. Rocca, P. V., Siegel, L. B., and Cupps, T. R. (1994). The concomitant expression of vasculitis and coagulopathy: Synergy for marked tissue ischemia. *J. Rheumatol.* **21**, 556–560.
9. Norden, D. K., Ostrov, B. E., Shafritz, A. B., and VonFeldt, J. M. (1995). Vasculitis associated with antiphospholipid syndrome. *Semin. Arthritis Rheum.* **24**, 273–281.
10. Lie, J. T., Kobayashi, S., Tokano, Y., and Hashimoto, H. (1995). Systemic and cerebral vasculitis coexisting with disseminated coagulopathy in systemic lupus erythematosus associated with the antiphospholipid syndrome. *J. Rheumatol.* **22**, 2173–2176.
11. Daly, D. (1945). Central nervous system in acute disseminated lupus erythematosus discrimination. *J. Nerv. Ment. Dis.* **102**, 461.
12. Bhathena, D. B., Sobel, B. J., and Migdal, S. D. (1981). Noninflammatory renal microangiopathy of systemic lupus erythematosus (lupus vasculitis). *Am. J. Nephrol.* **1**, 144–159.
13. Abramson, S. B., Given, W. P., Edelson, H. S., and Weissman, G. (1983). Neutrophil aggregation induced by sera from patients with active systemic lupus erythematosus. *Arthritis Rheum.* **26**, 630–636.
14. Abramson, S. B., Dobro, J., Eberle, M. A., *et al.* (1991). Acute reversible hypoxemia in systemic lupus erythematosus. *Ann. Intern. Med.* **114**, 941–947.
15. Argenbright, L., and Barton, R. (1993). Interactions of leukocyte integrins with intercellular adhesion molecule 1 in the production of inflammatory vascular injury in vivo: The Schwartzman phenomenon revisited. *J. Clin. Invest.* **89**, 259–272.
16. Jain, R., Chartash, E., Sushin, M., *et al.* (1994). Systemic lupus erythematosus complicated by thrombotic microangiopathy. *Semin. Arthritis Rheum.* **24**, 173–182.
17. Nesher, G., Hanna, V. E., Moore, T. L., *et al.* (1994). Thrombotic microangiopathic hemolytic anemia in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **24**, 165–172.
18. Fessler, B. J. (1997). Thrombotic syndromes and autoimmune diseases. *Rheum. Dis. Clin. North Am.* **23**, 461–479.
19. Toussirot, E., Figarella-Branger, D., Disdier, P., Harle, J. R., and Weiller, P. J. (1994). Association of cerebral vasculitis with a lupus anticoagulant. A case with brain pathology. *Clin. Rheumatol.* **13**, 624–627.
20. Sneller, M. C., and Fauci, A. S. (1997). Pathogenesis of vasculitis syndromes. *Med. Clin. North Am.* **81**, 221–242.
21. Miyata, M., Ito, O., Kobayashi, H., *et al.* (2001). CpG-DNA derived from sera in systemic lupus erythematosus enhances ICAM-1 expression on endothelial cells. *Ann. Rheum. Dis.* **60**, 685–689.
22. Takeuchi, T., Amano, K., Sekine, H., *et al.* (1983). Up-regulated expression and function of integrin adhesive receptors in systemic lupus erythematosus patients with vasculitis. *J. Clin. Invest.* **92**, 3008–3016.
23. Christian, C. L., and Sergent, J. S. (1976). Vasculitis syndromes: Clinical and experimental models. *Am. J. Med.* **61**, 385–392.
24. Dixon, F. J., Feldman, J. D., and Vasquez, J. J. (1976). Experimental glomerulonephritis. *J. Exp. Med.* **113**, 889.
25. Pincus, T., Haberkern, R., and Christian, C. L. (1968). Experimental chronic glomerulonephritis. *J. Exp. Med.* **127**, 819–832.
26. Henson, P. M., and Cochrane, C. G. (1971). Acute immune complex disease in rabbits. The role of complement and a leukocyte dependent release of vasoactive amines from platelets. *J. Exp. Med.* **133**, 554–571.
27. Fisher, E. R., and Bark, J. (1961). Effect of hypertension on vascular and other lesions of serum sickness. *Am. J. Pathol.* **39**, 665.
28. Abramson, S., Belmont, H. M., Hopkins, P., *et al.* (1987). Complement activation and vascular injury in systemic lupus erythematosus. *J. Rheumatol.* (14 Suppl.) **13**, 543–546.
29. Yancey, K. B., O'Shea, J., Chused, T., *et al.* (1985). Human C5a modulates monocyte Fc and C3 receptor expression. *J. Immunol.* **135**, 465–470.
30. Knicker, W., and Cochrane, C. (1965). Pathogenic factors in vascular lesions of experimental serum sickness. *J. Exp. Med.* **122**, 83.

31. Carvalho, D., Savage, C. O. S., Isenberg, D., *et al.* (1999). IgG anti-endothelial cell autoantibodies from patients with systemic lupus erythematosus or systemic vasculitis stimulate the release of two endothelial cell-derived mediators, which enhance adhesion molecule expression and leukocyte adhesion in an autocrine manner. *Arthritis Rheum.* **42**, 631–640.

31a. Springer, T. A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. *Cell* **76**, 301–314.

32. Wu, R., Svenungsson, E., Gunnarsson, I., *et al.* (1999). Antibodies to adult human endothelial cells cross-react with oxidized low-density lipoprotein and β2-glycoprotein I (β2-GPI) in systemic lupus erythematosus. *Clin. Exp. Immunol.* **115**, 561–566.

33. Cines, D. B., Lyss, A. P., Reeber, M., *et al.* (1984). Presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus. *J. Clin. Invest.* **73**, 611–625.

34. Kallenberg, C. G. (1993). Autoantibodies in vasculitis: Current perspectives. *Clin. Exp. Rheum.* **11**, 355–360.

35. van der Woude, F. J., Lobatto, S., Permin, H., *et al.* (1985). Autoantibodies against neutrophils and monocytes: Tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* **1**, 425–429.

36. Hoffman, G. S. (1996). ANCAs: Are they important? *J. Nephrol.* **9**, 216.

37. Nassberger, L., Sjoholm, A. G., Jonsson, H., *et al.* (1990). Autoantibodies against neutrophil cytoplasm components in systemic lupus erythematosus and in hydralazine induced lupus. *Clin. Exp. Immunol.* **81**, 380–383.

38. Pauzner, R., Urowitz, M., Gladman, D., and Gough, J. (1994). Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. *J. Rheumatol.* **21**, 1670–1673.

39. Schnabel, A., Csernok, E., Isenberg, D. A., *et al.* (1995). Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Prevalence, specificities and clinical significance. *Arthritis Rheum.* **38**, 633–637.

40. Spronk, P. E., Bootsma, H., Horst, G., *et al.* (1996). Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. *Br. J. Rheumatol.* **35**, 625–631.

41. Nassberger, L. (1996). Distribution of antineutrophil cytoplasmic autoantibodies in SLE patients with and without renal involvement. *Am. J. Nephrol.* **16**, 548–549.

42. Nishiya, K., Chikazawa, H., Nishimura, S., *et al.* (1997). Anti-neutrophil cytoplasmic antibody in patients with systemic lupus erythematosus is unrelated to clinical features. *Clin. Rheumatol.* **16**, 70–75.

43. Manolova, I., Dancheva, M., and Halacheva, K. (2001). Antineutrophil cytoplasmic antibodies in patients with systemic lupus erythematosus: Prevalence, antigen specificity and clinical associations. *Rheumatol. Int.* **20**, 197–204.

44. Galeazzi, M., Morozzi, G., Sebastiani, G. D., Bellisai, F., Marcolongo, R., Cervera, R., DeRamon Garrido, E., Fernandez-Nebro, A., Houssiau, F., Jedryka-Goral, A., Mathieu, A., Papasteriades, C., Piette, J. C., Scorza, R., and Smolen J. (1998). Anti-neutrophil cytoplasmic antibodies in 566 European patients with systemic lupus erythematosus: Prevalence, clinical associations and correlation with other autoantibodies. European Concerted Action on the Immunogenetics of SLE. *Clin. Exp. Rheumatol.* **16**, 541–546.

45. Jethwa, H. S., Nachman, P. H., Falk, R. J., and Jennette, J. C. (2000). False positive myeloperoxidase binding activity due to DNA/anti-DNA antibody complexes: A source for analytical error in serologic evaluation of anti-neutrophil cytoplasmic autoantibodies. *Clin. Exp. Immunol.* **121**, 544–550.

46. Hochberg, M. C., Boyd, R. E., Ahearn, J. M., *et al.* (1985). Systemic lupus erythematosus. A review of clinicolaboratory features and immunogenetic markers in 150 patients with emphasis on demographic subsets. *Medicine* **64**, 285–295.

47. Pistiner, M., Wallace, D. J., Nessim, S., *et al.* (1991). Lupus erythematosus in the 1980s: A survey of 570 patients. *Semin. Arthritis Rheum.* **21**, 55–64.

48. Cardinali, C., Caproni, M., Bernacchi, E., Amato, L., and Fabbri, P. (2000). The spectrum of cutaneous manifestations in lupus erythematosus—the Italian experience. *Lupus* **9**, 417–423.

49. Fessler, B. J., McGwin, G., Jr., Roseman, J. M., Bastian, H. M., Friedman, A., Lisse, J. R., Alarcon, G. S., Reveille, J. D., for the LUMINA Study Group. (2000). Cutaneous vasculitis (CV) in systemic lupus erythematosus (SLE): Association with Hispanic ethnicity and major organ system involvement. *Arthritis Rheum.* **43**, S253.

50. Feinglass, E. J., Arnett, F. C., Dorsch, C. A., *et al.* (1976). Neuropsychiatric manifestations of systemic lupus erythematosus: Diagnosis, clinical spectrum and the relationship to other features of the disease. *Medicine* **55**, 323–339.

51. Yasue, T. (1986). Livedoid vasculitis and central nervous system involvement in systemic lupus erythematosus. *Arch. Dermatol.* **122**, 66–70.

52. Callen, J. P., and Kingman, J. (1983). Cutaneous vasculitis in systemic lupus erythematosus—a poor prognostic indicator. *Cutis* **32**, 433–436.

53. Wysenbeek, A. J., Leibovici, L., Weinberger, A., and Guedj, D. (1993). Expression of systemic lupus erythematosus in various ethnic Jewish Israeli groups. *Ann. Rheum. Dis.* **52**, 268–271.

54. Tikly, M., Burgin, S., Mohanlal, P., Bellingan, A., and George, J. (1996). Autoantibodies in black South Africans with systemic lupus erythematosus: Spectrum and clinical associations. *Clin. Rheumatol.* **15**, 261–265.

55. Levy, Y., George, J., Hojnic, M., Ehrenfeld, M., Lorber, M., Bombardier, S., and Shoenfeld, Y. (1996). Comparison of clinical and laboratory parameters for systemic lupus erythematosus activity in Israelis versus Europeans. *Isr. J. Med. Sci.* **32**, 100–104.

56. Watson, R. (1989). Cutaneous lesions in systemic lupus erythematosus. *Med. Clin. North Am.* **73**, 1091–1111.

57. Provost, T. T., Talal, N., Bias, W., *et al.* (1988). Ro (SS-A) Positive Sjogren's/Lupus erythematosus overlap patients are associated with the HLA-DR3 and/or Drw6 phenotypes. *J. Invest. Dermatol.* **91**, 369–371.

58. Simmons-O'Brien, E., Chen, S., Watson, R., *et al.* (1995). 100 anti-Ro (SS-A) antibody positive patients: A ten year follow-up. *Medicine* **74**, 109–130.
59. Garcia-Carrasco, M., Ramos-Casals, M., Cervera, R., Trejo, O., Yague, J., Siso, A., Jimenez, S., De La Red, G., Font, J., and Ingelmo, M. (2001). Cryoglobulinemia in systemic lupus erythematosus: Prevalence and clinical characteristics in a series of 122 patients. *Semin. Arthritis Rheum.* **30**, 366–373.
60. Gammon, W. R., and Wheeler, C. E., Jr. (1979). Urticarial vasculitis. Report of a case and review of the literature. *Arch. Dermatol.* **115**, 76–80.
61. Provost, T. T., Zone, J. J., Synkowski, D., *et al.* (1980). Unusual cutaneous manifestations of systemic lupus erythematosus: Urticaria-like lesions: Correlation with clinical and serologic abnormalities. *J. Invest. Dermatol.* **75**, 495–499.
62. Asherson, R. A., D'Cruz, D., Stephens, C. J. M., *et al.* (1991). Urticarial vasculitis in a connective tissue disease clinic: Patterns, presentations, and treatment. *Semin. Arthritis Rheum.* **20**, 285–296.
63. Dubois, E. L., and Arterberry, J. D. (1962). Gangrene as a manifestation of systemic lupus erythematosus. *JAMA* **181**, 366.
64. Goldberg, D. P., Lewis, V. L., and Koenig, W. J. (1995). Antiphospholipid antibody antibody syndrome: A new cause of nonhealing skin ulcers. *Plast. Reconstr. Surg.* **95**, 837–841.
65. Barbaud, A. M., Gobert, B., Reichert, S., *et al.* (1994). Anticardiolipin antibodies and ulcerations of the leg. *J. Am. Acad. Dermatol.* **31**, 670–671.
66. Jindal, B. K., Martin, M. F., and Gayner, A. (1983). Gangrene developing after minor surgery in a patient with undiagnosed systemic lupus erythematosus and lupus anticoagulant. *Ann. Rheum. Dis.* **42**, 347–349.
67. Zeek, P. M., Smith, C. C., and Wheter, J. C. (1948). Studies of periarteritis nodosa III. The differentiation between the vascular lesions of periarteritis nodosa and hypersensitivity. *Am. J. Pathol.* **24**, 889.
68. Synkowski, D. R., and Provost, T. T. (1985). Cutaneous vasculitis in the LE patient. *Clin. Dermatol.* **3**, 88–95.
69. Johnson, R. T., and Richardson, E. P. (1968). The neurological manifestations of systemic lupus erythematosus: A clinico-pathological study of 24 cases and review of the literature. *Medicine* **47**, 337–369.
70. Ellis, S. G., and Verity, M. A. (1979). Central nervous system involvement in systemic lupus erythematosus. A review of neuropathological findings in 57 cases, 1955–1957. *Semin. Arthritis Rheum.* **8**, 212–221.
71. Sanders, C. M., and Hogenhust, W. A. H. (1986). Cerebral vasculitis as the presenting symptom of systemic lupus erythematosus. *Acta Neurol. Scand.* **74**, 75.
72. Karassa, F. B., Ioannidis, J. P. A., Touloumi, G., Boki, K. A., and Moutsopoulos, H. M. (2000). Risk factors for central nervous system involvement in systemic lupus erythematosus. *Q. J. Med.* **93**, 169–174.
73. Devinsky, O., Petito, C. K., and Alonso, D. R. (1988). Clinical and neuropathological findings in systemic lupus erythematosus: The role of vasculitis, heart emboli, and thrombotic thrombocytopenia purpura. *Ann. Neurol.* **23**, 380–384.
74. Hanly, J. G., Walsh, N. M. G., and Sangalang, V. (1992). Brain pathology in systemic lupus erythematosus. *J. Rheumatol.* **19**, 732–741.
75. Weiner, D. K., and Allen, N. B. (1991). Large vessel vasculitis of the central nervous system in systemic lupus erythematosus: Report and review of the literature. *J. Rheumatol.* **18**, 748–751.
76. Keeley, R. E., Stokes, N., Reyes, P., and Harik, S. I. (1980). Cerebral transmural angiitis and ruptured aneurysm: A complication of systemic lupus erythematosus. *Arch. Neurol.* **37**, 526.
77. Fody, E. P., Netsky, M. G., and Nrak, R. E. (1980). Subarachnoid spinal hemorrhage in a case of systemic lupus erythematosus. *Arch. Neurol.* **37**, 173–174.
78. Nagayma, Y., Kusudo, K., and Imura, H. (1989). A case of central nervous system lupus associated with ruptured cerebral berry aneurysm. *Jpn. J. Med.* **28**, 530–533.
79. Asai, A., Matsuitani, M., Kohro, T., *et al.* (1989). Multiple saccular cerebral aneurysms associated with systemic lupus erythematosus—case report. *Neurol. Med. Chir. (Tokyo)* **29**, 245.
80. Sakaki, T., Morimoto, T., and Utsumi, S. (1990). Cerebral transmural angiitis and ruptured cerebral aneurysms in patients with systemic lupus erythematosus. *Neurochirugia (Stuttg.)* **33**, 132–135.
81. Goldstein, I., and Wexler, D. (1932). Retinal vascular disease in acute lupus disseminated. *Arch. Ophthalmol.* **8**, 852.
82. Regan, C. D., and Foster, C. S. (1986). Retinal vascular diseases: Clinical presentation and diagnosis. *Int. Ophthalmol. Clin.* **26**, 25–53.
83. Cordes, F. C., and Aiken, S. D. (1947). Ocular changes in acute disseminated lupus erythematosus: Report of a case with microscopic finding. *Am. J. Ophthalmol.* **30**, 1541.
84. Graham, E. M., Spalton, D. J., Barnard, R. O., *et al.* (1985). Cerebral and retinal vascular changes in systemic lupus erythematosus. *Ophthalmology* **92**, 444–448.
85. Stafford-Brady, F. J., Urowitz, M. B., Gladman, D. D., and Easterbrook, E. (1988). Lupus retinopathy. Patterns, associations and prognosis. *Arthritis Rheum.* **31**, 1105–1110.
86. Ushiyama, O., Ushiyama, K., Koarada, S., *et al.* (2000). Retinal disease in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **59**, 705–708.
87. Andrianakos, A. A., Duffy, J., Suzuki, *et al.* (1975). Transverse myelopathy in systemic lupus erythematosus: Report of three cases and review of the literature. *Ann. Intern. Med.* **83**, 616–624.
88. McCombe, P. A., Mcleod, J. G., Pollard, J. D., *et al.* (1987). Peripheral sensorimotor and autonomic neuropathy associated with systemic lupus erythematosus. *Brain* **110**, 533–549.
89. Markusse, H. M., Vroom, T. M., Heurkens, A. H. M., and Vecht, C. J. (1991). Polyneuropathy as initial manifestation of necrotizing vasculitis and gangrene in systemic lupus erythematosus. *Neth. J. Med.* **38**, 204–208.

90. Hughes, R. A. C., Cameron, J. S., Hall, S. M., *et al.* (1982). Multiple mononeuropathy as the initial presentation of systemic lupus erythematosus—nerve biopsy and response to plasma exchange. *J. Neurol.* **228**, 239–247.
91. Bergemer, A. M., Fouquet, B., Goupille, P., and Valat, J. P. (1987). Peripheral neuropathy as the initial manifestation of systemic lupus erythematosus. Report of a case. *Sem. Hop. Paris* **63**, 1979.
92. Rechtand, E., Cornblath, D. R., Stern, B. J., and Meyerhoff, J. O. (1984). Chronic demyelinating polyneuropathy in systemic lupus erythematosus. *Neurology* **34**, 1375–1377.
93. Bailey, A. A., Sayre, G. P., and Clark, E. C. (1956). Neuritis associated with systemic lupus erythematosus. *Arch. Neurol. Psych*. **75**, 251.
94. Fayemi, A. O. (1976). Pulmonary vascular disease in systemic lupus erythematosus. *Am. J. Clin. Pathol.* **65**, 284–290.
95. Gross, M., Gasterly, J. R., and Earne, R. H. (1972). Pulmonary alterations in systemic lupus erythematosus. *Am. Rev. Respir. Dis.* **105**, 572.
96. Webb, W. R., and Gamsu, G. (1981). Cavitary pulmonary nodules with systemic lupus erythematosus: Differential diagnosis. *Am. J. Roentgenol.* **136**, 27–31.
97. Aitchinson, J. D., and Williams, A. W. (1956). Pulmonary changes in disseminated lupus erythematosus. *Ann. Rheum. Dis.* **15**, 26.
98. Myers, J. L., and Katzenstein, A. L. A. (1985). Microangiitis in lupus-induced pulmonary hemorrhage. *Am. J. Clin. Pathol.* **85**, 552–556.
99. Vitali, C., Bencivelli, W., Isenberg, D. A., *et al.* (1992). Disease activity in systemic lupus erythematosus: Report of the consensus study group of the European workshop for rheumatology research. I. A descriptive analysis of 704 European lupus patients. *Clin. Exp. Rheumatol.* **10**, 527–539.
100. Zizic, T. N., Classen, J. N., and Stevens, M. B. (1982). Acute abdominal complications in systemic lupus erythematosus and polyarteritis nodosa. *Am. J. Med.* **73**, 525–531.
101. Laing, T. J. (1988). Gastrointestinal vasculitis and pneumatosis intestinalis due to systemic lupus erythematosus: Successful treatment with pulse intravenous cyclophosphamide. *Am. J. Med.* **85**, 555–558.
102. Pollak, V. E., Grove, W. J., Kark, R. M., *et al.* (1958). Systemic lupus erythematosus simulating acute surgical condition of the abdomen. *N. Engl. J. Med.* **259**, 258.
103. Reynolds, J. C., Inman, R. D., Kimberly, R. P., *et al.* (1982). Acute pancreatitis in systemic lupus erythematosus: Report of twenty cases and a review of the literature. *Medicine* **61**, 25–32.
104. Serrano Lopez, M. C., Bango, M. Y., Bonet, E. L., *et al.* (1991). Acute pancreatitis and systemic lupus erythematosus: Necropsy of a case and review of the pancreatic vascular lesions. *Am. J. Gastroenterol.* **86**, 764–767.
105. Levitin, P. M., Sweet, D., Brunner, C. M., *et al.* (1977). Spontaneous rupture of the liver. An unusual complication of systemic lupus erythematosus. *Arthritis Rheum.* **20**, 748–750.
106. Haslock, I. (1974). Spontaneous rupture of the liver in systemic lupus erythematosus. *Ann. Rheum. Dis.* **33**, 482–484.
107. Matsumoto, T., Yoshimine, T., Shimouchi, K., *et al.* (1992). The liver in systemic lupus erythematosus: Pathologic analysis of 52 cases and review of Japanese autopsy registry data. *Hum. Pathol.* **23**, 1151–1158.
108. Huang, D. F., Yang, A. H., Lin, B. C., and Wang, S. R. (1995). Clinical manifestations of hepatic arteritis in systemic lupus erythematosus. *Lupus* **4**, 152–154.
109. Swanepoel, C. R., Floyd, A., Allison, H., *et al.* (1983). Acute acalculous cholecystitis complicating systemic lupus erythematosus: Case report and review. *Br. Med. J.* **286**, 251–252.
110. Newbold, K. M., Allum, W. H., Downing, R., *et al.* (1987). Vasculitis of the gall bladder in rheumatoid arthritis and systemic lupus erythematosus. *Clin. Rheumatol.* **6**, 287–289.
111. Shapeero, L., Myers, A., Oberkircher, P. E., and Miller, W. T. (1974). Acute reversible lupus vasculitis of the gastrointestinal tract. *Radiology* **112**, 569–574.
112. Ho, M. S., The, L. B., and Goh, H. S. (1987). Ischemic colitis in systemic lupus erythematosus—report of a case and review of the literature. *Ann. Acad. Med. Singapore* **16**, 501–503.
113. Koh, E. T., Boey, M. L., and Feng, P. H. (1992). Acute surgical abdomen in systemic lupus erythematosus—an analysis of 10 cases. *Ann. Acad. Med.* **21**, 833–838.
114. Reissman, P., Weiss, E. G., Teoth, T.-A., *et al.* (1994). Gangrenous ischemic colitis of the rectum: A rare complication of systemic lupus erythematosus. *Am. J. Gastroenterol.* **89**, 2234–2236.
115. Sultan, S. M., Ioannou, Y., and Isenberg, D. A. (1999). A review of gastrointestinal manifestations of systemic lupus erythematosus. *Rheumatology* **38**, 917–932.
116. Tsushima, Y., Uozumi, Y., and Yano, S. (1996). Reversible thickening of the bowel and urinary bladder wall in systemic lupus erythematosus: A case report. *Radiat. Med.* **14**, 95–97.
117. Ko, S.-F., Lee, T.-Y., Cheng, T.-T., *et al.* (1997). CT findings at lupus mesenteric vasculitis. *Acta Radiol.* **38**, 115–120.
118. Helliwell, T. R., Flook, D., Whitworth, J., and Day, D. W. (1985). Arteritis and venulitis in systemic lupus erythematosus resulting in massive lower intestinal hemorrhage. *Histopathology* **9**, 1103–1113.
119. Yamaguchi, M., Kumada, K., Sugiyama, H., *et al.* (1980). Hemoperitoneum due to a ruptured gastroepiploic artery aneurysms in systemic lupus erythematosus. *J. Clin. Gastroenterol.* **12**, 344–346.
120. Philips, J. C., and Howland, W. J. (1968). Mesenteric arteritis in systemic lupus erythematosus. *JAMA* **206**, 1569–1570.
121. Kistin, M. G., Kaplan, M. M., and Harrington, J. T. (1978). Diffuse ischemic colitis associated with systemic lupus erythematosus—response to subtotal colectomy. *Gastroenterology* **75**, 1147–1151.

122. Medina, F., Ayala, A., Jara, L. J., *et al.* (1997). Acute abdomen in systemic lupus erythematosus: The importance of early laparotomy. *Am. J. Med.* **103**, 100–105.
123. Gladman, D. D., Ross, T., Richardson, B., and Kulkarni, S. (1985). Bowel involvement in systemic lupus erythematosus: Crohn's disease or lupus vasculitis. *Arthritis Rheum.* **28**, 466–470.
124. Freiman, D., Chon, H. K., and Bilaniuk, L. (1975). Pneumatosis intestinalis in systemic lupus erythematosus. *Radiology* **116**, 563–564.
125. Cabrera, G. E., Scopelitis, E., Cuellar, M. L., *et al.* (1994). Pneumatosis cystoides intestinalis in systemic lupus erythematosus with intestinal vasculitis: Treatment with high dose prednisone. *Clin. Rheumatol.* **13**, 312–316.
126. Kleinman, P., Meyers, M. A., Abbott, G., and Kazam, E. (1976). Necrotizing enterocolitis with pneumatosis intestinalis in systemic lupus erythematosus and polyarteritis. *Radiology* **1221**, 595–598.
127. Kobayashi, K., Asakunah, H., Schinzawa, T., *et al.* (1989). Protein-losing enteropathy in systemic lupus erythematosus. Observations by magnifying endoscopy. *Dig. Dis. Sci.* **34**, 1924–1928.
128. Weiser, M. M., Andres, G. A., Brentjens, J. R., *et al.* (1981). Systemic lupus erythematosus and intestinal venulitis. *Gastroenterology* **81**, 570–579.
129. Matsumoto, T., Kobayashi, S., Shimizu, H., Nakajima, M., Watanbe, S., Kitami, N., Sato, N., Abe, H., Aoki, Y., Hoshi, T., and Hashimoto, H. (2000). The liver in collagen diseases: Pathologic study of 160 cases with particular reference to hepatic arteritis, primary biliary cirrhosis, autoimmune hepatitis and nodular regenerative hyperplasia of the liver. *Liver* **20**, 366–373.
130. Trambert, J., Reinitz, E., and Buchbinder, S. (1989). Ruptured hepatic artery aneurysms in a patient with systemic lupus erythematosus: Case report. *Cardiovasc. Interven. Radiol.* **12**, 32–34.
131. Grimbacher, B., Huber, M., Von Kempis, J., *et al.* (1998). Successful treatment of gastrointestinal vasculitis due to systemic lupus erythematosus with intravenous pulse cyclophosphamide: A clinical case report and review of the literature. *Br. J. Rheum.* **37**, 1023–1028.
132. Kong, T. Q., Kellum, R. E., and Haserick. (1962). Clinical diagnosis of cardiac involvement in systemic lupus erythematosus. *Circulation* **26**, 7.
133. Hejtmancik, M. R., Wright, J. C., Quint, R., *et al.* (1964). The cardiovascular manifestations of systemic lupus erythematosus. *Am. Heart J.* **68**, 119.
134. Bonfiglio, T. A., Botti, R. E., and Hagstrom, J. W. C. (1972). Coronary arteritis, occlusion and myocardial infarction due to systemic lupus erythematosus. *Am. Heart J.* **83**, 153–158.
135. Heibel, R. H., O'Toole, J. D., Curtiss, E. I., *et al.* (1976). Coronary arteritis in systemic lupus erythematosus. *Chest* **69**, 700–703.
136. Korbet, S. M., Schwartz, M. M., and Lewis, E. J. (1984). Immune complex deposition and coronary vasculitis in systemic lupus erythematosus. *Am. J. Med.* **77**, 141–146.
137. Straaton, K. V., Chatham, W. N., Reveille, J. D., *et al.* (1988). Clinically significant valvular heart disease in systemic lupus erythematosus. *Am. J. Med.* **85**, 645–650.
138. Homcy, C. J., Liberthson, R. R., Fallon, J. T., *et al.* (1982). Ischemic heart disease in systemic lupus erythematosus in the young patient: Report of 6 cases. *Am. J. Cardiol.* **49**, 478–484.
139. Douglas, W. A. C., and Gardner, M. A. H. (1998). Systemic lupus erythematosus with vasculitis confined to the coronary arteries. *Aust. N. Z. J. Med.* **30**, 1023.
140. James, T. N., Rupe, C. E., and Monto, R. W. (1965). Pathology of the cardiac conduction systems in systemic lupus erythematosus. *Ann. Intern. Med.* **63**, 402.
141. Tsumagari, T., Fukumoto, S., Kinjo, M., *et al.* (1985). Incidence and significance of intrarenal vasculopathies in patients with systemic lupus erythematosus. *Hum. Pathol.* **16**, 43–49.
142. Appel, G. B., Pirani, C. L., and D'agati, V. D. (1994). Renal vascular complications of systemic lupus erythematosus. *J. Am. Soc. Nephrol.* **4**, 1499–1515.
143. Appel, G. B., Silva, F. G., Pirani, C. L., *et al.* (1978). Renal involvement in systemic lupus erythematosus: A study of 56 patients emphasizing histologic classification. *Medicine* **57**, 371–410.
144. Hughson, M. D., Nadasdy, T., McCarty, G. A., *et al.* (1992). Renal thrombotic microangiopathy in patients with systemic lupus erythematosus and the antiphospholipid syndrome. *Am. J. Kidney Dis.* **20**, 150–158.
145. Bhuyan, U. N., Malaviya, A. N., Dash, S. C., and Malhotra, K. K. (1983). Prognostic significance of renal angiitis in systemic lupus erythematosus. *Clin. Nephrol.* **20**, 109–113.
146. Altieri, P., Pani, A., Bolasco, P., *et al.* (1992). Is renal vasculitis in patients with systemic lupus erythematosus a bad prognostic factor? *Contrib. Nephrol.* **99**, 72–78.
147. Weisman, M. H., McDonald, E. C., and Wilson, C. B. (1981). Studies of the pathogenesis of interstitial cystitis, obstructive uropathy and intestinal malabsorbtion in a patient with systemic lupus erythematosus. *Am. J. Med.* **70**, 875–881.
148. Baskin, L., Mee, S., Malthay, M., *et al.* (1989). Ureteral obstruction caused by vasculitis. *J. Urol.* **141**, 933–935.
149. Tripp, B. M., Chu, F., Halwani, F., and Hassouna, M. M. (1995). Necrotizing vasculitis of penis in systemic lupus erythematosus. *J. Urol.* **154**, 528–529.
150. Meyers, K. E. C., Pfieffer, S., Lu, T., and Kaplan, B. S. (2000). Genitourinary complications of systemic lupus erythematosus. *Pediatr. Nephrol.* **14**, 416–421.
151. Feriozzi, S., Muda, A. O., Amini, M., Faraggiana, T., and Ancarani, E. (1997). Systemic lupus erythematosus with membranous glomerulonephritis and uterine vasculitis. *Am. J. Kidney Dis.* **29**, 277–279.
152. Igarashi, T., Nagaoka, S., Matsunaga, K., *et al.* (1989). Aortitis syndrome (Takayasu's arteritis) associated with systemic lupus erythematosus. *J. Rheumatol.* **16**, 1579–1583.
153. Saxe, P. A., and Altman, R. D. (1990). Aortitis syndrome (Takayasu's arteritis) associated with SLE. *J. Rheumatol.* **17**, 1251–1252.

154. Kameyama, K., Kuramochi, S., Ueda, T., Kawada, S., Tominaga, N., Mimori, T., and Hata, J. (1999). Takayasu's aortitis with dissection in systemic lupus erythematosus. *Scand. J. Rheumatol.* **28**, 187–188.
155. Washiyama, N., Kazui, T., Takinami, M., Yamashita, K., Terada, H., Muhammad, B. A., and Miura, K. (2000). Surgical treatment of recurrent abdominal aortic aneurysm in a patient with systemic lupus erythematosus. *J. Vasc. Surg.* **32**, 209–212.
156. Saxe, P. A., and Altman, R. D. (1992). Takayasu's arteritis syndrome associated with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **21**, 295–305.
157. Rojo-Leyva, F., Ratliff, N., Cosgrove, D. M., and Hoffman, G. S. (2000). Study of 52 patients with idiopathic aortitis from a cohort of 1,204 surgical cases. *Arthritis Rheum.* **43**, 901–907.
158. Gladstein, G. S., Rynes, R., Parhami, N., and Bartholomew, L. E. (1979). Gangrene of a foot secondary to systemic lupus erythematosus with large vessel vasculitis. *J. Rheumatol.* **6**, 549–553.
159. Kaufman, J. L., Bancilla, E., and Slade, J. (1986). Lupus vasculitis with tibial artery thrombosis and gangrene. *Arthritis Rheum.* **29**, 1291–1292.
160. Bakker, F. C., Rauwerda, J. A., Moens, J. H., *et al.* (1989). Intermittent claudication and limb-threatening ischemia in systemic lupus erythematosus and in systemic lupus erythematosus-like disease: A report of two cases and review of the literature. *Surgery* **106**, 21–25.
161. Harmon, S. M., Oltmanns, K. L., and Min, K.-W. (1991). Large vessel occlusion with vasculitis in systemic lupus erythematosus. *South. Med. J.* **84**, 1150–1154.
162. Abramowsky, C. R., Vegas, M. E., Swinehart, G., *et al.* (1980). Decidual vasculopathy of the placenta in lupus erythematosus. *N. Engl. J. Med.* **303**, 668–672.
163. Hanly, J. G., Gladman, D. D., Rose, T. H., *et al.* (1988). Lupus pregnancy: A prospective study of placental changes. *Arthritis Rheum.* **31**, 358–366.
164. Grennan, D. M., McCormick, J. N., Wojtacha, D., *et al.* (1978). Immunological studies of the placenta in systemic lupus erythematosus. *Ann. Rheum. Dis.* **37**, 129–134.
165. Benirschke, K., and Driscoll, S. G. (1967). "The Pathology of the Human Placenta," p. 553. Springer-Verlag, New York.
166. Levy, Y., George, J., Hojnic, M., *et al.* (1996). Comparison of clinical and laboratory parameters for systemic lupus erythematosus activity in Israelis versus Europeans. *Isr. J. Med. Sci.* **32**, 100–104.
167. Tuffanelli, D. L., and Dubois, E. L. (1964). Cutaneous manifestations of systemic lupus erythematosus. *Arch Dermatol* **90**, 377.
168. Abu-Shakra, M., Urowitz, M. B., Gladman, D. D., and Gough, J. (1995). Mortality studies in systemic lupus erythematosus. Results from a single center. I. Causes of death. *J. Rheumatol.* **22**, 1259–1264.
169. Molina, J. F., Drenkard, C., Molina, J., *et al.* (1996). Systemic lupus erythematosus in males. A study of 107 Latin American patients. *Medicine* **75**, 124–130.
170. Abu-Shakra, M., Urowitz, M. B., Gladman, D. D., and Gough, J. (1995). Mortality studies in systemic lupus erythematosus. Results from a single center. II. Predictor variables for mortality. *J. Rheumatol.* **22**, 1265–1270.
171. Matolo, N. M., and Albo, D., Jr. (1971). Gastrointestinal complications of collagen vascular disease—surgical implications. *Am. J. Surg.* **122**, 678–682.
172. Nadorra, R. L., Nakazato, Y., and Landing, B. H. (1987). Pathologic features of gastrointestinal tract lesions in childhood-onset systemic lupus erythematosus: Study of 26 patients with review of the literature. *Pediatr. Pathol.* **7**, 245–259.

33

SYSTEMIC LUPUS ERYTHEMATOSUS AND THE LUNG

E. Clinton Lawrence

Systemic lupus erythematosus (SLE) is a systemic disease with protean manifestations affecting virtually every organ either directly or indirectly. Involvement of the lung and pleura in SLE may be as common as the renal involvement and more common than involvement of the central nervous system [1]. Fortunately, pulmonary derangements in SLE are not usually as life threatening as may be the renal or central nervous system complications. Nevertheless, pulmonary dysfunction does occur in SLE and may be a significant cause of morbidity and occasionally of mortality.

The reported frequency of pulmonary involvement in SLE varies greatly among published series, depending on the population studied and the referral patterns of the institution [2]. For example, parenchymal lung involvement attributed to SLE has ranged from as low as 0.9% [1] to a high of 98% [3]. Most series indicate that pulmonary involvement occurs 50% or more of the time in SLE [4], with autopsy series reporting even greater frequencies of involvement [5]. However, clinically inapparent pulmonary impairment may occur in the majority of cases, since abnormalities of pulmonary testing have been reported in 88% of unselected cases in one series [6]. The most frequently noted abnormality was the diffusing capacity for carbon monoxide, which was reduced in 72% of cases [6]. However, there was no correlation between the pulmonary abnormalities and other measures of lupus activity [6]. Additionally, one prospective study has shown only a low rate of pulmonary function deterioration over time [7].

In addition to primary pulmonary disorders attributed to SLE, secondary pulmonary complications, especially infections [1, 2], may occur as a consequence of disease in other organs or of treatment. Clearly, then, pulmonary derangements in SLE may frequently challenge the diagnostic and therapeutic acumen of physicians from a variety of disciplines.

PRIMARY PULMONARY INVOLVEMENT IN SYSTEMIC LUPUS ERYTHEMATOSUS

The term pulmonary involvement in SLE, as used in this chapter, refers to abnormalities not only of the lungs themselves but also of contiguous structures involved in normal respiration. In this context, distinct clinical entities have been described that correlate with anatomic disease of the diaphragm, the pleura, the lung parenchyma (including interstitium, bronchioles, and alveoli), and vasculature. Each of these disease processes is discussed in greater detail.

Diaphragmatic Involvement

It has long been recognized and shown radiographically that certain patients with SLE may exhibit progressive loss of lung volumes because of insidious and progressive basilar atelectasis accompanied by elevation of both hemidiaphragms [8]. Whether this syndrome of so-called shrinking or vanishing lungs was due

to abnormalities within the lungs (i.e., atelectasis) or to abnormalities of the diaphragms was not clear until Gibson *et al.* [9] measured diaphragmatic function in unselected patients with SLE. Abnormalities of maximal transdiaphragmatic pressures during maximal inspiratory effort were present in four of the five individuals who had elevation of the diaphragms. These and other findings [10–13] indicated that weakness of respiratory muscles, possibly as part of a more generalized myopathy or mysositis or even diaphragmatic fibrosis [12], might explain the syndrome of vanishing lungs.

However, incrimination of diaphragmatic weakness as the primary factor in the pathogenesis of shrinking lungs has been challenged by Laroche *et al.* [13], who found decreases in maximal transdiaphragmatic pressure caused by incomplete activation of the diaphragm during a maximal voluntary effort in only 3 of 12 patients with SLE. These observations led them to invoke a restriction in chest-wall compliance to explain the syndrome.

Although the role of the diaphragm in the shrinking lungs syndrome remains controversial, the clinical picture is characteristic. Patients thus afflicted often complain of dyspnea that is out of proportion to objective impairment of pulmonary function. The sensation of dyspnea may be exacerbated by the recumbent position when the diaphragm pushes further into the chest cavity. Such patients may find at least partial alleviation of their discomfort by sleeping with the head of their bed elevated. Rarely, patients may suffer from muscle weakness resulting from an association of myasthenia gravis and SLE; such patients may respond to anticholinesterase drugs such as edrophonium-chloride (Tensilon). It is not clear from the literature whether corticosteroid and immunosuppressive therapy improve diaphragmatic performance in affected patients. Fortunately, such treatments may not be needed for diaphragmatic disease per se, because Gibson *et al.* [9] reported that three of their five patients remained stable over 4–6 years.

Pleural Involvement

Involvement of the pleura may occur in as many as 50% of SLE patients [1, 2, 5] either as pleuritis with chest pain or as frank effusion. The pathogenesis of the pleuritis appears to be lymphocyte and plasma cell infiltration of the pleura [2] although granulomas have also been described in biopsy specimens [14]. In patients with known SLE, pleural effusion in the presence of disease activity elsewhere may be due to lupus involvement of the pleura, particularly when the effusion is bilateral, as is often the case. The other major diagnostic consideration in established SLE is infection, particularly in patients who have been receiving immunosuppressive drugs. Thoracentesis with examination of the pleural fluid is essential to clarify the diagnosis [15].

Although effusions related to SLE or infection may share certain characteristics, other differences may be appreciated (see Table 1). With infected pleural effusions, bacteria may be seen with Gram stain or grown from culture. The white blood cell (WBC) count is usually much higher in bacterially infected effusions than in effusions caused by SLE and shows a predominance of neutrophils [16], whereas either a lymphocytic or a neutrophilic predominance has been described with SLE [17]. Tuberculous effusions may be similar to SLE effusions, with a lower total white blood cell count and a lymphocytic predominance [16, 18]. With grossly infected fluids (empyemas), the glucose and pH levels are typically low [19, 20], whereas these values are characteristically normal in SLE effusions [21]. The features that most readily distinguish SLE effusions from infected effusions are immunologic, such as reduced levels of complement and the presence of immune complexes, anti-DNA antibodies, and occasionally lupus erythematosus (LE) cells [22–25]. The same characteristics serve to differentiate pleural effusions in SLE from effusions seen with pulmonary embolus with infarction and from malignant effusions. Pleural fluid findings that may be considered diagnostic of SLE include (1) LE cells, (2) pleural fluid ANA titer greater than or equal to 1:160, and (3) a pleural fluid/serum ANA ratio of greater than 1 [26].

Early in the course of the disease process, it is sometimes difficult to differentiate SLE from rheumatoid arthritis (RA). Pathologically, evidence of pleural involvement is present in the majority of patients with either disease process [4]. Clinically, however, the two disorders vary a great deal. For example, the majority of patients with SLE complain of pleuritic chest pain and dyspnea, whereas the pleural disease in patients with RA is often asymptomatic [4]. Effusions in RA more often affect men and are associated with subcutaneous rheumatoid nodules, high-titer rheumatoid factor, and parenchymal lung disease [27–31].

Analysis of the pleural fluid is helpful in distinguishing between the two processes. Thus the glucose is normal in SLE but is almost always decreased in RA [30, 31], so that the finding of a pleural fluid glucose level of less than 30 mg/dl in the absence of infection or malignancy is virtually diagnostic of RA [30, 32, 33]. Complement levels, both total hemolytic complement (CH100) and C3 and C4, may be decreased in either disease and thus are helpful in differentiating effusions

TABLE 1 Differential between SLE and Other Causes of Pleural Effusions

	SLE	RA	Pulmonary infection	Pulmonary embolus[a]	Malignancy
Symptoms	Chest pain Dyspnea	May be asymptomatic	Fever Chest pain	Chest pain Dyspnea	Varies
Pleural fluid character	Usually clear (may be hemorrhagic)	Clear	Clear to cloudy	Clear to bloody	Clear to bloody
Protein	Increased	Increased	Increased	Increased (usually)	Increased
Glucose	Normal	Decreased (often)	Decreased (often)	Normal	May be decreased
pH	Normal	Normal	Decreased (empyema)	Normal	May be decreased
WBC	Increased (<10,000/mm^3)	Increased (<10,000/mm^3)	Increased (>10,000/mm^3)	Increased	Increased
Cell predom	PMN[b] or lymph	PMN or lymph	PMN (lymph with Tb)	PMN (usually)	Varies
Complement					
C_3	Decreased	Decreased	Normal	Normal	Normal
C_4	Decreased	Decreased	Normal	Normal	Normal
Total	Decreased	Decreased	Normal	Normal	Normal
Immune complex					
C_1q	Often positive	Always positive	Negative	Negative	Negative
RFm[c]	Rarely positive	Usually positive	Negative	Negative	Negative
Raji	Often positive	Usually positive	Negative	Negative	Negative
Anti-DNA	Often positive	Negative	Negative	Negative	Negative
LE[d] cells	Occasionally positive	Negative	Negative	Negative	Negative

[a] Sec Reference 160.
[b] PMN, polymorphonuclear.
[c] RFm, monoclonal antirheumatoid factor by radioimmunoassay [22].
[d] LE, lupus erythematosus.

caused by connective tissue disease from those resulting from other causes of pleural effusions, but they are of no value in separating SLE from rheumatoid arthritis. Immune complexes may be present in either disease with enough overlap so that it would be difficult to establish a diagnosis firmly solely by the presence of immune complexes in the pleural space regardless of the assay employed [22]. The presence of either anti-DNA antibodies or LE cells in the pleural fluid is diagnostic of SLE [22, 23]; LE cells may also be seen in the pleural fluid with drug-induced SLE [34].

Once infection and other causes of the effusions have been excluded, treatment may be directed at the lupus effusion. In the absence of systemic disease activity, treatment with nonsteroidal anti-inflammatory drugs such as indomethacin or corticosteroids may be tried. Other immunosuppressive agents such as cyclophosphamide or azathioprine are rarely indicated for SLE pleuritis alone, but may be considered for systemic involvement such as renal disease. Pleural effusions caused by SLE usually respond to therapy or resolve spontaneously. Occasionally, however, chronic pleuritis ensues and may require pleurectomy to achieve symptomatic relief [35, 36].

Parenchymal Lung Involvement

Acute Lupus Pneumonitis

Involvement of the lung parenchyma in SLE has been appreciated since the time of Osler [37]. Subsequently, a variety of reports have documented cases of pneumonitis in the absence of detectable infection in patients with SLE [38–41]. The term *acute lupus pneumonitis* has been applied to individuals with an abrupt febrile pneumonitic process in whom infection has been excluded (see Fig. la).

Matthay and co-workers [41] reported 12 such cases from a pool of 102 SLE patients over a 6-year period (11.7% incidence). The clinical presentation was suggestive of bacterial pneumonia with fever, pleuritic chest pain, cough with hemoptysis, and dyspnea as prominent features. However, cultures for pathogens were negative, and LE preparations or ANA studies were positive. Only 50% of these cases were diagnosed as having SLE before admission. Despite treatment with corticosteroids either alone or in combination with azathioprine, 50% were left with persistent infiltrates with pulmonary function studies severely restricted in one patient.

The pathologic changes in acute lupus pneumonitis are generally nonspecific, with evidence of acute alveolar wall injury, alveolar hemorrhage, alveolar edema, and hyaline membrane formation (see Fig. 1b) [38–42]. A small vessel vasculitis has been suggested to be distinctive for SLE [43]; diffuse interstitial lymphoid infiltrates suggestive of lymphocytic interstitial pneumonitis have also been described [41]; depositions within the lung of immune complexes of IgG, C3, and DNA have been reported [44, 45].

Acute lupus pneumonitis may respond to treatment with corticosteroids alone [46] or to azathioprine when corticosteroids are ineffective [47]. The addition of plasmapheresis to immunosuppressive therapy may be warranted for refractory cases of fulminant acute lupus pneumonitis [48–50]. Nonetheless, the mortality and morbidity of acute lupus pneumonitis are extremely high [41].

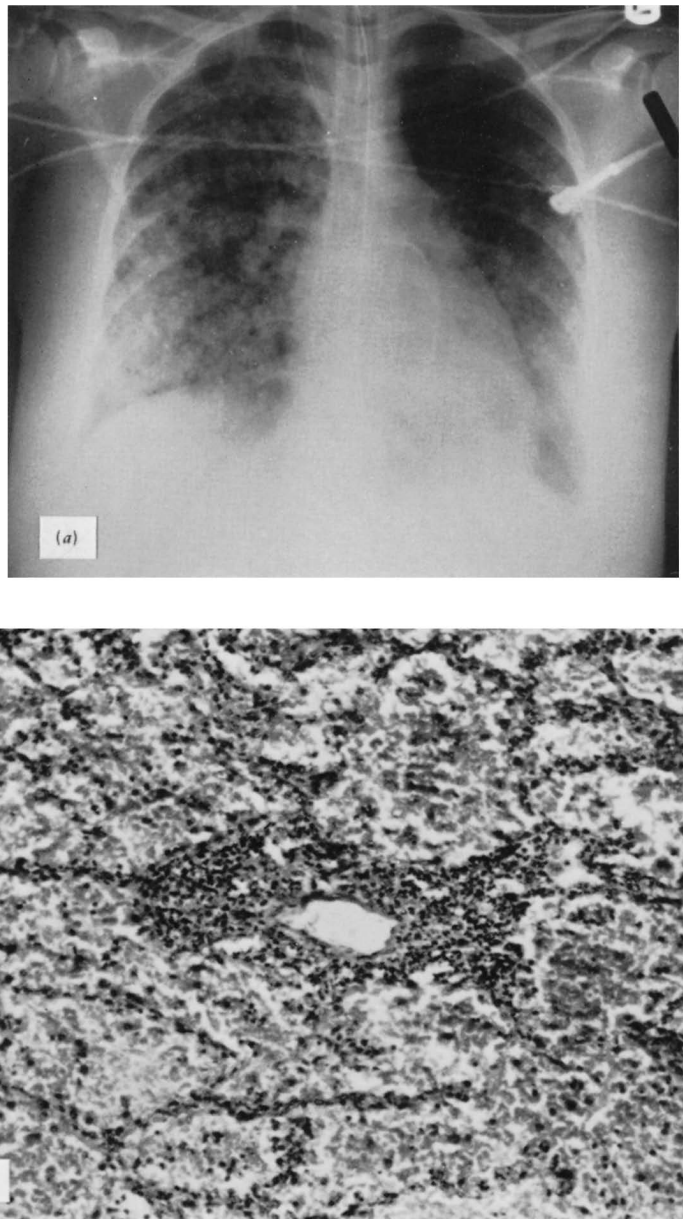


FIGURE 1 Acute lupus pneumonitis. (*a*) Chest *x* ray showing diffuse alveolar infiltrates in a patient with SLE. (*b*) Open lung biopsy specimen showing perivascular inflammatory infiltration (vasculitis) and alveolar hemorrhage. Alveolar septae are well preserved. (H&E, × 250.) (Courtesy of S. Donald Greenberg, M.D.)

Chronic (Fibrotic) Lupus Pneumonitis

In addition to the acute pulmonary disease associated with SLE, some patients may develop a more chronic syndrome characterized by progressive dyspnea and diffuse interstitial infiltrates (see Fig. 2) [46]. The incidence of interstitial disease in SLE varies from 0 to 9% in reported series [51, 52], indicating this to be an uncommon feature of SLE. In a series of 18 cases with diffuse interstitial lung disease and SLE reported by Eisenberg *et al.* [53], 7 (39%) initially presented with pulmonary symptoms, but other cases developed at various stages of SLE. As might be expected, pulmonary function studies showed a restrictive pattern with reduction in lung volumes and in the diffusion capacity for carbon monoxide. All patients in their series exhibited positive LE preparations, and most had ANAs as well. In addition, most suffered from other stigmata of SLE, including arthritic complaints and skin, renal, or central nervous system involvement. An association of interstitial pneumonitis in SLE with anti-Ro (SSA) antibodies has been described [54, 55]. A trend toward an increased occurrence of other autoantibodies may also be present [56].

The differential diagnosis of diffuse pulmonary infiltrates developing in patients with established SLE is limited mainly to infection (particularly when immunosuppressive therapy has been employed) and to SLE-associated fibrosis of the lungs. However, the diagnostic possibilities are more numerous in patients with interstitial infiltrates before the onset of the more classic features of SLE. When challenged with such cases physicians try to limit the diagnostic possibilities through clinical exclusion of such conditions as cardiogenic and noncardiogenic pulmonary edema (i.e., congestive heart failure and the adult respiratory distress syndrome, respectively).

To distinguish between the various causes of interstitial lung diseases, specialized procedures are required. As indicated in Figure 3, bronchoscopy with bronchoalveolar lavage to exclude infection is the diagnostic procedure of choice; lung tissue may also be obtained with transbronchial lung biopsy. Major diagnoses that may be established include (1) infection, particularly opportunistic ones; (2) cancer, particularly lymphangitic carcinomatosis or bronchioalveolar cell carcinoma; (3) sarcoidosis (defined *clinically* as the finding of characteristic noncaseating granulomas in a patient with a compatible clinical presentation in whom other causes of granulomas have been excluded); (4) interstitial fibrosis; and (5) no pathologic diagnosis [57]. When either of the latter two results from transbronchial lung biopsy are encountered, the clinician is faced with deciding whether to act on the available

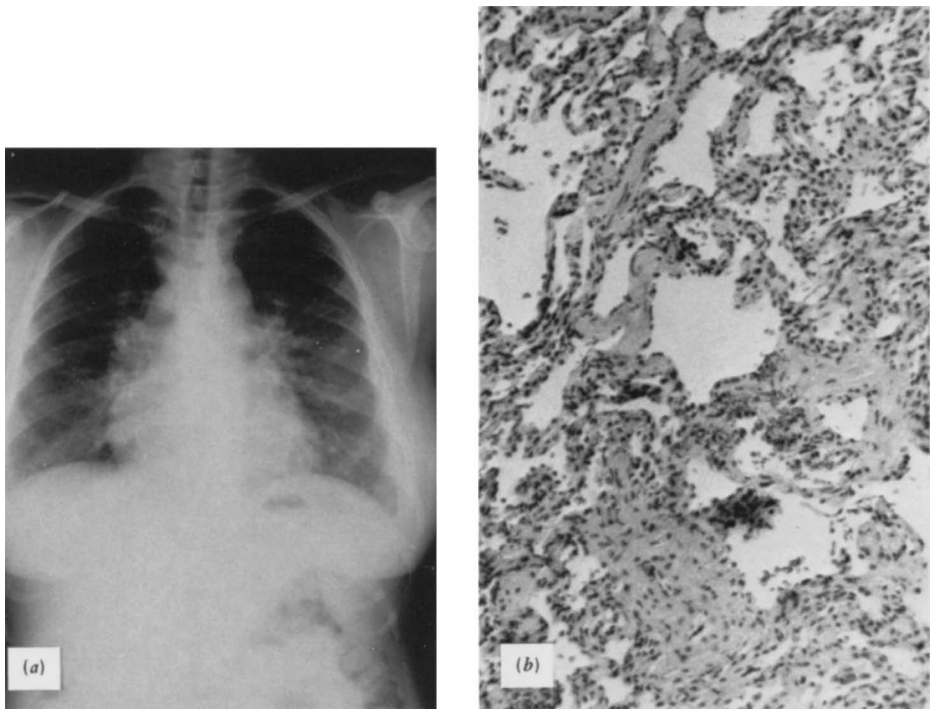


FIGURE 2 Interstitial lung disease associated with SLE. (*a*) Chest *x* ray showing diffuse interstitial infiltrates in a woman with established SLE. (*b*) Transbronchial biopsy specimen shows mild interstitial fibrosis (H&E, × 250.) (Courtesy of Carlox A. Mattioli, M.D.)

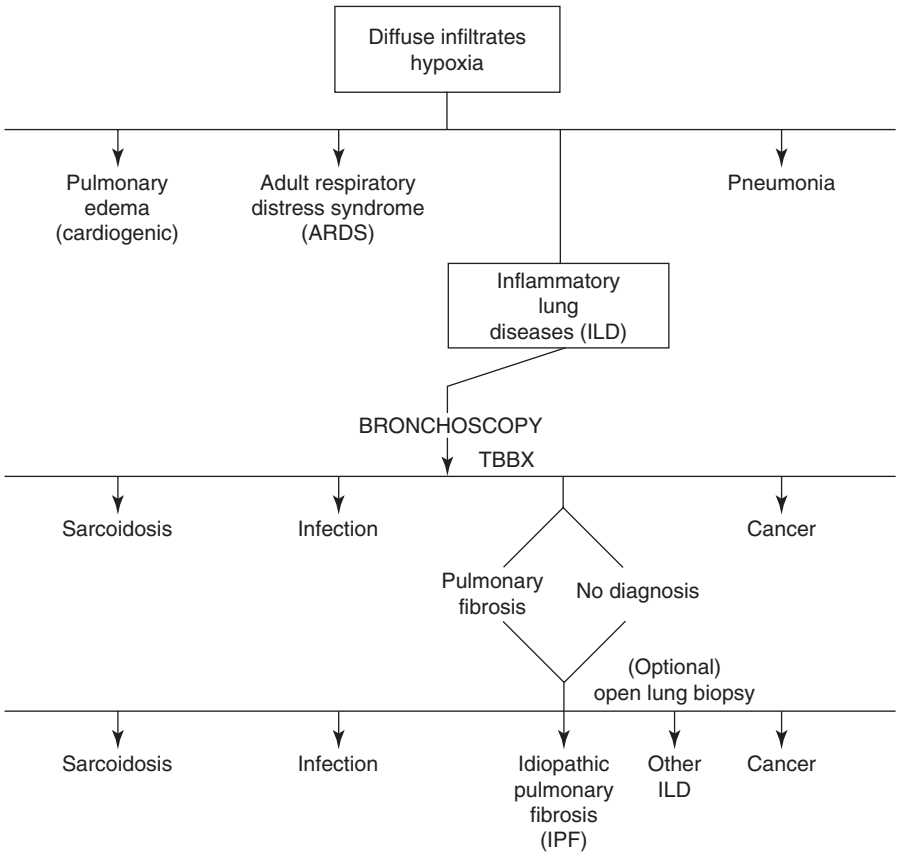


FIGURE 3 Clinical approach to the patient with pulmonary infiltrates and hypoxia. TBBX, Transbronchial lung biopsy.

information or to proceed to lung biopsy via either thoracotomy or video-assisted thoracoscopy (VAT). Some would advocate proceeding directly to thoracotomy or VAT whereas others rarely send patients to open lung biopsy, arguing that the risk relative to transbronchial lung biopsy is higher and the additional information to be gained is small. The type of patient involved should influence the approach taken. Thus, in nonimmunocompromised individuals, the additional yield from open lung biopsies compared with transbronchial biopsies may be low [58]. Others have argued for open lung biopsy in critically ill immunocompromised patients [59, 60]. Because of the many variables involved, the invasive diagnostic approach to any given patient must be carefully individualized. In SLE, the main value of either transbronchial or open lung biopsies is to exclude other diagnostic possibilities, particularly infections, since the histological features of chronic lung involvement in SLE are nonspecific (see Fig. 2b) [42].

When the more classic features of SLE precede the pulmonary involvement, differentiation from infection rather than other causes of neutrophilic alveolitis becomes the critical issue [61]. In cases in which the pulmonary involvement is the presenting feature, however, it may be difficult to implicate SLE initially. For example, the clinical, radiographic, laboratory, and immunologic features of idiopathic pulmonary fibrosis and SLE-associated chronic fibrosis are very similar [62, 63]. Indeed, idiopathic pulmonary fibrosis is usually distinguished from SLE by the absence of extrapulmonary manifestations [63]. Perhaps the most dramatic illustration of how difficult it may be to diagnose SLE when the initial abnormalities are limited to the lungs was a patient who was initially reported as having idiopathic pulmonary hemosiderosis with immune complex deposition in the lungs [64] who later exhibited more classic features of SLE [65].

The chronic interstitial lung disease of SLE, while infrequent, may have a poor prognosis [66]. The response to corticosteroids may be disappointingly poor, similar to the response of idiopathic pulmonary fibrosis [67, 68], which resembles SLE in its pulmonary manifestations. There are insufficient data with which to

access the possible usefulness of cytotoxic therapy or of plasmapheresis singly or in combination for the treatment of SLE-associated pulmonary fibrosis. One attempted heart–lung transplant in a 16-year-old male with SLE and chronic pulmonary fibrosis was unsuccessful [69]. However, the patient also suffered from multisystem disease and was moribund at the time of transplantation.

New therapeutic modalities being used for idiopathic pulmonary fibrosis could potentially be useful for SLE-associated pulmonary fibrosis. For example, α-interferon was effective in halting disease progression in a small series of European patients with idiopathic pulmonary fibrosis [70]; additional studies are underway in the United States.

Pulmonary Vascular Involvement

Diffuse Alveolar Hemorrhage

Diffuse alveolar hemorrhage may be considered as a manifestation of acute lupus pneumonitis, but because of its distinctive features is herein treated as a separate clinical entity. A report of 19 cases and review of the literature [71] demonstrated that diffuse alveolar hemorrhage affects predominantly young women (median age 27) and can be the initial manifestation of SLE in from 11 to 20% of patients. Hemoptysis eventually appeared in all 19 cases but was present on admission in only 42% of the cases vs 66% of cases previously reported. Pulmonary capillaritis was found in 8 of 10 lung tissue samples, representing a far higher percentage of capillaritis than noted in previous series. Concurrent lupus nephritis was found in the majority of cases (14/15) and associated infections were present in more than half of patients with diffuse alveolar hemorrhage. The overall mortality rate is approximately 50%, with increased mortality associated with the presence of infection, the requirement for mechanical ventilation, and usage of cyclophosphamide for acute diffuse alveolar hemorrhage episodes.

The diagnosis of diffuse alveolar hemorrhage may be suspected during bronchoscopy with bronchoalveolar lavage, as the aspirated fluids become progressively more bloody. Microscopically, bronchoalveolar lavage may demonstrate red blood cells, either free or within alveolar macrophages [72]; hemosiderin-laden macrophages indicate bleeding of 48 h or greater duration [73]. The appropriate treatment of diffuse alveolar hemorrhage is high-dose intravenous methylprednisolone in the range of 15 mg/kg daily for 3 days. Plasmapheresis appears to not improve survival whereas cyclophosphamide treatment is associated with a worsened outcome.

Pulmonary Hypertension

Severe pulmonary artery hypertension with resultant cor pulmonale may occur as an isolated disease (primary pulmonary hypertension) [74–77] or in association with the connective tissue diseases [78–84]. In the latter category, pulmonary hypertension is more commonly associated with scleroderma [78, 79] and the mixed connective diseases than with either RA [81] or SLE [82, 83]. However, several reports have documented the development of pulmonary hypertension in patients with either established or quiescent SLE [82–87]. Despite the sensation of breathlessness, patients are not profoundly hypoxemic, and the chest X ray is characteristically clear; pulmonary function studies show a restricted pattern with a reduced diffusion capacity for carbon monoxide [87]. Interestingly, Raynaud's phenomenon, which occurs in both primary pulmonary hypertension [75–77] and the pulmonary hypertension associated with scleroderma, is also common in SLE patients with pulmonary hypertension [82–85]. Thus, pulmonary vascular hyperresponsiveness with vasoconstriction, rather than vasculitis, is likely involved in the pulmonary hypertension of SLE. Indeed, vasculitis is rarely seen in lung biopsy specimens or autopsy lung tissues from SLE patients with pulmonary hypertension.

The prognosis for severe pulmonary hypertension, whether primary or associated with SLE or other forms of connective tissue disease, is poor [74–88]. Until more recently, the medical treatment for both primary pulmonary hypertension and pulmonary hypertension associated with connective tissue diseases had been unsatisfactory. However, continuous infusion epoprostenol (Prostacyclin) has been shown to prolong survival and improve performance status and hemodynamics in primary pulmonary hypertension [89] and to improve performance and hemodynamic parameters in pulmonary hypertension associated with connective tissue diseases, especially scleroderma [90] and SLE [91]. Endothelin receptor antagonists, oral agents that are effective in patients with both primary and scleroderma-associated pulmonary hypertension [92], hold promise for SLE-associated pulmonary hypertension as well.

One successful treatment for primary pulmonary hypertension, as well as pulmonary hypertension secondary to congenital heart disease (Eisenmenger's complex), has been heart–lung or lung transplantation [93, 94]. Indeed, one of two patients initially reported from Papworth Hospital at Cambridge, United Kingdom, survived heart–lung transplantation for SLE-associated pulmonary hypertension [88], but a subsequent report from this center cautioned against lung

transplantation in SLE patients, especially those with anti-cardiolipin antibodies [95]. Long-term survival has been reported following heart–lung transplantation in another SLE patient transplanted for pulmonary hypertension [96]. Thus, while systemic diseases have in general been considered contraindications for lung or heart–lung transplantation, selected patients with SLE may be considered for some form of lung transplantation on a case-by-case basis.

Thromboembolic Disease

The occurrence of anti-phospholipid antibodies in patients with SLE may predispose them to recurrent thromboses of both the arterial and venous systems, along with thrombocytopenia and increased fetal loss [97–99]. The presence of lupus anticoagulant increases the risk for deep venous thrombosis with or without pulmonary embolism by sixfold, while anti-cardiolipin antibodies increases the risk twofold [100]. The incidence of anti-phospholipid antibodies in SLE patients may be as high as 41% [101]. Deep venous thrombosis occurred in 10/24 patients (41%) and pulmonary embolism in one patient [101]. Thus, thromboembolic disease should be considered in all SLE patients with appropriate presentation, especially those with anti-phospholipid antibodies. Treatment may require anticoagulation with placement of an inferior vena cava filter because of the ongoing risk of recurrent thromboembolism. Additionally, chronic thromboembolic disease, with or without anti-phospholipid antibodies, is one cause of pulmonary hypertension in SLE [88]; therefore, all SLE patients with pulmonary hypertension should be evaluated for this possibility.

Pulmonary Disorders Associated with SLE

Infections

Infections, particularly pulmonary ones, are extremely common in immunocompromised patients [102]. Immunocompromised people are susceptible to common and uncommon bacterial organisms as well as to unusual (opportunistic) infections with fungi, viruses, and protozoa [102]. Patients with SLE are similarly at risk for a variety of infections [103, 104].

Treatment with corticosteroids and other immunosuppressive drugs is certainly implicated in many of these infectious problems, but some evidence exists for abnormalities of immune defense in SLE in the absence of immunosuppressive therapy [105–108]. For example, neutrophils are defective in both chemotaxis [105] and phagocytosis [106]. A serum inhibitor of complement-derived chemotactic factor activity may also contribute to increased susceptibility to infection in SLE [108]. Alveolar macrophage function is also impaired [109]. For all these reasons, SLE patients may be at risk of severe infections with common organisms, such as *streptococcus pneumoniae* [110].

In an effort to reduce the incidence of pneumococcal infections, investigators at the National Institutes of Health have shown that SLE patients can be successfully immunized against pneumococci, although with less of an antibody response than seem with controls [110]. SLE patients may also be safely and successfully immunized against influenza [111]. However, the efficacy of either pneumococcal or influenzal vaccines in preventing infection is unproven. For this reason, the decision of whether to immunize SLE patients with either or both vaccines must be determined on an individual basis. Certainly, annual influenzal vaccination is recommended for immunocompromised individuals unless contraindications to vaccination exist.

The presentation of pulmonary infections in SLE may be dramatic or more subtle, especially when corticosteroids mask the febrile response. Although primary (noninfectious) acute lupus pneumonitis may be indistinguishable clinically from bacterial pneumonia (i.e., fever, chest pain, hemoptysis, and pulmonary infiltrates), the presumption of infection *must* always prevail until evidence for the lack of infection has accumulated. As discussed earlier, procedures such as bronchoscopy with bronchoalveolar lavage and/or transbronchial lung biopsy or even lung biopsy via thoracotomy or video-assisted thoracoscopy may be needed to determine whether the process is infectious. The use of sheathed-brush sterile catheters [112, 113] or quantitative cultures of bronchoalveolar lavage fluids [112] may allow non-contaminated sampling of lower respiratory secretions for stains and cultures. It must be emphasized that empiric therapy with broad-spectrum antibiotics is indicated *immediately* in such cases, even if appropriate cultures cannot be obtained initially.

Other Associated Conditions

Uremic Pulmonary Edema

Systemic lupus erythematosus patients may present with clinical and radiographic signs of pulmonary edema, including bilateral pleural effusions and confluent fluffy perihilar infiltrates. This usually occurs in the setting of fluid overload and uremia from progressive renal failure [4]. The anatomic findings of pulmonary edema have also been ascribed to infection, aspiration, increased intracranial pressure, or congestive heart failure [42]. Obviously, many of these conditions may coexist in critically ill patients with SLE.

Postpartum Pneumonitis

Systemic lupus erythematosus is primarily a disease affecting young women of childbearing age. Many of these women with established SLE become pregnant and have uneventful pregnancies, while others may have exacerbations of their disease process [114]. However, as many as one-half of SLE patients suffer a major flare during the postpartum period [115]. This can occur up to 8 weeks after delivery, with worsening lupus as the most common abnormality seen [115]. Cases of acute lupus pneumonitis developing in the postpartum period have been described [115, 116]. The clinical presentation and high mortality are similar to cases unrelated to pregnancy [41]. Anti-phospholipid antibodies may be involved in some cases of postpartum pneumonitis [99].

Bronchiolitis/Bronchiolitis Obliterans with Organizing Pneumonia (BOOP)

Although a restricted pattern of pulmonary function is characteristically associated with SLE [6, 117, 118], obstruction to airflow has also been described [119] and attributed to bronchiolitis found in open lung biopsy materials. Bronchiolitis was also described in 1 of 12 of the Matthay *et al.* [41] cases and has been reported in both RA [120] and in scleroderma [121]. Clinically, patients experience cough, dyspnea, and wheezing [122]. The response to corticosteroid therapy is variable, with five of six RA patients dying [120] despite such measures. The SLE patient reported by Kinney and Angelillo [119], however, is said to have improved with corticosteroids. Bronchiolitis obliterans with organizing pneumonia (BOOP) has rarely been reported in SLE [123] and is usually responsive to corticosteroids.

Sarcoidosis

Sarcoidosis and SLE are dissimilar clinically, yet they share certain impairments of immunologic function. For example, humoral immune function is augmented in both diseases with hyperglobulinemia and circulating immune complexes, while T-cell activity is impaired [124, 125]. Several cases of the concurrence of SLE and sarcoidosis have been reported [125–130]. It is unclear whether these two disease processes occur in the same individual by coincidence or through some common mechanisms related to immune complex deposition or defective T-cell function [127].

Lymphocytic Interstitial Pneumonia

The usual form of interstitial lung disease in SLE is nonspecific [42] and is similar to Liebow's usual interstitial pneumonia [131]. However, cases have been reported in which the histological appearance has been consistent with lymphocytic interstitial pneumonia [132, 133], which represents another subset of the interstitial pneumonias as classified by Liebow [131]. The lung's interstitium in lymphocytic interstitial pneumonia is diffusely infiltrated by mature lymphocytes and plasma cells. Patients with lymphocytic interstitial pneumonia are characteristically ill with fever, cough, and dyspnea.

Lymphoma and Pseudolymphoma

Patients with SLE have an increased incidence of lymphomas [134–137] and Kaposi's sarcoma has been reported [138]. Additionally, focal masslike accumulations of lymphocytes and plasma cells in the lungs are termed *pseudolymphomas* when the process is localized to the lungs [139, 140]. A case of pseudolymphoma occurring in SLE has been reported [141]. Thus lymphocytic interstitial pneumonia and pseudolymphoma may represent variations in the spectrum of a premalignant but benign process.

Management

The overall approach to the various pulmonary aspects of SLE has been discussed in the preceding sections. To reiterate, the basic tenets of the management of SLE-related pulmonary problems are as follows:

1. Assume infection to be present until proven otherwise.
2. Establish a definitive diagnosis whenever possible.
3. Treat with broad-spectrum antibodies until infection is excluded.
4. Move quickly to immunosuppressive therapy when infection is excluded or in patients not responding to antimicrobial therapy.
5. Consider the anti-phospholipid syndrome in patients with thromboembolic disease.
6. Consider vasodilator therapies including prostacyclins and endothelin receptor antagonists for the treatment of pulmonary hypertension.

Although immunosuppressive therapy or plasmapheresis [142, 143] alone may improve many of the manifestations of SLE, even more impressive results are obtained when immunosuppressive therapy is coupled with plasmapheresis [144]. There are little published data on the efficacy of such combined therapy on the pulmonary complications of SLE, except for reports of dramatic improvement in patients with fulminant acute lupus pneumonitis [48–50]. Because this pulmonary complication has an inordinately high mortality rate [41] further consideration of cytotoxic therapy com-

bined with plasmapheresis in acute lupus pneumonitis seems warranted. Lung transplantation may be a therapeutic option for highly selected SLE patients with pulmonary fibrosis or pulmonary hypertension.

References

1. Dubois, E. L., and Tuffanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus. *JAMA* **19**, 104.
2. Turner-Stokes, L., and Turner-Warwick, M. (1982). Intrathoracic manifestations of SLE. *Clin. Rheum. Dis.* **8**, 229.
3. Gross, M., Esterly, Jr., and Earle, R. H. (1972). Pulmonary alterations in systemic lupus erythematosus. *Am. Rev. Respir. Dis.* **105**, 572.
4. Hunninghake, G. W., and Fauci, A. S. (1979). Pulmonary involvement in the collagen vascular diseases. *Am. Rev. Respir. Dis.* **119**, 231.
5. Miller, L. R., and Greenberg, S. D. (1985) Lupus lung. *Chest* **88**, 265
6. Silberstein, S. L., Barland, P., Grayzel, A. I., *et al.* (1980). Pulmonary dysfunction in systemic lupus erythematosus: Prevalence classification and correlation with other organ involvement. *J. Rheumatol.* **7**, 187.
7. Eichacker, P. Q., Pinsker, K., Epstein, A., *et al.* (1988). Serial pulmonary function testing in patients with systemic lupus erythematosus. *Chest* **94**, 129.
8. Hoffbrand, B. I., and Beck, E. R. (1965). Unexplained dyspnea and shrinking lungs in systemic lupus erythematosus. *Br. Med. J.* **1**, 273.
9. Gibson, G. J., Edmonds, J. P., and Hughes, G. R. V. (1977). Diaphragm function and lung involvement in systemic lupus erythematosus. *Am. J. Med.* **63**, 926.
10. Worth, H., Grahn, S., Lakomek, H. J., *et al.* (1988). Lung function disturbances versus respiratory muscle fatigue in patients with systemic lupus erythematosus. *Respiration* **53**, 81.
11. Martens, J., Demedts, M., Vanmeenen, M. T., and Dequeker, J. (1983). Respiratory muscle dysfunction in systemic lupus erythematosus. *Chest* **84**, 170.
12. Rubin, L. A., and Urowitz, M. B. (1983). Shrinking lung syndrome in SLE—a clinical pathologic study. *J. Rheumatol.* **10**, 973.
13. Laroche, C. M., Mulvey, D. A., Hawkins, P. N., *et al.* (1989). Diaphragm strength in the shrinking lung syndrome of systemic lupus erythematosus. Q. J. Med. **71**, 429.
14. Datta, S. K., Gandhi, V. C., Lee, H. J., *et al.* (1972). Granuloma in systemic lupus erythematosus. *S. Afr. Med. J.* **46**, 1514.
15. Light, R. W., Macgregor, M. I., Luchsinger, P. C., *et al.* (1972). Pleural effusions: The diagnostic separation of transudates and exudates. *Ann. Intern. Med.* **77**, 507.
16. Light, R. W., Erozan, Y. S., and Ball, W. C., Jr. (1973). Cells in pleural fluid. *Arch. Intern. Med.* **132**, 854.
17. Reda, M. G., and Baigelman, W. (1980). Pleural effusion in systemic lupus erythematosus. *Acta Cytol.* **24**, 553.
18. Berger, H. W., and Mejia, E. (1973). Tuberculosis pleurisy. *Chest* **63**, 88.
19. Potts, D. E., Levine, D. C., and Sahn, S. A. (1976). Pleural fluid pH in parapneumonic effusions. *Chest* **70**, 328.
20. Potts, D. E., Willcox, M. A., Good, J. T., Jr., *et al.* (1978). The acidosis of low-glucose pleural effusions. *Am. Rev. Respir. Dis.* **117**, 665.
21. Carr, D. T., Lillington, G. A., and Mayne, J. G. (1970). Pleural-fluid glucose in systemic lupus Erythematosus. *Mayo. Clin. Proc.* **45**, 409.
22. Halla, J. T., Schrohenloher, R. E., and Volanakis, J. E. (1980). Immune complexes and other laboratory features of pleural effusions: A comparison of rheumatoid arthritis, systemic lupus erythematosus and other diseases. *Ann. Intern. Med.* **92**, 748.
23. Hunder, G. G., McDuffie, F. C., and Hepper, M. G. G. (1972). Pleural fluid complement in systemic lupus erythematosus and rheumatoid arthritis. *Ann. Intern. Med.* **76**, 357.
24. Small, P., Frank, H., Kreisman, H., *et al.* (1982). An immunological evaluation of pleural effusions in systemic lupus erythematosus. *Ann. Allergy* **49**, 101.
25. Pandya, M. R., Agus, B., and Grady, R. F. (1976). In vivo LE phenomenon in pleural fluid. *Arthritis Rheum.* **19**, 962.
26. Good, J. T., Jr., King., T. E., Antony, V. B., and Sahn, S. A. (1983). Lupus pleuritis. Clinical features and pleural fluid characteristics with special reference to pleural fluid antinuclear antibodies. *Chest* **84**, 714.
27. Horler, A. R., and Thompson, M. (1959). The pleural and pulmonary complications of rheumatoid arthritis. *Ann. Intern. Med.* **51**, 1179.
28. Emerson, P. A. (1956). Pleural effusion complicating rheumatoid arthritis. *Br. Med. J.* **1**, 428.
29. Mason, R. M., and Steinberg, V. L. (1958). Systemic manifestations of rheumatoid arthritis and the pararheumatic diseases: II. Rheumatoid arthritis. *Ann. Phys. Med.* **4**, 265.
30. Carr, D. T., and Power, M. H. (1960). Pleural fluid glucose with special reference to its concentration in rheumatoid pleurisy with effusion. *Dis. Chest.* **37**, 321.
31. Carr, D. T., and Mayne, J. G. (1962). Pleurisy with effusion in rheumatoid arthritis, with reference to the low concentration of glucose in pleural fluid. *Am. Rev. Respir. Dis.* **85**, 345.
32. Light, R. W., and Ball, W. C. (1973). Glucose and amylase in pleural effusions, *JAMA* **225**, 257.
33. Light, R. W. (1981). Evaluating the patient with pleural effusions. *J. Respir. Dis.* **62**, 89.
34. Carel, R. S., Shapiro, M. S., Shoham, D., *et al.* (1977). Lupus erythematosus cells in pleural effusion: The initial manifestations of procainamine-induced lupus erythematosus. Chest **72**, 670.
35. Bell, R., and Lawrence, D. S. (1979). Chronic pleurisy in systemic lupus erythematosus treated with pleurectomy. *Br. J. Dis. Chest.* **73**, 314.
36. Elborn, J. S., Conn, P., and Roberts, S. D. (1987). Refractory massive pleural effusion in systemic lupus erythematosus treated by pleurectomy. *Ann. Rheum. Dis.* **46**, 77.

37. Osler, W. (1904). On the visceral manifestations of the erythema group of skin diseases. *Am. J. Med. Sci.* **127**, 1.
38. Rakov, H. L., and Taylor, J. S. (1942). Acute disseminated lupus erythematosus without cutaneous manifestations and with heretofore undescribed pulmonary lesions. *Arch. Intern. Med.* **70**, 88.
39. Israel, H. L. (1953). The pulmonary manifestations of disseminated lupus erythematosus. *Am. J. Med. Sci.* **226**, 387.
40. Olsen, E. G. J., and Lever, J. V. (1972). Pulmonary changes in systemic lupus erythematosus. *Br. J. Dis. Chest* **66**, 71.
41. Matthay, R. A., Schwarz, M. I., Petty, T. L., *et al.* (1974). Pulmonary manifestations of systemic lupus erythematosus: Review of twelve cases of acute lupus pneumonitis. *Medicine* **54**, 397.
42. Haupt, H. M., Moore, G. W., and Hutchins, G. M. (1981). The lung in systemic lupus erythematosus: Analysis of the pathogenic changes in 120 patients. *Am. J. Med.* **71**, 791.
43. Myers, J. L., and Katzenstein, A. A. (1986). Microangiitis in lupus-induced pulmonary hemorrhage. *Am. J. Clin. Pathol.* **85**, 552.
44. Churg, A., Franklin, W., Chan, K. L., *et al.* (1980). Pulmonary hemorrhage and immune-complex deposition in the lung: Complications in a patient with systemic lupus erythematosus. *Arch. Pathol. Lab. Med.* **104**, 388.
45. Inoue, T., Kanayama, Y., Ohe, A., *et al.* (1979). Immunopathologic studies of pneumonitis in systemic lupus erythematosus. *Ann. Intern. Med.* **91**, 30.
46. Harvey, A. M., Shulman, L. E., Tumulty, P. A., *et al.* (1954). Systemic lupus erythematosus: Review of literature and clinical analysis of 138 cases. *Medicine* **33**, 291.
47. Matthay, R. A., Hudson, L. D., and Petty, T. L. (1973). Acute lupus pneumonitis: Response to azathioprine therapy. *Chest* **63**, 117.
48. Millman, R. P., Cohen, T. B., Levinson, A. I., *et al.* (1981). Systemic lupus erythematosus complicated by acute pulmonary hemorrhage: Recovery following plasmapheresis and cytotoxic therapy. *J. Rheumatol.* **8**, 1021.
49. Isbister, J. P., Ralston, M., and Hayes, J. M. (1981). Fulminant lupus pneumonitis with acute renal failure and RBC aplasia. Successful management with plasmapheresis and immunosuppression. *Arch. Intern. Med.* **141**, 1081.
50. Brasington, R. D., and Furst, D. E. (1985). Pulmonary disease in systemic lupus pneumonitis. *Clin. Exp. Rheum.* **3**, 269.
51. Bulgrin, J. G., Dubois, E. L., and Jacobson, G. (1960). Chest roentgenographic changes in systemic lupus erythematosus. *Radiology* **74**, 42.
52. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85.
53. Eisenberg, H., Dubois, E. L., Sherwin, R. P., *et al.* (1973). Diffuse interstitial lung disease in systemic lupus erythematosus. *Ann. Intern. Med.* **79**, 37.
54. Hedgpeth, M. T., and Boulware, D. W.(1988). Interstitial pneumonitis in antinuclear antibody-negative systemic lupus erythematosus: A new clinical manifestation and possible association wtih anti-Ro (SSA) antibodies. *Arthritis Rheum.* **31**, 545.
55. Boulware, D. W., and Hedgpeth, M. T. (1989). Lupus pneumonitis and anti-SSA (Ro) antibodies. *J. Rheumatol.* **16**, 479.
56. Turner-Stokes, L., Haslam, P., Jones, M., *et al.* (1982). Autoantibody and idiotype profile of lung involvement in autoimmune rheumatic disease. *Ann. Rheum. Dis.* **49**, 160.
57. Lawrence, E. C. (1982). Determining the effectiveness of therapy for interstitial lung disease. *Respir. Ther.* **12**, 15.
58. Wilson, R. K., Fechner, R. E., Greenberg, S. D., *et al.* (1978). Clinical implications of a nonspecific transbronchial biopsy. *Am. J. Med.* **54**, 252.
59. Greenman, R. L., Goodall, P. T., and King, D. (1975). Lung biopsy in immunocompromised hosts. *Am. J. Med.* **59**, 488.
60. Springmeyer, S. C., Silvestri, R. C., Sale, G. E., *et al.* (1982). The role of transbronchial biopsy for the diagnosis of diffuse pneumonias in immunocompromised marrow transplant recipients. *Am. Rev. Respir. Dis.* **126**, 763.
61. Reynolds, H. Y., Fulmer, J. D., Kazmierowski, J. A., *et al.* (1977). Analysis of cellular and protein content of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J. Clin. Invest.* **59**, 165.
62. Crystal, R. G., Gadek, J. E., Ferrans, V. J., *et al.* (1981). Interstitial lung disease: Current concepts of pathogenesis, staging and threapy. *Am. J. Med.* **70**, 542.
63. American Thoracic Society. (2000). Idiopathic pulmonary fibrosis: Diagnosis and treatment. *Am. J. Respir. Crit. Care Med.* **161**, 646.
64. Elliott, M. L., and Kuhn, C. (1970). Idiopathic pulmonary hemosiderosis: Ultrastructural abnormalities in the capillary walls. *Am. Rev. Respir. Dis.* **102**, 895.
65. Kuhn, C., and Elliott, M. L. (1972). Systemic lupus erythematosus in a patient with ultrastructural lesions of the pulmonary capillaries previously reported in the review as due to idiopathic pulmonary hemosiderosis. *Am. Rev. Respir. Dis.* **106**, 931.
66. Holgate, S. T., Glass, D. N., Haslam, P., *et al.* (1975). Respiratory involvement in systemic lupus erythematosus: A clinical and immunological study. *Clin. Exp. Immunol.* **24**, 385.
67. Carrington, C. B., Gaensler, E. A., Coutu, R. E., *et al.* (1978). Natural history and treated course of usual and desquamative interstitial pneumonia. *N. Engl. J. Med.* **298**, 801.
68. NHLBI Workshop Summary. (1999). Pharmcological therapy for idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care. Med.* **160**, 1771.
69. Lawrence, E. C., Holland, V. A., Berger, M. B., *et al.* (1988). Lung transplantation: An emerging technology. *Texas Med.* **84**, 61.
70. Ziesche, R., Hofbauer, E., Wittman, K., Petkov, V., and Block, L. H. (1999). A preliminary study of long-term treatment with interferon gamma-1b and low-dose

prednisolone in patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* **341**, 1264.
71. Zamora, M. R., Warner, M. L., Tuder, R., and Schwarz, M. I. (1997). Diffuse alveolar hemorrhage and systemic lupus erythematosus: Clinical presentation, histology, survival and outcome. *Medicine* **76**, 192.
72. Drew, L., Finley, T., and Golde, D. (1977). Diagnostic lavage and occult pulmonary hemorrhage in thrombocytopenic immunocompromised patients. *Am. Rev. Respir. Dis.* **116**, 215.
73. Sherman, J., Winnie, G., Thomassen, M. J., Abdul-Karim, F., and Boat, T. (1984). Time course of hemosiderin production and clearance by human pulmonary macrophages. *Chest* **86**, 409.
74. Wood, P. (1956). "Diseases of the Heart and Circulation." Lippincott, Philadelphia.
75. Walcott, G., Burchell, H. B., and Brown, A. L. (1970). Primary pulmonary hypertension. *Am. J. Med.* **39**, 70.
76. Rawson, A. J., and Woske, H. M. (1960). A study of etiological factors in so-called primary pulmonary hypertension. *Arch. Intern. Med.* **105**, 81.
77. Wagenvoort, C. A., and Wagenvoort, N. (1970). Primary pulmonary hypertension. *Circulation* **142**, 1163.
78. Salerni, R., Rodman, G. P., Leon, F. L., *et al.* (1977). Pulmonary hypertension in the CREST syndrome variant of progressive systemic sclerosis (scleroderma). *Ann. Intern. Med.* **86**, 394.
79. Young, R. H., and Mark, G. (1978). Pulmonary vascular changes in scleroderma. *Am. J. Med.* **64**, 998.
80. Jones, M. B., Osterholm, R. K., Wilson, R. B., *et al.* (1978). Fatal pulmonary hypertension and resolving immune-complex glomerulonephritis in mixed connective tissue disease. *Am. J. Med.* **65**, 855.
81. Fraser, R. G., and Pare, J. A. P. (1978). Rheumatoid disease of the lungs and pleura. In "Diagnosis of Diseases of the Chest" (R. G. Fraser, Ed.) Saunders, Philadelphia.
82. Cummings, P. (1973). Primary pulmonary hypertension and SLE. *N. Engl. J. Med.* **288**, 1078.
83. Sergent, J. S., and Lockshin, M. D. (1973). Primary pulmonary hypertension and SLE. *N. Engl. J. Med.* **288**, 1078.
84. Kanemoto, N., Gonda, N., Katsu, M., *et al.* (1975). Two cases of pulmonary hypertension with Raynaud's phenomenon: Primary pulmonary hypertension and systemic lupus erythematosus. *Jpn. Heart J.* **16**, 354.
85. Nair, S. S., Askare, A. D., Popelka, C. G., *et al.* (1980). Pulmonary hypertension and systemic lupus erythematosus. *Arch. Intern. Med.* **140**, 109.
86. Santini, D., Fox, D., Kloner, R. A., *et al.* (1980). Pulmonary hypertension in systemic lupus erythematosus: Hemodynamics and effects of vasodilator therapy. *Clin. Cardiol.* **3**, 406.
87. Perez, H. D., and Kramer, N. (1981). Pulmonary hypertension in systemic lupus erythematosus: Report of four cases and review of the literature. *Semin. Arthritis Rheum.* **11**, 177.
88. Asherson, R. A., Higenbottam, T. W., Dinh, Xuan, *et al.* (1990). Pulmonary hypertension in a lupus clinic: Experience with twenty-four patients. *J. Rheumatol.* **17**, 1292.
89. Barst, R. J., Rubin, L. J., Long, W. A., *et al.* (1966). A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. *N. Engl. J. Med.* **334**, 296.
90. Badesch, D. B., Tapson, V. F., McGoon, M. D., *et al.* (2000). Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. *Ann. Intern. Med.* **132**, 425.
91. Robbins, I. M., Gaine, S. P., Schilz, R., Tapson, V. F., Rubin, L. J., and Loyd, J. E. (2000). Epoprostenol for treatment of pulmonary hypertension in patients with systemic lupus erythematosus. *Chest* **117**, 14.
92. Channick, R. N., Simonneau, G., Sitbon, O., *et al.* (2001). Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: A randomised placebo-controlled study. *Lancet* **358**, 1119.
93. Reitz, B. A., Wallwork, J. L., Hunt, S. L., *et al.* (1982). Heart-lung transplantation: Successful therapy for patients with pulmonary vascular disease. *N. Engl. J. Med.* **306**, 557.
94. Hosenpud, J. D., Bennett, L. E., Keck, B. M., Boucek, M. M., and Novick, R. J. (2001). The registry of the international society for heart and lung transplantation: Eighteenth official report—2001. *J. Heart Lung Transplant.* **20**, 805.
95. Yeatman, M., McNeil, K., Smith, J. A., *et al.* (1966). Lung transplantation in patients with systemic diseases: An eleven-year experience at Papworth Hospital. *J. Heart Lung Transplant.* **15**, 144.
96. Levy, R. D., Guerraty, A. J., Yacoub, M. H., and Loertscher, R. (1993). Prolonged survival after heart–lung transplantation in systemic lupus erythematosus. *Chest* **104**, 1903.
97. Asheron, R. A., Khamashta, M. A., Ordi-Ros, J., *et al.* (1989). The "primary" phospholipid syndrome: Major clinical and serologic features. *Medicine* **68**, 366.
98. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticrdiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. *Ann. Intern. Med.* **112**, 682.
99. Asherson, R. A., and Cervera, R. (1995). Review: Antiphospholipid antibodies and the lung. *J. Rheumatol.* **22**, 62.
100. Wahl, D. G., Guillemin, F., de Maistre, E., *et al.* (1997). Risk for venous thrombosis related to antiphospholipid antibodies in systemic lupus erythematosus—a meta-analysis. *Lupus* **6**, 467.
101. Komatireddy, G. R., Wang, G. S., Sharp, G. C., and Hoffman, R. W. (1997). Antiphospholipid antibodies among anti-U1-70 kDa autoantibody positive patients with mixed connective tissue disease. *J. Rheumatol.* **24**, 319.
102. Matthay, R. A., and Greene, W. H. (1989). Pulmonary infections in the immunocompromised patient. *Med. Clin. North Am.* **64**, 529.
103. Lee, P., Urowitz, M. B., Bookman, A. A. M., *et al.* (1977). Systemic lupus erythematosus: A review of 100 cases

with reference to nephritis, the nervous system, infections, aseptic necrosis, and prognosis. *Q. J. Med.* **181**, 1.
104. Staples, P. J., Gerding, D. N., Decker, J. L., *et al.* (1974). Incidence of infection in systemic lupus erythematosus. *Arthritis Rheum.* **17**, 1.
105. Clark, R. A., Kimbal, H. R., and Decker, J. L. (1974). Neutrophil chemotaxis in systemic lupus erythematosus. *Ann. Rheum. dis.* **33**, 167.
106. Brandt, L., and Hedberg, H. (1969). Impaired phagocytosis by peripheral blood granulocytes in systemic lupus erythematosus. *Scand. J. Haematol.* **6**, 348.
107. Landry, M. (1977). Phagocyte function and cell-mediated immunity in systemic lupus erythematosus. *Arch. Dermatol.* **113**, 147.
108. Perez, H. D., Andron, R. I., and Goldstein, I. M. (1979). Infection in patients with systemic lupus erythematosus: Association with a serum inhibitor of complement derived chemotactic activity. *Arthritis Rheum.* **22**, 1326.
109. Wallaert, B., Aerts, C., Bart, F., *et al.* (1987). Alveolar macrophage dysfunction in systemic lupus erythematosus. *Am. Rev. Respir. Dis.* **136**, 293.
110. Klippel, J. H., Karsh, J., Stahl, N. I., *et al.* (1979). A controlled study of pneumococcal polysaccharide vaccine in systemic lupus erythematosus. *Arthritis Rheum.* **22**, 1321.
111. Williams, G. W., Steinberg, A. D., Reinertsen, J. L., *et al.* (1978). Influenza immunization in systemic lupus erythematosus: A double blind trial. *Ann. Intern. Med.* **88**, 729.
112. Fagon, J. Y., Chastre, J., Hance, A. J., *et al.* (1988). Detection of nosocomial lung infection in ventilated patients. Use of a protected specimen brush and quantitive culture techniques in 147 patients. *Am. Rev. Respir. Dis.* **138**, 110.
113. Wimberly, N. E., Faling, L. J., and Bartlett, J. G. (1979). A fiberoptic bronchoscopy technique to obtain uncontaminated lower airway secretions for bacterial culture. *Am. Rev. Respir. Dis.* **119**, 337.
114. Zurier, R. B. (1975). Systemic lupus erythematosus and pregnancy. *Clin. Rheum. Dis.* **1**, 613.
115. Cryer, P. E., and Kissane, J. M. (1978). Systemic lupus erythematosus with postpartum pulmonary disease. *Am. J. Med.* **64**, 1047.
116. Myers, S. A., Podczaski, E., and Freese, U. (1980). Acute lupus pneumonitis in the puerperium: A case report and literature review. *J. Reprod. Med.* **25**, 285.
117. Huang, C. T., Hennigar, G. R., and Lyons, H. R. (1965). Pulmonary dysfunction in systemic lupus erythematosus. *N. Engl. J. Med.* **272**, 288.
118. Gold, W. M., and Jennings, D. B. (1966). Pulmonary function in patients with systemic lupus erythematosus. *Am. Rev. Respir. Dis.* **93**, 556.
119. Kinney, W. W. and Angellilo, V. A. (1982). Bronchiolitis in systemic lupus erythematusus. *Chest* **82**, 646.
120. Epler, G. R., Snider, G. L., Gaensler, E. A., *et al.* (1979). Bronchiolitis and bronchitis in connective tissue disease: A possible relationship to the use of penicillamine. *JAMA* **242**, 528.
121. Gosink, B. B., Friedman, P. J., and Liebow, A. A. (1973). Bronchiolitis obliterans: Roentgenologic–patholigic correlations. *Am. J. Roentgenol.* **117**, 816.
122. Wohl, M., and Chernick, V. (1978). Bronchiolitis: State of the art. *Am. Rev. Respir. Dis.* **118**, 759.
123. Min, J. K., Hong, Y. S., Park, S. H., *et al.* (1997). Bronchiolitis obliterans organizing pneumonia as an initial manifestation in patients with systemic lupus erythematosus. *J. Rheumatol.* **24**, 2254.
124. Decker, J. L., Steinberg, A. D., Reinertsen, J. L., *et al.* (1979). Systemic lupus erythematosus: Evolving concepts. *Ann. Intern. Med.* **91**, 587.
125. Daniele, R. P., Dauber, J. H., and Rossman, M. D. (1980). Immunologic abnormalities in sarcoidosis. *Ann. Intern. Med.* **92**, 406.
126. Harrison, G. N., Lipham, M., Elguindi, A. S., *et al.* (1979). Acute sarcoidosis occurring during the course of systemic lupus erythematosu. *South. Med. J.* **72**, 1387.
127. Hunter, T., Arnott, J. E., and McCarthy, D. S. (1980). Features of systemic lupus erythematosus and sarcoidos occurring together. *Arthritis Rheum.* **23**, 364.
128. Needleman, S. W., Silber, R. A., Von Brecht, J. H., *et al.* (1982). Systemic lupus erythematosus complicated by disseminated sarcoidosis. *Am. J. Clin. Pathol.* **78**, 105.
129. Schnabel, A., Barth, J., Schubert, F., and Gross, W. L. (1996). Pulmonary sarcoidosis coexisting with systemic lupus erythematosus. *Scand. J. Rheumatol.* **25**, 109.
130. Dong, X., Michelis, M. A., Wang, J., Bose, R., DeLange, T., and Reeves, W. H. (1998). Autoantibodies to DEK oncoprotein in a patient with systemic lupus erythematosus and sarcoidosis. *Arthritis Rheum.* **41**, 1505.
131. Liebow, A. A. (1975). Definition and classification of interstitial pneumonias in human pathology. In "Progress in Respiration Research Volume 8. Alveolar Interstitum of the Lung: Pathological and Physiological Aspects" (F. Basset and R. Georges, Eds.), Karger, New York.
132. Benisch, B., and Peison, B. (1979). The association of lymphocytic interstitial pneumonia and systemic lupus erythematosus. *Mt. Sinai J. Med.* **46**, 398.
133. Yood, R. A., Steigman, D. M., and Gill, L. R. (1995). Lymphocytic interstitial pneumonitis in a patient with systemic lupus erythematosus. *Lupus* **4**, 161.
134. Mellemkjaer, L., Andersen, V., Linet, M. S., *et al.* (1997). Non-Hodgkin's lymphoma and other cancers among a cohort of patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 761.
135. Nived, O., Bengtsson, A., Jonsen, A., Sturfelt, G., and Olsson, H. (2001). Malignancies during follow-up in an epidemiologically defined systemic lupus erythematosus inception cohort in southern Sweden. *Lupus* **10**, 500.
136. Cibere, J., Sibley, J., and Haga, M. (2001). Systemic lupus erythematosus and the risk of malignancy. *Lupus* **10**, 394.
137. Sultan, S. M., Ioannou, Y., and Isenberg, D. A. (2000). Is there an association of malignancy with systemic lupus erythematosus? An analysis of 276 patients under long-term review. *Rheumatology* **39**, 1147.
138. Greenfield, D. I., Trinh, P., Fulenwider, A., and Barth, W. F. (1986). Kaposi's sarcoma in a patient with SLE. *J. Rheumatol.* **13**, 637.

139. Saltzstein, S. L. (1963). Pulmonary malignant lymphomas and psuedolymphomas. *Cancer* **16**, 928.
140. Greenberg, S. D., Heisler, J. G., Gyorkey, F., *et al.* (1972). Pulmonary lymphoma versus pseudolymphoma. A perplexing problem. *South. Med. J.* **65a**, 775.
141. Yum, M. N., Ziegler, J. R., Walker, P. D., *et al.* (1979). Pseudolymphoma of the lung in a patient with systemic lupus erythematosus. *Am. J. Med.* **66**, 172.
142. Blaszcyk, M., Chorzelski, T., Daszynski, J., *et al.* (1981). Plasmapheresis in the treatment of systemic lupus erythematosus. *Arch. Immunol. Ther. Exp.* **29**, 769.
143. Wei, N., Klippel, J. H., Huston, D. P., *et al.* (1983). Randomized trial of plasma exchange in mild systemic lupus erythematosus. *Lancet* **1**, 17.
144. Huston, D. P., White, M. J., Mattioli, C., *et al.* (1983). A controlled trial of Plasmapheresis and cyclophosphamide therapy of lupus nephritis. *Arthritis Rheum.* **26**, 33.
145. Bynum, L. J., and Wilson III, J. E. (1976). Characteristics of pleural effusions associated with pulmonary embolism. *Arch. Intern. Med.* **136**, 159.

34

NONHEPATIC GASTROINTESTINAL MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Iris Dotan
Lloyd Mayer

When considering the "systemic" nature of lupus erythematosus, the gastrointestinal (GI) tract tends to be overlooked, not by the disease itself but by the primary physician. William Osler's description [1] in 1895 of significant gastrointestinal crises in "erythema exudativum multiforme" illustrates that the GI tract can overshadow many of the other aspects of systemic lupus erythematosus (SLE). In general, the major GI complications of SLE are less frequently seen than the skin rash, arthritis, and nephritis that commonly bring patients to clinical attention. However, if we look at various series of patients with SLE [2–15], we find that there are quite a number of listed signs and symptoms related to the GI tract (Table 1). For example, in the largest such series, by Dubois and Tuffanelli [5], of 520 patients studied, 53% had nausea or vomiting, 49% complained of anorexia, and 19% had abdominal pain. Although many of these symptoms are nonspecific and may relate to other systemic manifestations of SLE (e.g., nausea and vomiting secondary to uremia) or may be side effects of drug therapy, some may represent inapparent GI tract disease.

As just suggested, a major problem in recognizing GI tract disease as a complication of SLE is that much of the same GI pathology can be induced by the various medications used in SLE treatment. For example, there are controversies about lupus pancreatitis and about liver disease caused directly by SLE vs aspirin-induced focal necrosis or steroid-induced fatty liver or pancreatitis. Another problem is that many of the symptoms related to the GI tract are not as severe as the more pressing arthritis or nephritis and thus tend to be glossed over. In this chapter, a review of the diverse GI disorders seen as sequelae of SLE is presented, beginning at the beginning: the oral cavity. GI ailments related to therapy for SLE and SLE induced by drugs prescribed for GI disorders are considered in a separate section.

ORAL CAVITY

Clinical Features

The GI tract extends from the mouth to the anus, so involvement of the oral cavity in SLE should be considered a GI manifestation. Oral lesions are so central and common a sign in SLE that presence of oral ulceration is one of the criteria in the diagnosis of SLE [16]. They tend to precede severe systemic disease flares [17]. Involvement of the oral cavity is reported to occur in 7–57% of SLE patients [2, 6, 8–12, 14, 18]. Oral lesions are in general of three types: erythematous, discoid, and ulcerative. According to one study [12] erythematous

TABLE 1 GI-Related Symptoms Reported (as Percentages) in SLE Patients

Author	Number of patients	Anorexia	Vomiting nausea	Diarrhea	Abdominal pain	Hemorrhage	Oral lesions	Esoph. dysphagia	Serositis, ascites	Pancreatitis	Gastroesoph ulcers	Gastro SI ulcers, LI ulcer	Splenomegaly
Jessar *et al.* [2]	44	—	18	18	22	—	18 ulcers	—	—	—	—	—	27
Jessar *et al.* [2]	168	—	13	13	17	—	15 ulcers	—	—	—	—	—	17
Harvey *et al.* [3]	138	—	11/14	8	10	5	—	5/6	—	—	0	2/4	—
Brown *et al.* [4]	87	—	8	3	2	—	—	—	—	—	—	4/2	—
Dubois and Tuffanelli [5]	520	49	53	6	19	6	—	—/15	—/11	—	1.5	0/<1	9
Estes and Christian [6]	150	—	—	—	—	<1	?	—	—	<1	<1	2/0	18
Fries and Holman [7]	—	36	27/36	25	34	10	—	—/5	—	—	—	—	—
Ropes [8]	99	—	—	—	62	—	41	3/22	63 autopsy/8	9 autopsy	22	1/0	67
Urman *et al.* [9]	182	—	—	—	—	—	40 ulcers	—	—	—	—	—	—
Al Rawi *et al.* [10]	67	—	—	—	—	—	52	—	—	—	—	—	18
Jonsson *et al.* [11]	51	—	—	—	—	—	45	—	—	—	—	—	—
Tsianos *et al.* [12]	25	—	—	—	—	—	57	—	—	—	—	—	—
Nadorra *et al.* [13]	26 autopsy	8	27	19	65	46	—	—/4	42/90	23	33	23	—
Burge *et al.* [14]	53	—	—	—	—	—	36 ulcers	—	—	—	—	—	—
Castrucci *et al.* [15]	18	—	—	—	—	—	—	72 (manometric abnormalities)	—	—	—	—	—

lesions occur most often (35%), followed by discoid (16%) and ulcerative (6%) lesions. Another study [9] found 9% of patients to have more than one lesion at a time. The lesions may merge into one another and may be associated with edema and petechiae [11].

Discoid Lesions

Discoid lesions may be painful. They are characterized by a central area of erythema with white dots surrounded by rays of white striae and telangiectasia, are firm on palpation, and have a well-defined elevated border [11–19]. They are found in decreasing frequency in the buccal mucosa, gingiva, labial mucosa, and vermilion border [9, 11, 19] and are generally seen in patients with active disease [11]. They may be ulcerated and are infected by yeast in about 50% of cases [19].

Ulcers

Ulcers are usually shallow, 1–2 cm in diameter, tend to occur in crops, and are most commonly found on the hard palate (89% of cases) [9]. They may also be found on the buccal mucosa and extend into the pharynx in about one-third of patients [9]. Involvement of the nasal and laryngeal mucosa has also been described [20–22]. Oral ulcers often present with pain, a burning sensation, soreness, dry mouth, and when extending into the pharynx, with odynophagia and even dysphagia [7]. These lesions may persist for years [11, 23, 24] or may be intermittent, displaying cyclical remissions and exacerbations [7]. As with discoid lesions, oral ulcers tend to occur with disease flares [11, 17, 24]. The presence of oral ulcers has been considered by some to be a poor prognostic sign. One study [7] reported patients with oral ulcers to have a higher mortality than patients without oral ulcers. This has not been corroborated by a second study [9].

Erythematous Lesions

Erythematous lesions are typically painless, flat, red lesions with poorly defined borders. They are occasionally accompanied by edema and petechiae. They most commonly occur on the hard palate [11].

Pathology–Pathophysiology

The histopathology and immunopathology of the discoid lesions in SLE are similar to those of discoid lupus erythematosus (DLE) except that the inflammatory infiltrate found deep in the connective tissue is more diffuse and less intense in SLE [11, 19, 24, 25]. Keratotic plugging, pseudoepitheliomatous hyperplasia, and epithelial cell islands within the connective tissue are also found. The inflammatory infiltrate found in the connective tissue is typically lymphohistiocytic with an occasional plasma cell, and the inflammatory infiltrate is typically separated from the epithelium by an eosinophilic free zone. The infiltrating cells are predominantly $CD4^+$ and $CD8^+$ T cells distributed in the lamina propria, submucosa, and occasionally in the epithelium. Less than 5% of the infiltrating inflammatory cells express interleukin-2 receptors or transferrin receptors [26]. Epithelial cell changes are seen and include the presence of colloid bodies, multinucleated cells, occasional nuclear hyperchromatism, and rarely dysplasia. Some epithelial cells express major histocompatibility class II antigens with HLA-DR being more commonly present than HLA-DP. No HLA-DQ is seen. Liquefaction degeneration of the basal epithelial cell layers is also noted [26]. Hyalinization of the subepithelial connective tissue may occur, as can edema of the lamina propria [11, 19, 24, 25]. Immunoglobulin and complement as well as fibrinogen is deposited in the basement membrane zone and is found in both discoid lesions and in clinically normal mucosa of SLE patients [11, 19, 27–32].

Ultrastructurally, the lesions show cytomorphologic features peculiar to keratinizing epithelium. Dyskeratotic cells and filamentous bodies, which probably correspond to the colloid bodies seen on light microscopy, are present [19, 33–37].

As is suggested by the occasional presence of dysplastic epithelial cells in discoid lesions (see earlier), it is important to be aware that cancer can develop in long-standing discoid lesions of the lip at the vermilion border [11].

Erythematous and ulcerative lesions show nonspecific changes, including liquefaction degeneration of the basal layer and a diffuse subepithelial inflammatory infiltrate [11, 19, 24]. Erythematous lesions also show subepithelial and epithelial inflammation, disturbed epithelial maturation, and acanthosis [11]. On immunopathology, IgM deposits are noted in the basement membrane [11].

Diagnosis

As a significant proportion of oral lesions may be asymptomatic, careful examination of the oral cavity is mandatory in all lupus patients. Besides the difficulty in distinguishing discoid lesions from lichen planus and leukoplakia, there may be difficulty in distinguishing SLE oral lesions from monilia, erythema multiforme, pemphigus, and herpetic lesions [38–40]. Bacterial, fungal, and viral cultures, as well as detailed drug

history, can help in these cases. In addition, the finding of immunoglobulin and complement deposition in the basement membrane in discoid lesions (see earlier) may be useful as a distinguishing feature, as immunoglobulin deposition is rare in lichen planus and leukoplakia [19]. Schiodt [25] described five features that may distinguish discoid lesions from the similar appearing lesions of lichen planus and leukoplakia: (1) hyperkeratosis with keratotic plugs; (2) atrophy of the rete processes; (3) edema of the lamina propria; (4) deep inflammatory infiltrate; and (5) thick, patchy, or continuous periodic acid Schiff positive deposits in the basement membrane zone. Presence of two or more of these criteria was specific and sensitive for the diagnosis of SLE discoid lesions.

Therapy

There are no well-controlled trials demonstrating efficacy of therapy of any of these lesions [19]. Treatments that have been recommended for oral ulcerations include oral and topical steroids [19]. For discoid lesions, antimalarials [9, 21, 39, 41] have been tried, as have oral and topical steroids [41, 42] and dapsone [43, 44]. Treatment directed at controlling the SLE flare itself may often prove helpful in ameliorating the oral lesions. Antimicrobial, antiviral, or antifungal therapy should be used where infection is suspected or proven. Drug withdrawal, such as the withdrawal of hydralazine (shown to resolve oral ulcerations in at least one patient with SLE) [45], may, on occasion, prove useful.

SLE and Sjögren's Syndrome

Sjögren's syndrome has been associated with SLE in 13–20% of patients [46–48]. A higher percentage of sicca symptoms associated with SLE has been reported, but as the criteria for Sjögren's syndrome classification ***were not met*** this probably is an overestimation [49, 50]. Though Sjögren's syndrome usually appears in later stages of the disease, it may precede the diagnosis of SLE [51]. Compared to patients with SLE alone, those with secondary Sjögren's syndrome tend to exhibit fewer systemic manifestations, especially renal involvement [52].

Other Oral Lesions

Other oral lesions have been seen in lupus patients. Acute necrotizing ulcerative gingivitis has been associated with SLE [24, 53]. It is manifested by ulceration, bleeding, facial swelling, dysphagia, halitosis, and even sloughing of the gingival tissue with loss of teeth. Steroids may contribute to the severity of the disease. Treatment includes (1) reduction in steroid dose; (2) oral, topical, and if severe, intravenous antibiotics against usual mouth flora or against a specific organism if one is identified; and (3) topical gentian violet. Jaworski *et al*. [53] suggested that altered host immune mechanisms, such as decreased chemotaxis of polymorphonuclear cells, in addition to further suppression of the immune system by immunosuppressive agents used in the treatment of SLE, probably contribute to tip the host–organism balance in favor of the symbiotic organism. All SLE patients especially those taking steroids or other immunosuppressive drugs, should be instructed to maintain excellent oral hygiene, and dentists should be alerted to the greater risks of postoperative bleeding and infection in this group [54].

Other rare lesions include Raynaud's phenomenon of the tip of the tongue [55] and tense bullae of the oral cavity [56]. The bullae resolve with steroid therapy. A case of oral hairy leukoplakia has also been documented [57]. SLE patients may also get yeast infections or oral herpes simplex virus, especially during exacerbations or steroid therapy [7, 11, 21]. In addition, drugs used in the treatment of SLE may cause oral lesions. For example, antimalarials have been reported to produce brown or blue–gray pigmentation of the oral mucosa [58].

ESOPHAGUS

The incidence of esophageal disease is difficult to ascertain. The reports on the incidence of symptomatic esophageal disease vary widely (1.5–22%) [3, 5, 7, 8, 13]. One problem is the fact that no correlation between symptomatology and pathology exists [15]. Compounding this problem is the possibility that some patients with mixed connective tissue disease, with known esophageal pathology, may have been misclassified as SLE in the earlier literature.

Clinical Features

Patients with SLE may present with dysphagia, heartburn typical of acid reflux, as well as atypical chest pain. Esophagitis with ulcerations may be seen in about 3.5% of patients [3, 8], rarely with perforation [13]. Mucosal bridging, a finding occasionally seen in patients with a history of peptic esophagitis, has also been reported [59]. In addition, corticosteroids or immunosuppressive therapy can predispose patients to *Candida* esophagitis, and patients may present with oral candidiasis and odynophagia.

Pathology–Pathophysiology

Controversy exists as to the etiology of the esophagitis and chest pain seen in lupus patients. Some consider these findings to be peptic in origin. Evidence for this comes from the findings of abnormal esophageal motility, specifically hypoperistalsis and aperistalsis in as many as 72% of lupus patients [15, 60–65], that might result in decreased clearance of refluxed acid. Others have found a high incidence of gastroesophageal reflux [15]. The type of dysmotility seen varies. In one study of unselected SLE patients studied by manometry, X-ray, and history, 14% had aperistalsis in the lower two-thirds of the esophagus, lower esophageal sphincter, or both (i.e., the smooth muscle portion of the esophagus) consistent with a progressive systemic sclerosis picture; 14% had a motility disorder of the upper one-third of the esophagus (i.e., the skeletal muscle portion of the esophagus) consistent with a dermatomyositis picture; and two had involvement of the entire esophagus [61].

This has led other authors to suggest that the pathologic findings in the lupus esophagus are vasculitic rather than peptic in origin. Castrucci *et al*. [15] hypothesized that the hypoperistalsis or aperistalsis found in the esophagus of SLE patients may be due to an inflammatory reaction in the esophageal muscle or to ischemic vasculitic damage of the Auerbach plexus. Harvey *et al*. [3] noted arteritis in the esophagus of four SLE patients in association with esophageal ulcers. A large proportion of patients with esophageal ulcers in these studies had been taking steroids, which suggests that these may have been stress ulcers.

More recently, a lesser incidence of involvement of the lower esophageal sphincter (LES) in SLE has been reported [66]. In contrast to systemic sclerosis patients, 81.8% of whom had LES abnormalities, 30% classified as severe, none of the SLE patients evaluated had severe involvement of the LES. This may explain the interesting observation that the complications of acid reflux so pronounced in other collagen vascular diseases such as progressive systemic sclerosis are not noted in SLE.

Despite manometric and histologic evidence of esophageal disease, the lack of correlation of abnormalities to symptoms makes one wonder about the significance of these findings. With the exception of the occasional esophageal ulcer [5, 6, 8, 13], which only rarely has been reported to perforate, esophageal symptoms are generally not severe.

Diagnosis

Esophagitis and esophageal ulcer may be diagnosed by esophagogastroduodenoscopy or by barium esophagram. Esophageal dysmotility may be detected by manometric studies or occasionally on barium esophagogram.

Therapy

As it is not clear whether the esophagitis and ulcerations found are vasculitic in origin, peptic in origin, or a complication of steroid therapy, it is difficult to determine the appropriate mode of therapy. Empiric proton-pump inhibitors, H_2 blockers, or other antacid therapy in patients taking steroids is controversial. A controlled trial of antireflux therapy in patients with SLE would greatly help determine whether such esophageal lesions truly are peptic in nature and help to decide whether antiacid and antireflux therapy should be routinely recommended to SLE patients. Treatment of the lupus itself may help if in fact the esophageal lesions are vasculitic in origin. A trial of calcium channel blockers for esophageal dysmotility may be of benefit. Patients with *Candida* esophagitis will require appropriate antifungal therapy, including nystatin or clotrimazole troches for mild infections and fluconazole for more severe cases.

STOMACH AND DUODENUM

Clinical Features

Lupus patients with intestinal complications referable to the stomach or duodenum may have gastric or duodenal ulcer disease. Abdominal pain is the most common symptom, often described as burning, boring, or nagging. Pain is usually epigastric and relieved by food or antacids. Nausea and vomiting may occur with gastric ulcer or duodenal ulcer complicated by gastric outlet obstruction. A small proportion of patients may present with bleeding or perforation. On physical examination, abdominal tenderness or fecal occult blood may be found, but the exam is often nonspecific. Corticosteroid use may mask the signs and symptoms of disease.

Pathology–Pathophysiology

The actual incidence of ulcer disease in SLE has never been directly addressed. In Brown *et al*.'s review [4], it is mentioned that 5% (4 of 85) of patients had ulcer disease unrelated to therapy, while Ropes [8] quotes a figure of 20% (7 of 35). Dubois and Tuffanelli [5] cite a 1.5% incidence of ulcer perforation, which resulted in death in one patient taking steroids. While the role of *Helicobacter pylori* and nonsteroidal anti-inflammatory agents in the etiology of gastroduodenal ulcers is now well established [67, 68], in SLE patients

the possibility still exists that such ulcers are a direct consequence of lupus. One group reported a case of giant gastric ulceration associated with the anti-phospholipid antibody syndrome, which resolved on treatment with anticoagulants and corticosteroids [69]. In a review of the digestive tract findings in connective tissue disorders, Siurala *et al*. [70] performed gastric biopsies on 17 patients with SLE. Four showed superficial gastritis, and an additional 8 showed varying degrees of atrophic gastritis. There appeared to be no correlation to therapy, and of note was the presence of similar histologic findings in 8 of 20 control patients with celiac disease. No data were available as to the presence of anti-parietal cell antibodies, which have been seen in these patients [71] and may relate to the atrophic gastritis. Nadorra *et al*. [13] found heterotopic calcification of the superficial gastric mucosa in 3 of 36 autopsies of patients with childhood-onset SLE, albeit 2 of the 3 had been on hemodialysis. Thus there does not appear to be sufficient data to determine whether SLE is causative in the development of peptic ulcer disease.

Okayasu *et al*. [72] performed an autopsy study in Japan examining the risk of cancer in patients with connective tissue disorders. Although it was found that dermatomyositis was strongly associated with the development of gastric carcinoma, no such association was found for SLE either in the stomach or elsewhere. Thus there appears to be no link between SLE and the development of gastric malignancy.

Diagnosis

The diagnosis of gastric or duodenal ulcer may be made on clinical grounds or by esophagogastroduodenoscopy or upper GI series. *Helicobacter pylori* infection may be detected through serology for IgG and IgA anti-*Helicobacter* antibodies, by [^{13}C] or [^{14}C]-urea breath test following an oral urea load, or with endoscopic biopsy using urease or histologic examination.

Therapy

It is reasonable to treat all documented benign gastric and duodenal ulcers with proton-pump inhibitors, H_2 blockers, or other antiacid therapy, and it has been argued that it is reasonable to give prophylactic therapy to those patients taking steroids, aspirin, or nonsteroidal anti-inflammatory drugs. Misoprostol may also be considered for prophylaxis, although the complication of misoprostol-induced diarrhea (up to 40% of patients) may be worse than the symptoms of peptic ulcer disease. Special consideration should be given to bleeding peptic ulcers in SLE patients. Hiraishi *et al*. [73] reported a patient with SLE who presented with severe upper gastrointestinal bleeding as a result of gastric ulcer. The bleeding did not stop with conventional antiulcer therapy or by surgery in which an antral ulcer penetrating into the ileum with histologic stigmata of vasculitis was found. The bleeding was controlled only after pulse methylprednisolone therapy was initiated, stressing the issue of etiology-oriented therapy.

Other Gastric Manifestations

Watermelon Stomach

A case of iron deficiency anemia due to chronic blood loss attributed to watermelon-stomach in an SLE patient with anti-cardiolipin antibodies was reported by Archimandritids *et al*. [74]. This rare condition of gastric antral vascular ectasia is associated with connective tissue disorders in 62% of cases. Treatment with methylprednisolone and subsequently hydroxychloroquine in addition to iron supplements was effective in controlling the anemia.

SMALL AND LARGE INTESTINE

Some of the more relevant and potentially dangerous GI complications of SLE occur in the small and large intestines. Although some complications described earlier (such as aspirin-induced gastritis or ulcer) may be related to therapy, complications seen in the small and large intestines (such as vasculitis of the bowel with concomitant bleeding perforation) are clearly lupus related.

Vasculitis

Clinical Features

Patients with vasculitis may present with any of a variety of abdominal pain syndromes. They may present with nondescript bloating, nausea, and diarrhea or may have abrupt and massive GI hemorrhage or acute abdominal pain. Lupus-related symptoms referable to the intestine may be insidious or acute, localized or diffuse, benign or catastrophic. Steroid use may cloak symptoms. One must maintain a high index of suspicion when the lupus patient reports abdominal complaints in order to avert disaster.

Vasculitis of the small or large intestine is the most serious GI complication of SLE [21]. It may present as GI hemorrhage, occurring in 46% of cases in the Nadorra *et al*. [13] study and resulting in death in one-half of these cases, therefore accounting for about 25% of the deaths in their series. It may also present as an acute abdomen secondary to intestinal ischemia or infarction with perforation [75–80]. In the Zizic *et al*. study [77], for example, 5 of 107 SLE patients seen in

their clinic died from intestinal perforation, making it the major cause of death in their series. In general, the outcome with perforation is dismal, resulting in death in more than two-thirds of cases [81] when the outcome was known.

Patients with intestinal vasculitis may present with less severe symptoms such as nausea, vomiting, bloating, diarrhea, and fever on the basis of intestinal ischemia with or without ileus, infection, obstruction, or malabsorption [75, 79, 82, 83]. These symptoms are often insidious, may be masked by corticosteroids, often precede the more serious complications of perforation and hemorrhage, and should alert the clinician to the possibility of an approaching disaster. Unexplained acidosis, hypotension, abdominal distention, or bowel dilation on X-ray should alert the clinician to the possibility of a perforated viscus. Absence of bowel sounds and presence of guarding are not reliably found [84].

Additionally, those SLE patients with peripheral vasculitis, circulating rheumatoid factor, central nervous system involvement, and thrombocytopenia appear to be more at risk for developing an acute abdominal event according to one study [77], although others dispute this [84].

Pathology–Pathophysiology

Grossly, the appearance of the bowel ranges from segmental edema [4, 21, 82, 85] to discrete ulceration [86–88] to gangrene [88] and perforation [75–80]. Histologically both small vessel arteritis [13, 76, 79, 80, 89] and venulitis [79, 88, 90] have been seen, although larger vessels may be involved as in polyarteritis nodosa [21] (Fig. 1). Lesions of the larger vessels tend to produce more necrosis and gangrene. Associated histologic findings include atrophy and degeneration of the media of small arteries, fibrinoid necrosis of vessel walls, old thrombosis, phlebitis, and monocyte infiltrate in the lamina propria. Acute and chronic inflammatory infiltrate is seen, often confined to the mucosa but occasionally transmural [13].

Pneumatosis cystoides intestinalis, a usually benign entity that consists of intramural air in the intestine, has multiple etiologies and may be seen in SLE [91] as a possible result of vasculitis. In one case this finding was associated with a cecal perforation [78] whereas it was benign in others [91, 92].

Diagnosis

In those patients with the more insidious onset of symptoms, appropriate studies can be obtained, such as barium studies (or gastrografin when perforation is suspected) or endoscopy looking for signs of ischemia secondary to vasculitis (such as thumbprinting, ulcerations, and pallor) (Fig. 2). Computed tomography (CT) may reveal nonspecific findings such as bowel wall thickening and ascites [93, 94]. Arteriography may play a role in the diagnosis of ischemia secondary to vasculitis as reported in one case [89], whereas a second report [95] describes the use of an [111] indium granulocyte scan to identify 4 of 5 lupus patients with gastrointestinal symp-

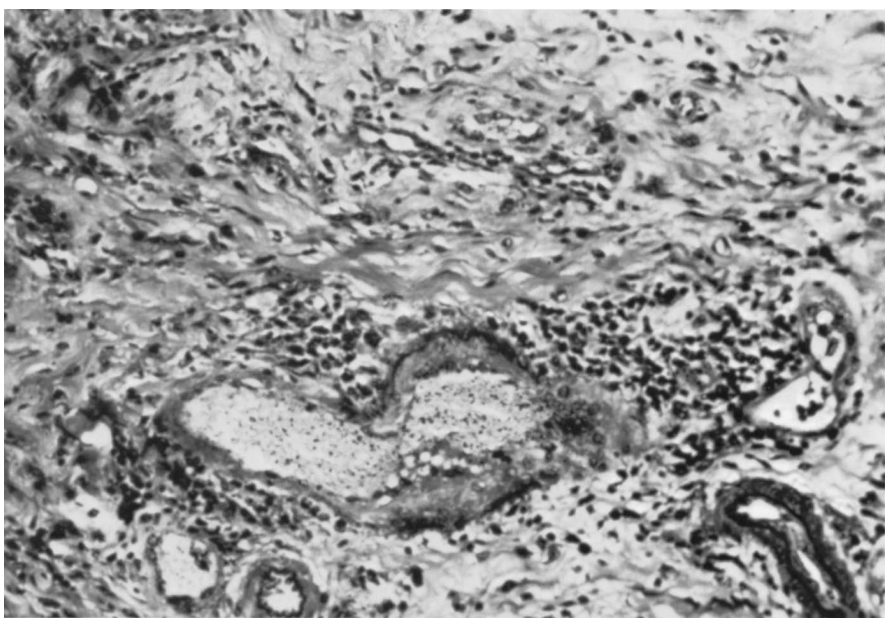


FIGURE 1 A cut section of a sigmoid biopsy from the patient whose X ray is depicted in Fig. 2. Note the perivascular lymphocytic infiltrate and destruction of a large artery wall. (Courtesy of Dr. David Sachar.)

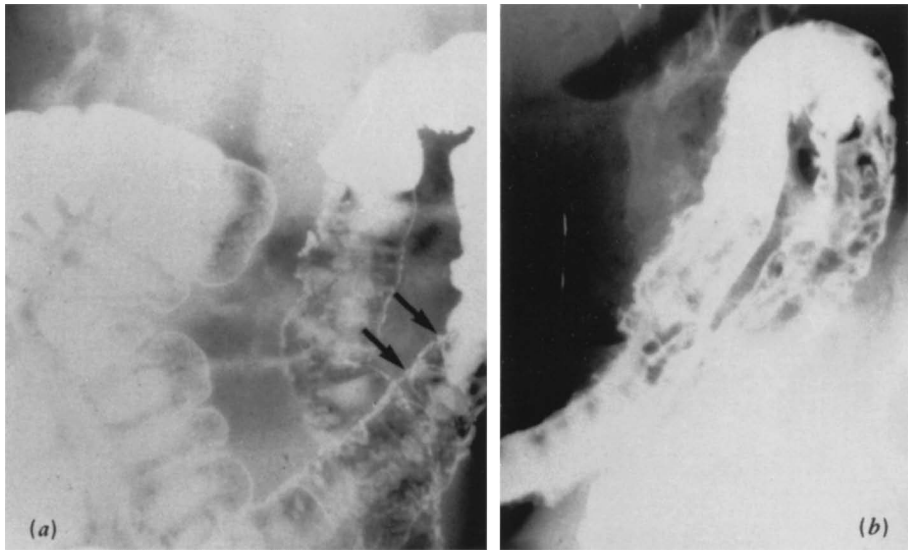


FIGURE 2 Barium enema X ray presentation of a patient with acute lupus vasculitis of the bowel. There are discrete ulcers (collar button ulcers—arrows (*a*)) seen as well as increased secretions ("graying" of the barium (*b*)). (Courtesy of Dr. David Sachar.)

toms who were then determined to have vasculitis. These latter tests have not been generally used. In those patients presenting with acute abdominal pain, CT findings may assist in the diagnosis of mesenteric ischemia. Byun *et al*. [96] retrospectively examined 39 CT examinations performed on 33 patients with acute abdominal pain and report that 31 (79%) had signs of ischemic bowel disease, defined by at least three of the following: bowel wall thickening, target sign (thickened bowel wall with peripheral rim enhancement or an enhancing inner and outer rim with hypoattenuation in the center), dilatation of intestinal segments, engorgement of mesenteric vessels, and increased attenuation of mesenteric fat. This suggests that CT may be useful for detecting the primary cause of gastrointestinal symptoms, particularly in the patient presenting with acute abdominal pain. In those patients presenting with an acute abdomen, emergency exploratory laparotomy is in order.

Therapy

Intestinal perforation or infarction as well as small bowel ischemia requires emergency surgery for resection of the involved area, as mortality is high in untreated cases. Early laparotomy has been shown to improve survival [97]. High-dose intravenous steroids are commonly used, and in patients who do not rapidly improve, cyclophosphamide can be added [98]. Large bowel ischemia may be treated expectantly with blood products, fluids, and antibiotics when appropriate. As with small bowel ischemia, high-dose prednisone is the most common and recommended treatment and was reported to resolve one case of pneumatosis cystoides intestinalis in a lupus patient with vasculitis [88]. In case this fails, high-dose cyclophosphamide was successful in the treatment of one patient with peritonitis and colitis due to intestinal vasculitis [100].

Other SLE-Related Intestinal Diseases

Intestinal Thrombosis

In addition to vasculitis-associated intestinal ischemia and infarction, infarction and ischemia have also been seen in the lupus intestine in association with thrombosis. Lupus patients with the anti-phospholipid antibody syndrome seem to be especially at risk. Asherson *et al*. [101] describe a case of bowel infarction necessitating colectomy. Histologic examination of the specimen revealed the presence of thrombus in branches of the inferior mesenteric artery with the total absence of any vasculitis. The patient's serum demonstrated antibodies to phospholipid (the lupus anticoagulant) and to cardiolipin, leading the authors to theorize that a thrombotic diathesis, on the basis of the presence of these antibodies, precipitated the bowel infarction. Another group has reported a case of repeated intestinal ulcerations secondary to thrombosis in a patient with lupus and high serum anti-phospholipid antibody levels [102]. Diagnosis and therapy are similar to those for intestinal vasculitis, and include appropriate

anticoagulation or antithrombotic medication. Plasmapheresis for patients with anti-phospholipid antibody syndrome may be of some benefit [98].

Inflammatory Bowel Disease

An association between SLE and both ulcerative colitis [86, 103, 104] and Crohn's disease [105, 106] has been suggested. Although this may imply that some lupus patients may have intestinal findings secondary to inflammatory bowel disease rather than vasculitis, there is no convincing proof. In most disputed cases, the pathology seemed most likely to be vasculitic and the association with inflammatory bowel disease, when seen, to be coincidental [104]. Diagnosis is made by clinical history in addition to typical barium X-ray findings or colonoscopic or histologic appearance, or both. Treatment includes sulfasalazine, 5-aminosalicylate derivatives, steroids, and azathioprine/6-mercaptopurine.

Drug-Related Disease

As with the other parts of the GI tract, drug therapy may contribute to intestinal disease in SLE. For example, Khoury [108] describes a case of an SLE patient who developed ulcerative proctitis twice, each time after starting ibuprofen, which remitted when the ibuprofen was stopped. NSAIDs have been shown to trigger the onset of UC in predisposed patients so this may be the unmasking of an underlying disease. One must always be aware that patients with inflammatory bowel disease may develop drug-induced SLE from drug therapy such as rafliximab (anti-TNF) and sulfasalazine [109]. Diagnosis may be suggested by history. Treatment includes withdrawal of the offending drug.

Protein-Losing Enteropathy

There are 24 reported cases of protein-losing enteropathy in association with SLE [110–116]. It is defined as excessive transintestinal mucosal loss of plasma proteins. It typically occurs in young women. Clinically, one may see diarrhea and typical manifestations of hypoproteinemia such as peripheral edema. It is characterized by coarse, thickened folds in the stomach and small bowel with or without ulcerations. The diagnosis is suspected in patients with hypoproteinemia and no other obvious source of protein loss and may be confirmed by radioisotopic studies or 24-h stool α_1-anti-trypsin clearance [115]. In some cases [110, 116–119] the enteropathy preceded the diagnosis of SLE. The reported cases responded to low-dose steroid therapy, and it was postulated that immune complex deposition led to increased permeability of intestinal capillaries and subsequently of the epithelial lining cells [111], analogous to glomerular basement membrane deposition in lupus nephritis.

Fat Malabsorption

Malabsorption of fat, with or without carbohydrate malabsorption, has also been described [69, 83, 120–124]. Clinically it is manifest by the presence of voluminous oily, foul smelling stools and weight loss in a patient with adequate oral intake. Diagnosis is suggested by a positive staining of stool sample with Sudan black and may be confirmed by elevated levels of fat in a 72-h stool collection or by ^{14}C-Triolein breath test. Pathologically, Weisman *et al*. [121] reported widening and blunting of the villi and immune complex deposition in their case of fat malabsorption. This lesion responded dramatically to steroids with reversal of the histologic picture.

In another report [124] two cases of fat malabsorption in SLE patients are described that are attributed to lupus-induced gut hypomotility with subsequent bacterial overgrowth. One would expect antibiotics to improve fat malabsorption in this case.

It is also theoretically possible, though unproven, that serositis leading to adhesions and partial bowel obstruction as described by Miller *et al*. [125] might lead to stasis and bacterial overgrowth with subsequent fat malabsorption and that lupus- or drug-induced pancreatitis [21, 83, 126–131] might lead to pancreatic insufficiency with concomitant fat malabsorption. Treatment of antibiotic- and steroid-unresponsive cases would include low-fat diet, lipid-soluble vitamin (A, E, D, K) supplements, cholestyramine, and judicious use of antidiarrheal agents.

Gluten-Sensitive Enteropathy (Celiac Sprue)

The coexistence of lupus and celiac sprue has been rarely reported [132]. Siurula *et al*. [70] noted nonspecific inflammatory changes in villus blunting on random small bowel biopsy specimens and likened these to celiac sprue. Davies and Marks [133] reported the case of a patient with SLE who developed dermatitis herpetiformis, a dermatologic disease with a known association to celiac sprue. This suggests the possibility of an association between SLE and celiac disease in these patients. In fact, several patients with collagen vascular disease and protein malabsorption with diarrhea, including one patient with SLE, have reportedly responded to a gluten-free diet [69, 122]. Classically, the diagnosis of celiac sprue is made by the presence of flattened small intestinal villi on biopsy that normalize on gluten-free diet and recur with reintroduction of

gluten into the diet. However, many lupus patients with celiac-like changes on intestinal biopsy do not respond to a gluten-free diet. Immunohistologic studies reveal deposition of C3 in the capillary walls of the small intestine [134]. Steroids may prove effective in resistant cases.

Chronic Intestinal Pseudo-Obstruction

Several small case-series reported the association of chronic intestinal pseudo-obstruction (CIPO) with lupus [135–138]. This complication, characterized by ineffective intestinal propulsion and manifested by abdominal pain and distention, bloating, vomiting, and constipation reflects a dysfunction of the visceral smooth muscle or the enteric nervous system. Out of 19 cases reported, 9 had CIPO as the initial presentation of SLE. Interestingly, in 12/19 there was bilateral ureterohydronephrosis [138], suggesting a central smooth muscle motility problem of neuropathic or myogenic origin. The diagnosis is made by radiographic studies showing fluid levels and dilated bowel, without an organic cause for obstruction, and by manometric studies. Treatment is with high-dose steroids, parenteral nutrition, promotility agents, and maintenance immunosuppression. Perlemuter *et al*. [139] used octreotide, 50 μg twice daily, when the previous measures failed, with no recurrence of CIPO. Even with aggressive therapy, CIPO remains a serious complication of SLE associated with a mortality of 26% (5/19 patients).

Amyloidosis

Amyloidosis in association with lupus has very rarely been reported [140, 141]. Patients may present with recurrent diarrhea and GI bleeding.

PERITONEAL CAVITY

Clinical Features

The presence of peritoneal inflammation has been well documented in autopsy and surgical studies as well as in several case reports [7, 8, 83, 125, 131, 142–147] in as many as 63% of patients [7]. Peritoneal inflammation may present in a number of ways, ranging from an acute abdomen to painless ascites. Overt signs may not be present due to the masking effect of steroids.

Pathology–Pathophysiology

The inflammatory change in the peritoneum is presumably due to deposition of immune complexes in mesenteric vessels [148–150] and can result in abdominal pain compatible with an acute abdomen [83, 151] or can be painless [129, 142, 143]. Patchy serosal and peritoneal plaques have been observed [144]. Peritoneal inflammation can also result in ascites, which in various series is present in 8–88% of cases [5, 8, 13]. Paracentesis usually reveals an exudative effusion with white blood cells [152] that is sterile when cultured. Lupus erythematosus (LE) cells were found in about one-third of the patients in one study [153]. Interestingly, the ascitic fluid has been shown to have high DNA-binding activity and low total complement, supporting a role for immune complexes in the development of ascites. Ascites can also be due to the nephrotic syndrome or constrictive pericarditis, both of which should be considered first before ascribing the ascites to peritoneal inflammation. Lastly, Budd-Chiari syndrome (thrombosis of the portal vein and potentially the inferior vena cava) as a consequence of clotting diathesis in SLE patients with lupus anticoagulant has also been reported to produce ascites [154] and should be considered in the differential diagnosis.

The presence of ascites increases the patient's susceptibility to spontaneous bacterial peritonitis. This complication has been reported in SLE [155, 156]. Conn [157] suggests that it is relatively more common in patients with "disseminated" SLE than in patients with alcoholic cirrhosis. The great majority of cases of spontaneous bacterial peritonitis develop in the hospital, usually more than 1 week after admission [157]. In one of the SLE patients reported to have developed spontaneous bacterial peritonitis, *Streptococcus pneumoniae* was the causative organism [151].

Lupus patients with renal failure on continuous ambulatory peritoneal dialysis are obviously at risk for bacterial peritonitis. For example, Allais *et al*. [142] reported a case of *Listera monocytogenes* in a lupus patient on continuous ambulatory peritoneal dialysis. Abdominal abscesses have also been found [13, 91]. Perforation of the intestine in a patient with SLE can also lead to peritonitis [77].

Hemoperitoneum has also occurred in SLE [158]. In this case, the patient had a ruptured gastroepiploic aneurysm, which showed evidence of vasculitis (fibrinoid necrosis with destruction of muscle and elastic fibers in the vasa vasorum as well as disintegration of the connective tissue layer of the tunica media). A fall prior to the development of the hemoperitoneum was probably the precipitating event. McCollum *et al*. [159] have also reported on the development of a ruptured aneurysm in a patient with SLE, in this case the hepatic artery.

Diagnosis

The value of paracentesis is obvious in the lupus patient with ascites or signs of peritonitis. Zizic *et al*. [77] emphasized the urgency in evaluation and diagnosis of such patients, maintaining a high index of suspicion, because many of the gross signs of peritonitis such as rebound tenderness and guarding may be masked by the high steroid doses that these patients are often taking.

Therapy

Once a diagnosis is made, appropriate therapy should be initiated. In the case of SLE-induced peritonitis, increasing the dose of steroids has been shown to be effective [152]. In the presence of ascites, steroids alone appear to be less effective and some have suggested that the addition of cyclophosphamide improves outcome [149]. For bacterial peritonitis, antibiotics should be instituted, and for bowel perforation or aneurysm rupture, emergency surgery is imperative.

Serositis may also lead to other complications. For example, as noted earlier, Miller *et al*. [125] described a case of small bowel obstruction secondary to extensive small bowel adhesions in a patient with SLE serositis.

PANCREAS

Clinical Features

Pancreatitis in association with SLE has been recognized for some time [72, 83, 127, 130, 131, 158, 160, 161] although the incidence is variable, ranging from 1% to 23% [6, 8, 13]. In most reported cases, it occurs in the setting of SLE with multiorgan involvement. Clinically, the presenting symptoms of acute pancreatitis in SLE are no different than those found in non-SLE patients: severe abdominal pain, often radiating to the back; nausea; and vomiting. X rays frequently reveal a localized ileus of either the small bowel or colon (sentinel loop). The activity of the underlying SLE appears to be greater in those patients with pancreatitis than in those without [126, 162].

Complications of pancreatitis are not common. Fistulae and abscess have not been noted. Pseudocyst formation [91, 162] and resolution [163] have been reported. Three-fourths of the patients in the Pollak *et al*. [84] series with pancreatitis died. The signs and symptoms of chronic pancreatitis are more subtle and may include evidence of diabetes and fat malabsorption.

Pathology–Pathophysiology

Because steroids as well as other drugs used in SLE such as azathioprine have been implicated in pancreatitis [131, 164–167] there have been some questions as to whether SLE in and of itself can cause pancreatitis. The weight of evidence points toward an actual vasculitic lesion as etiologic in some cases of lupus pancreatitis [127]. Several cases occurred in the presteroid era [83, 130] and in patients not taking steroids or immunosuppressives [126, 162]. Furthermore, in some patients the pancreatitis resolved while either maintaining or increasing their steroid dose [162]. Four of 20 patients in the Reynolds *et al*. [162] review had no predisposing factor for their pancreatitis other than SLE, and histologically, steroid-induced pancreatitis differs from the vasculitic picture of pancreatitis seen in the available animal models thus far [168, 169]. A role for antiphospholipid and anti-cardiolipin antibodies, seen in some patients with lupus, has been suggested [170, 171].

Ropes [8] described the pancreas in three lupus patients as containing, respectively, (1) moderate fibrosis and lymphocytic infiltration; (2) ectasia of the acini; and (3) acute and chronic inflammatory changes in the vessels with fibrinoid necrosis. The absence of clinically evident pancreatitis in Ropes' study [8] concurrent with the finding of pancreatitis in 6% of his autopsied cases, suggests the possibility of subclinical pancreatitis in lupus patients. Further evidence for this comes from the Tsianos *et al*. [12] study in which 30.5% (11 of 36) of SLE patients who had no abdominal pain or other gastrointestinal symptoms while at the time of blood drawing had hyperamylasemia. The authors suggest that asymptomatic pancreatic damage in SLE may occur frequently and that the hyperamylasemia in these patients probably reflects a slow, subclinical, inflammatory process of the pancreas. Another possibility is hyperamylasemia from small bowel perforation.

Diagnosis

Acute pancreatitis may be diagnosed by typical symptoms and blood work. The serum amylase level is elevated, but amylase isoenzymes, serum lipase, and amylase/creatinine clearance ratio (>5.5) are suggested to differentiate pancreatic from salivary hyperamylasemia and the hyperamylasemia seen in renal disease [172]. In addition, an inflamed, edematous pancreas on sonogram or computed tomographic scan can confirm the diagnosis.

Therapy

If steroids or azathioprine are suspected to be the inciting agent, they should be withdrawn. Treatment of

uncomplicated acute pancreatitis should include fluid support, analgesics, and placing the pancreas at rest by allowing no food by mouth.

GALLBLADDER

Gallbladder disease is distinctly uncommon in SLE patients. Four cases have been reported [13, 173–175] and in one of these cases [173] occurred in a patient with increased activity of disease and high levels of circulating immune complexes. Histologically, the gallbladder in this case revealed an acute arteritis with periarterial fibrosis, consistent with SLE vasculitis. These changes are similar to those seen in other vasculitic syndromes such as polyarteritis nodosa, in which gallbladder disease is more common [176]. It is interesting that the gallbladder is often involved in polyarteritis nodosa but rarely in SLE. This may suggest differences in the type of immune complexes generated in these two diseases.

SPLEEN

Splenomegaly, usually asymptomatic, has been reported in various series of SLE with a frequency of 9–67% [2, 5, 6, 9, 10]. Symptomatic disease of the spleen in association with SLE may also occur. Asherson *et al*. [101] reported a patient with lupus anticoagulant whose clotting diathesis led to splenic artery thrombosis with subsequent splenic infarction, requiring splenectomy. One case of spontaneous splenic rupture and hemoperitoneum in a girl who was later diagnosed with lupus has also been described [177]. Ropes' examination [8] of autopsy specimens from patients with SLE revealed the spleen to contain fibrosis, hemosiderosis, congestion, acute and chronic inflammation, onion skin thickening of the arterioles, splenic infarcts, and, in one case a caseating granuloma consistent with tuberculosis. Rare cases of asplenism have also been reported [178].

DRUG-RELATED PATHOLOGY

Corticosteroids and aspirin are two therapeutic agents used in SLE that are fraught with significant side effects. As has been discussed, they can be implicated in many pathologic findings in the GI tract. Steroids may cause pancreatitis, fatty liver, possibly peptic ulcer disease, and may increase the risk of diverticular perforation [19]. Aspirin and nonsteroidal anti-inflammatory drugs are equally implicated in a variety of disorders such as gastritis, gastric ulcer, and liver disease. *However, there are no lupus-specific data on nonhepatic gastrointestinal side effects of either nonsteroidal anti-inflammatory drugs or the selective Cox-2 inhibitors* [179]. Antihypertensives, such as methyldopa, have been implicated in chronic active hepatitis, and thiazides are etiologic in some cases of pancreatitis. Azathioprine, used in the treatment of SLE, can cause pancreatitis, typically presenting within the first 3 weeks of therapy. Ibuprofen has been implicated in a case of colitis in a patient with SLE [107]. Also of interest is a reported case of procainamide-induced lupus with pancreatitis [180]. It is unclear whether the pancreatitis was caused by the drug itself or by the lupus syndrome. Antimalarials, also used in the treatment of SLE, may cause blue, gray, or brown pigmentation of the buccal mucosa [51]. Cyclophosphamide can cause stomatitis. In general, as has been suggested by Hoffman and Katz [81], if any GI complication arises one should think of a drug-related event before proceeding to a more extensive workup.

In addition to various lupus drugs causing GI symptoms, there are various drugs used in GI diseases that may cause drug-induced SLE. Infliximab therapy has been associated with the development of ANAs and anteds DNA antibodies. In some patients, a lupus like illness was described. Trimethoprim-sulfa and tetracycline, both used in patients with infectious diarrhea and in Crohn's disease [181] may do this. Sulfasalazine and olsalazine have also been implicated, and IgG against the (H2A H2B)–DNA complex has been documented in two cases [182–184]. It may be difficult to determine which is the primary disease. The symptoms usually resolve on cessation of the offending agents.

CONCLUSIONS

Approach to SLE Patients Presenting with Abdominal Pain

From this overview, several conclusions can be drawn that we hope can guide therapeutic decisions in the future. The most serious GI complications of SLE are those associated with abdominal pain. As noted previously, medication effects should always be considered first, since they are probably more common than the serious GI complications. If a drug-related effect is ruled out, the appropriate laboratory evaluation (including CBC, ESR, serum amylase and lipase levels, serous lactate urinalysis and stool guaiac in addition to disease activity-related serologies) should be initiated. Abdominal X rays, looking for a paralytic ileus or free air secondary to perforation, should be followed by a diagnostic paracentesis, if warranted. Ascites should always be tapped to rule out the possibility of concomitant spontaneous bacterial peritonitis or the much

rarer hemoperitoneum. Computed tomography is valuable in assessing both lupus-related as well as unrelated abdominal events, as it should be kept in mind that SLE patients presenting with abdominal pain syndromes may have conventional gastrointestinal illnesses [184]. Quick surgical intervention is mandatory if there is evidence of perforation or gangrene, as it appears that earlier surgery, even in sicker patients, yields a better prognosis. If paracentesis and X rays are negative, then these patients fall into Dubois' category [21] of potentially reversible ischemia and should be treated with increasing doses of steroids. These patients should respond rapidly.

As more studies are performed, we should gain a better knowledge of subcategories of patients and their responses to various treatment protocols. Until then it would be prudent to be more aggressive in treating these patients, since current knowledge supports this course.

References

1. Osler, W. (1895). On the visceral complications of erythema exudativum multiforme. *Am. J. Med. Sci.* **110**, 629.
2. Jessar, R. A., Lamont-Havers, R. W., and Ragar, C. (1953). Natural history of lupus erythematosus disseminatus. *Ann. Intern. Med.* **38**, 717.
3. Harvey, A. M., Shulman, L. E., Tumulty, P. A., *et al*. (1954). Systemic lupus erythematosus: Review of the literature and clinical analysis of 138 cases. *Medicine* **33**, 291.
4. Brown, C. H., Shirey, E. K., and Haserick, J. R. (1956). Gastrointestinal manifestations of systemic lupus erythematosus. *Gastroenterology* **31**, 649.
5. Dubois, E. L., and Tuffanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus. *JAMA* **190**, 104.
6. Estes, D., and Christian, C. L. (1971). The natural history of systemic lupus erythematosus by prospective analysis. *Medicine* **50**, 85.
7. Fries, J. F., and Holman, H. R. (1975). "Systemic Lupus Erythematosus: A Clinical Analysis." Saunders, Philadelphia.
8. Ropes, M. W. (1976). "Systemic Lupus Erythematosus." Harvard Univ. Press, Cambridge, Massachusetts.
9. Urman, J. D., Lowenstein, M. B., Abeles, M., and Weinstein, A. (1978). Oral mucosal ulceration in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 58–61.
10. Al Rawi, Z., Al Shaarbaf, H., Al Raheem, E., and Khalifa, S. J. (1983). Clinical features of early cases of systemic lupus erythematosus in Iraqui patients. *Br. J. Rheumatol.* **22**, 165–171.
11. Jonsson, R., Heyden, G., Westberg, N. G., and Nyberg, G. (1984). Oral mucosal lesions in systemic lupus erythematosus—a clinical, histopathological and immunopathological study. *J. Rheumatol.* **11**, 38–42.
12. Tsianos, E. B., Tzioufas, A. G., Kita, M. D., Tsolas, O., and Moutsopoulos, H. M. (1984). Serum isoamylases in patients with autoimmune rheumatic diseases. *Clin. Exp. Rheumatol.* **2**, 235–238.
13. Nadorra, R. L., Nakazato, Y., and Landing, B. H. (1987). Pathologic features of gastrointestinal tract lesions in childhood-onset systemic lupus erythematosus: Study of 26 patients, with review of the literature. *Pediatr. Pathol.* **7**, 245–259.
14. Burge, S. M., Frith, P. A., Juniper, R. P., and Wojnarowska, F. (1989). Mucosal involvement in systemic and chronic cutaneous lupus erythematosus. *Br. J. Dermatol.* **121**, 727–741; Fries, J. F., and Holman, H. R. (1975). Systemic lupus erythematosus: A clinical analysis. *Major Probl. Intern. Med.* **6**, v–199.
15. Castrucci, G., Alimandi, L., Fichera, A., Altomonte, L., and Zoli, A. (1990). [Changes in esophageal motility in patients with systemic lupus erythematosus: An esophago-manometric study]. *Minerva Dietol. Gastroenterol.* **36**, 3–7.
16. Cohen, A. S., Reynolds, W. F., Franklin, E. C., *et al*. (1971). Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21**, 643.
17. Jorizzo, J. L., Salisbury, P. L., Rogers, III, R. S., Goldsmith, S. M., Shar, G. G., Callen, J. P., Wise, C. M., Semble, E. L., and White, W. L. (1992). Oral lesions in systemic lupus erythematosus. Do ulcerative lesions represent a necrotizing vasculitis? *J. Am. Acad. Dermatol.* **27**, 389–394.
18. Yell, J. A., Mbuagbaw, J., and Burge, S. M. (1996). Cutaneous manifestations of systemic lupus erythematosus. *Br. J. Dermatol.* **135**, 355–362.
19. Schiodt, M. (1984). Oral manifestations of lupus erythematosus. *Int. J. Oral Surg.* **13**, 101–147.
20. Snyder, G. G., McCarthy, R. E., Toomey, J. M., and Rothfield, N. F. (1974). Nasal septal perforation in systemic lupus erythematosus. *Arch. Otolaryngol.* **99**, 456–457.
21. DuBois, E. L. (1974). "Lupus Erythematosus. A Review of the Current Status of Discoid and Systemic Lupus Erythematosus and Their Variants." Univ. of Southern California Press, Los Angeles.
22. Gilliam, J. N., and Cheatum, D. E. (1973). Immunoglobulins in the larynx in systemic lupus erythematosus. *Arch. Dermatol.* **108**, 696.
23. Wilson, H. A., Hamilton, M. E., Spyker, D. A., Brunner, C. M., O'Brien, W. M., Davis, J. S., and Winfield, J. B. (1981). Age influences the clinical and serologic expression of systemic lupus erythematosus. *Arthritis Rheum.* **24**, 1230–1235.
24. Andreasen, J. O., and Pousen, H. E. (1964). Oral manifestations in discoid and systemic lupus erythematosus. *Acta Odontol. Scand.* **22**, 389.
25. Schiodt, M. (1984). Oral discoid lupus erythematosus (III). A histopathologic study of sixty-six patients. *Oral Surg. Oral Med. Oral Pathol.* **57**, 281–293.
26. Sanchez, R., Jonsson, R., Ahlfors, E., Backman, K., and Czerkinsky, C. (1988). Oral lesions of lupus erythematosus patients in relation to other chronic inflammatory oral diseases: An immunologic study. *Scand. J. Dent. Res.* **96**, 569–578.
27. Schiodt, M., Andersen, L., Shear, M., and Smith, C. J. (1981). Leukoplakia-like lesions developing in patients

with oral discoid lupus erythematosus. *Acta Odontol. Scand.* **39**, 209–216.
28. Schiodt, M., Dabelsteen, E., Ullman, S., and Halberg, P. (1974). Deposits of immunoglobulins and complement in oral lupus erythematosus. *Scand. J. Dent. Res.* **82**, 603–607.
29. Jonsson, R., Nyberg, G., Kristensson-Aas, A., and Westberg, N. G. (1983). Lupus band test in uninvolved oral mucosa in systemic lupus erythematosus. *Acta Med. Scand.* **213**, 269–273.
30. Laskaris, G., Perissos, A., and Varelzidis, A. (1978). Immunofluorescent oral mucosa test in lupus erythematosus. *Iatriki* **33**, 424.
31. Piamphongscant, T., and Chaikittisilpa, S. (1979). Mucocutaneous lupus erythematosus. *J. Med. Assoc. Thai.* **12**, 661.
32. Schiodt, M., Holmstrup, P., Dabelsteen, E., and Ullman, S. (1981). Deposits of immunoglobulins, complement, and fibrinogen in oral lupus erythematosus, lichen planus, and leukoplakia. *Oral Surg.* **51**, 603–608.
33. Schiodt, M., and Anderson, L. (1980). Ultrastracture of oral discoid lupus erythematosus. *Acta Dermatol. Venereol.* **60**, 99.
34. Ueki, H. (1969). Hyaline bodies in subepidermal papillae. Immunohistochemical studies in several dermatoses. *Arch. Dermatol.* **100**, 610.
35. Hashimoto, K. (1976). Apoptosis in lichen planus and several other dermatoses. Intradermal cell death with filamentous degeneration. *Acta Dermatol. Venereol.* **56**, 187.
36. Ebner, H., and Gebhart, W. (1972). Beitrag sur histochemie und ultrastructure der sogennannten hyalinen bzw. Kolloiden korperchen. *Arch. Dermatol. Forsch.* **242**, 153.
37. Ebner, H., and Gebhart, W. (1977). Light and electron microscopic studies on colloid and other cytoid bodies. *Clin. Exp. Dermatol.* **2**, 311–322.
38. Herschfus, L. (1972). Lupus erythematosus. *J. Oral Med.* **27**, 12–18.
39. O'Neill, S., Thomson, J., Strong, A., and Lang, W. (1977). Systemic lupus erythematosus presenting as a recurrent sore throat and oral ulceration: A case report. *Br. J. Dermatol.* **96**, 211–213.
40. Jonsson, R. (1983). "Oral Manifestations of Systemic Lupus Erythematosus." Thesis, University of Goteborg, Faculty of Odontology.
41. Cummings, N. A. (1971). Oral manifestations of connective tissue disease. *Postgrad. Med. J.* **49**, 134.
42. Edwards, M. B., and Gayford, J. J. (1971). Oral lupus erythematosus. Three cases demonstrating three variants. *Oral Surg. Oral Med. Oral Pathol.* **31**, 332–342.
43. Jakes, J. T., Dubois, E. L., Quismorio, F. P., Jr. (1982). Antileprosy drugs and lupus erythematosus. *Ann. Intern. Med.* **97**, 788.
44. Ruzicka, T., and Goerz, G. (1981). Dapsone in the treatment of lupus erythematosus. *Br. J. Dermatol.* **104**, 53–56.
45. Neville, E., Graham, P. Y., and Brewis, R. A. (1981). Orogenital ulcers, SLE and hydralazine. *Postgrad. Med. J.* **57**, 378–379.
46. Sultan, S. M., Ioannou, Y., and Isenberg, D. A. (1999). A review of gastrointestinal manifestations of systemic lupus erythematosus. *Rheumatology* **38**, 917–932.
47. Andonopoulos, A., Skopouli, F., Dimou, G., Drosos, A., and Moutsopoulos, H. (1990). Sjogren syndrome in systemic lupus erythematosus. *J. Rheumatol.* **17**, 202–204.
48. Moutsopoulos, H., Webber, B., Vlagopoulos, T., Chused, T., and Decker, J. (1979). Differences in the clinical manifestations of sicca syndrome in the presence and absence of rheumatoid arthritis. *J. Rheumatol.* **22**, 1152–1158.
49. Ramos-Niembro, F., and Alarcon-Segovia, D. (1979). Development of sicca symptoms in systemic lupus erythematosus. *Arthritis Rheum.* **22**, 935–936.
50. Alarcon-Segovia, D., Ibanez, G., Velazquez-Forero, F., Hernandez-Ortiz, J., and Gonzalez-Jimerez, Y. (1974). Sjogren's syndrome in systemic lupus erythematosus. *Ann. Intern. Med.* **81**, 557–583.
51. Zufferey, P., Meyer, O., Bourgeois, P., Vayssairat, M., and Kahn, M. (1995). Primary systemic Sjogren's syndrome (SS) preceding systemic lupus erythematosus: A retrospective study of 4 cases in a cohort of 55 SS patients. *Lupus* **4**, 23–27.
52. Grennan, D. M., Ferguson, M., Ghobarey, A. E., Williamson, J., Mavrikakis, M., Dick, W. C., and Buchanan, W. W. (1977). Sjogren's syndrome in SLE: Part 1 The frequency of the clinical and subclinical features of Sjogren's syndrome in patients with SLE. *N. Z. Med. J.* **86**, 374–376.
53. Jaworski, C. P., Koudelka, B. M., Roth, N. A., and Marshall, K. J. (1985). Acute necrotizing ulcerative gingivitis in a case of systemic lupus erythematosus. *J. Oral Maxillofac. Surg.* **43**, 43–46.
54. Greenberg, M. S. (1977). "Burket's Oral Medicine. Diagnosis and Treatment." Lippincott, Philadelphia.
55. DaCunha Bang, F., Lang Wantzin, G., and Dahl Christensen, J. (1985). Raynaud's phenomenon with oral manifstatons in systemic lupus erythematosus. *Dermatologica* **170**, 263.
56. Wanke, N. C., Bruce, M. C., De Aguilar, E. J., and Pinto, F. M. (1984). [Bullous eruption in systemic lupus erythematosus]. *Med. Cutan. Ibero Lat. Am.* **12**, 209–213.
57. Miranda, C., and Lozada-Nur, F. (1996). Oral hairy leukoplakia in an HIV-negative patient with systemic lupus erythematosus. *Compend. Contin. Ed. Dent.* **17**, 408–410, 412.
58. Tannenbaum, L., and Tuffanelli, D. L. (1980). Antimalarlial agents. *Arch. Dermatol.* **116**, 587.
59. Hano, T., Tomoda, J., Harada, H., *et al*. (1986). A case of systemic lupus erythematosus with esophaggeal mucosal bridge. *Nippon Shokakibuyo Gakkai Zasshi* **83**, 2598.
60. Stevens, M. B., Hookman, P., Siegal, C. I., *et al*. (1964). Aperistalsis of the esophagus in patients with connective disorders and Raynuald's phenomenon. *N. Engl. J. Med.* **270**, 1218.
61. Ramirez-Mata, M., Reyes, P. A., Alarcon-Segovia, D., *et al*. (1974). Esophgeal motility in systemic lupus erythematosus. *Dig. Dis.* **19**, 132.

62. Tetelman, M., and Keech, M. K. (1966). Esophgeal motility in systemic lupus erythematosus, rheumatoid artheritis and scleroderma. *Radiology* **86**, 1041.
63. Clark, M., and Fountain, R. B. (1967). Oesophgeal motility in connective tissue disease. *Br. J. Dermatol.* **79**, 449.
64. Turner, R., Rittenberg, G., Lipshutz, W., *et al*. (1973). Esophageal dysfunction in collagen disease. *Am. J. Med. Sci.* **265**, 191.
65. Gould, D. M., and McAfee, J. G. (1958). A review of roentgen findings in systemic lupus erythematosus. *Am. J. Med. Sci.* **235**, 596.
66. Lapadula, G., Muolo, P., Semeraro, F., Covelli, M., Brindicci, D., Cuccorese, G, *et al*. (1994). Esophageal motility disorders in the rheumatic diseases. A review of 150 patients. *Clin. Exp. Rheumatol.* **12**, 512–521.
67. Van Der Hulst, R. W., and Tytgat, G. N. (1996). *Helicobacter pylori* and peptic ulcer disease. *Scand. J. Gastroenterol.* **220** (Suppl), 10–18.
68. Hawkey, C. J. (1990). Nonsteroidal anti-inflammatory drugs and peptic ulcer disease. *Br. Med. J.* **300**, 278.
69. Kalman, D. R., Khan, A., Romain, P. L., and Nompleggi, D. J. (1996). Giant gastric ulceration associated with antiphospholipid antibody syndrome. *Am. J. Gastroenterol.* **91**, 1244–1247.
70. Siurala, M., Julkunen, H., Tolvonen, S., *et al*. (1965). Digestive tract in collagen diseases. *Acta Med. Scand.* **178**, 13.
71. Kuknel, H. Personal communication.
72. Okayasu, I., Mizutani, H., Kurihara, H., and Yanagisawa, F. (1984). Cancer in collagen disease. A statistical analysis by reviewing the Annual of Pathological Autopsy Cases [Nippon Boken Shuho] in Japan. *Cancer* **54**, 1841.
73. Hiraishi, H, Konishi, T., Ota, S., Shimada, T., Terano, A., and Sugimoto, T. (1999). Massive gastrointestinal hemorrhage in systemic lupus erythematosus. *Am. J. Gastroenterol.* **94**, 3349–3353.
74. Archimandritis, A., Tsirantonaki, M., and Tzivras, M. (1996). Watermelon stomach in a patient with vitiligo and systemic lupus erythematosus (letter). *Clin. Exp. Rheumatol.* **14**, 227–228.
75. Moriuchi, J., Ichikawa, Y., Takaya, M., Shimizu, H., Tokunaga, M., Eguchi, T., Izumi, M., Ohta, W., Katsuoka, Y., and Nakajima, I. (1989). Lupus cystitis and perforation of the small bowel in a patient with systemic lupus erythematosus and overlapping syndrome. *Clin. Exp. Rheumatol.* **7**, 533–536.
76. Gore, R. M., Marn, C. S., Ujiki, G. T., Craig, R. M., and Marquardt, J. (1983). Ischemic colitis associated with systemic lupus erythematosus. *Dis. Colon Rectum* **26**, 449–451.
77. Zizic, T. M., Shulman, L. E., and Stevens, M. B. (1975). Colonic perforations in systemic lupus erythematosus. *Medicine* **54**, 411.
78. Kleinman, P., Mayers, M. A., Abbott, G., *et al*. (1976). Necrotizing enterocolitis with pneumatosis intestinalis in systemic lupus erythematosus and polyarteritis. *Radiology* **121**, 595.
79. Papa, M. Z., Shiloni, E., and McDonald, H. D. (1986). Total colonic necrosis. A catastrophic complication of systemic lupus erythematosus. *Dis. Colon Rectum* **29**, 576–578.
80. Ho, M. S., Teh, L. B., and Goh, H. S. (1987). Ischaemic colitis in systemic lupus erythematosus—report of a case and review of the literature. *Ann. Acad. Med. Singapore* **16**, 501–503.
81. Hoffman, B. I., and Katz, W. A. (1980). The gastrointestinal manifestations of systemic lupus erythematosus: A review of the literature. *Semin. Arthritis Rheum.* **9**, 237–247.
82. Boulter, M., Brink, A., Mathias, C., Peart, S., Stevens, J., Stewart, G., and Unwin, R. (1987). Unusual cranial and abdominal computed tomographic (CT) scan appearances in a case of systemic lupus erythematosus (SLE). *Ann. Rheum. Dis.* **46**, 162–165.
83. Mader, R., and Schonfeld, S. (1990). [Malabsorption in systemic lupus erythematosus]. *Harefuah* **118**, 572–573.
84. Pollak, V. E., Grove, W. J., Kark, R. M., *et al*. (1958). Systemic lupus erythematosus simulating acute surgical condition of the abdomen. *N. Engl. J. Med.* **259**, 258.
85. Bruce, J., and Sircus, W. (1959). Disseminated lupus erythematosus of the alimentary tract. *Lancet* **1**, 795.
86. Philips, J. C., and Howland, W. J. (1968). Mesenteric arteritis in systemic lupus erythematosus. *JAMA* **206**, 1569.
87. Tsuchiya, M., Okazaki, I., Asakura, H., *et al*. (1975). Radiographic and endoscopic features of colonic ulcers in Systemic lupus erythematosus. *Am. J. Gastroenterol.* **64**, 277.
88. Helliwell, T. R., Flook, D., Whitworth, J., and Day, D. W. (1985). Arteritis and venulitis in systemic lupus erythematosus resulting in massive lower intestinal haemorrhage. *Histopathology* **9**, 1103–1113.
89. Kistin, M. G., Kaplan, M. M., and Harrington, J. T. (1978). Diffuse ischemic colitis associated with systemic lupus erythematosus—response to subtotal colectomy. *Gastroenterology* **75**, 1147–1151.
90. Weiser, M. M., Andres, G. A., Brentjens, J. R., Evans, J. T., and Reichlin, M. (1981). Systemic lupus erythematosus and intestinal venulitis. *Gastroenterology* **81**, 570–579.
91. Heiberg, E., Wolverson, M. K., Sundaram, M., and Shields, J. B. (1988). Body computed tomography findings in systemic lupus erythematosus. *J. Comput. Tomogr.* **12**, 68–74.
92. Morrison, W. J., and Siegelman, S. S. (1976). Pneumatosis intestinalis in association with connective tissue disease. *South. Med. J.* **69**, 1536–1539.
93. Kirshy, D. M., Gordon, D. H., and Atweh, N. A. (1991). Abdominal computed tomography in lupus mesenteric arteritis. *Comput. Med. Imaging. Graph.* **15**, 369–372.
94. Ko, S. F., Lee, T. Y., Cheng, T. T., Ng, S. H., Lai, H. M., Cheng, Y. F., and Tsai, C. C. (1997). CT findings at lupus mesenteric vasculitis. *Acta Radiol.* **38**, 115–120.
95. Keshavarzian, A., Saverymuttu, S. H., Chadwick, V. S., Lavender, J. P., and Hodgson, H. J. (1984). Noninvasive investigation of the gastrointestinal tract in collagen-vascular disease. *Am. J. Gastroenterol.* **79**, 873–877.
96. Byun, J. Y., Ha, H. K., Yu, S. Y., Min, J. K., Park, S. H., Kim, H. Y., Chun, K. A., Choi, K. H., Ko, B. H., and Shinn,

K. S. (1999). CT features of systemic lupus erythematosus in patients with acute abdominal pain: Emphasis on ischemic bowel disease. *Radiology* **211**, 203–209.
97. Medina, F., Ayala, A., Jara, L. J., Becerra, M., Miranda, J. M., and Fraga, A. (1997). Acute abdomen in systemic lupus erythematosus: The importance of early laparotomy. *Am. J. Med.* **103**, 100–105.
98. Petri, M. (1996). Gastrointestinal manifestations. In "The Clinical Management of Systemic Lupus Erythematosus" (P. H. Schur, Ed.), 2nd ed., pp. 127–140. Lippincott-Raven, Philadelphia.
99. Cabrera, G. E., Scopelitis, E., Cuellar, M. L., Silveira, L. H., Mena, H., and Espinoza, L. R. (1994). Pneumatosis cystoides intestinalis in systemic lupus erythematosus with intestinal vasculitis: Treatment with high dose prednisone. *Clin. Rheumatol.* **13**, 312–316.
100. Grimbacher, B., Huber, M., von Kempis, J., Kalden, P., Uhl, M., Kohler, G., Blum, H. E., and Peter, H. H. (1998). Successful treatment of gastrointestinal vasculitis due to systemic lupus erythematosus with intravenous pulse cyclophosphamide: A clinical case report and review of the literature. *Br. J. Rheumatol.* **37**, 1023–1028.
101. Asherson, R. A., Morgan, S. H., Harris, E. N., Gharavi, A. E., Krausz, T., and Hughes, G. R. (1986). Arterial occlusion causing large bowel infarction—a reflection of clotting diathesis in SLE. *Clin. Rheumatol.* **5**, 102–106.
102. Sasamura, H., Nakamoto, H., Ryuzaki, M., Kumagai, K., Abe, S., Suzuki, H., Hirakata, M., Tojo, T., Handa, M., and Yamamoto, M. (1991). Repeated intestinal ulcerations in a patient with systemic lupus erythematosus and high serum antiphospholipid antibody levels. *South. Med. J.* **84**, 515–517.
103. Stevens, H. P., Ostlere, L. S., and Rustin, M. H. (1994). Systemic lupus erythematosus in association with ulcerative colitis: Related autoimmune diseases. *Br. J. Dermatol.* **130**, 385–389.
104. Garcia-Porrua, C., Gonzalez-Gay, M. A., Lancho, A., and Alvarez-Ferreira, J. (1998). Systemic lupus erythematosus and ulcerative colitis: An uncommon association. *Clin. Exp. Rheum.* **16**, 511.
105. Johnson, D. A., Diehl, A. M., Finkelman, F. D., and Cattau, E. L., Jr. (1985). Crohn's disease and systemic lupus erythematosus. *Am. J. Gastroenterol.* **80**, 869–870.
106. Gladman, D. D., Ross, T., Richardson, B., and Kulkarni, S. (1985). Bowel involvement in systemic lupus erythematosus: Crohn's disease or lupus vasculitis? *Arthritis Rheum.* **28**, 466–470.
107. Ishikawa, O., Miyachi, Y., Fujita, K., Takenoshita, S., Nagamachi, Y., and Hirato, J. (1995). Ulcerative colitis associated with preceding systemic lupus erythematosus. *J. Dermatol.* **22**, 289–291.
108. Khoury, M. I. (1989). Ulcerative proctitis in juvenile systemic lupus erythematosus after ibuprofen treatment. *J. Rheumatol.* **16**, 217–218.
109. Clementz, G. L., and Dolin, B. J. (1988). Sulfasalazine-induced lupus erythematosus. *Am. J. Med.* **84**, 535–538.
110. Pachas, W. N., Linscheer, W. G., and Pinals, R. S. (1971). Protein-losing enteropathy in systemic lupus erythematosus. *Am. J. Gastroenterol.* **55**, 162–167.
111. Trentham, D. E., and Masi, A. T. (1976). Systemic lupus erythematosus with a protein-losing enteropathy. *JAMA* **236**, 287–288.
112. Tsukahara, M., Matsuo, K., and Kojima, H. (1980). Protein-losing enteropathy in a boy with systemic lupus erythematosus. *J. Pediatr.* **97**, 778–780.
113. Waldaman, T. A., Wochner, R. D., and Strober, W. (1969). The role of the gastrointestinal tract in plasma protein metabolism. *Am. J. Gastroenterol.* **46**, 275.
114. Murao, S., Taooka, Y., Yamanishi, Y., Mukuzono, H., Aoi, K., Isibe, Y., and Yamana, S. (1994). [Protein-losing enteropathy and cerebral infarction associated with systemic lupus erythematosus]. *Ryumachi* **34**, 59–63.
115. Perednia, D. A., and Curosh, N. A. (1990). Lupus-associated protein-losing enteropathy. *Arch. Intern. Med.* **150**, 1806–1810.
116. Northcott, K. A., Yoshida, E. M., and Steinbrecher, U. P. (2001). Primary protein losing enteropathy in anti-double-stranded DNA disease: The initial and sole clinical manifestation of occult systemic lupus erythematosus? *J. Clin. Gastroenterol.* **33**, 340–341.
117. Chung, U., Oka, M., Nakagawa, Y., Nishishita, T., Sekine, N., Tanaka, Y., Harada, S., Igarashi, T., and Ogata, E. (1992). A patient with protein-losing enteropathy associated with systemic lupus erythematosus. *Intern. Med.* **31**, 521–524.
118. Molina, J. F., Brown, R. F., Gedalia, A., and Espinoza, L. R. (1996). Protein losing enteropathy as the initial manifestation of childhood systemic lupus erythematosus. *J. Rheumatol.* **23**, 1269–1271.
119. Sunheimer, R. L., Finck, C., Mortazavi, S., McMahon, C., and Pincus, M. R. (1994). Primary lupus-associated protein-losing enteropathy. *Ann. Clin. Lab. Sci.* **24**, 239–242.
120. Bazinet, P., and Marin, G. A. (1971). Malabsorption in systemic lupus erythematosus. *Am. J. Dig. Dis.* **16**, 460–466.
121. Weisman, M. H., McDanald, E. C., and Wilson, C. B. (1981). Studies of the pathogenesis of interstitial cystitis, obstructive uropathy, and intestinal malabsorption in a patient with systemic lupus erythematosus. *Am. J. Med.* **70**, 875–881.
122. Rustai, A. K., and Peppercorn, M. A. (1988). Gluten-sensitive enteropathy and systemic lupus erythematosus. *Arch. Intern. Med.* **148**, 1583–1584.
123. Kurlander, D. J., and Kirsner, J. B. (1964). The association of chronic "non-specific" inflammatory bowel disease with lupus erythematosus. *Ann. Intern. Med.* **60**, 799.
124. (1989). Severe acquired hypocholesterolemia: Two case reports. *Nutri. Rev.* **47**, 202.
125. Miller, M. H., Urowitz, M. B., Gladman, D. D., and Tozman, E. C. (1984). Chronic adhesive lupus serositis as a complication of systemic lupus erythematosus. Refractory chest pain and small-bowel obstruction. *Arch. Intern. Med.* **144**, 1863–1864.
126. Mekori, Y. A., Yaretzky, A., Schneider, M., and Klajman, A. (1980). Pancreatitis in systemic lupus erythematosus—a case report and review of the literature. *Postgrad. Med. J.* **56**, 145–147.

127. Baron, M., and Brisson, M. L. (1982). Pancreatitis in systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1006–1009.
128. Herskowitz, L. J., Olansky, S., and Lang, P. G. (1979). Acute pancreatitis associated with long-term azathioprine therapy. Occurrence in a patient with systemic lupus erythematosus. *Arch Dermatol.* **115**, 179.
129. Kawanishi, H., Rudolph, E., and Bull, F. E. (1973). Azathioprine induced acute pancreatitis. *N. Engl. J. Med.* **289**, 357.
130. Reifenstein, E. C., Reifenstein, E. C., Jr., and Reifenstein, G. H. (1939). Variable symptom complex of undetermined etiology with total termination including conditions described as visceral erythema group (Osler) disseminated lupus erythematosus, atypical verrucous endocarditis (Libman-Sacks), fever of unknown origin (Christian) and a diffuse peripheral vascular disease (Baehr and others). *Arch. Intern. Med.* **63**, 553.
131. Paulino-Netto, A., and Dreiling, D. A. (1960). Pancreatitis in disseminated lupus erythematosus. A case report. *J. Mt. Sinai Hosp.* **27**, 291.
132. Komatireddy, G. R., Marshall, J. B., Aqel, R., Spollen, L. E., and Sharp, G. C. (1995). Association of systemic lupus erythematosus and gluten enteropathy. *South. Med. J.* **88**, 673–676.
133. Davies, M. G., and Marks, R. (1976). Simultaneous systemic lupus erythematosus and dermatits herpetiformis. *Arch. Dermatol.* **112**, 1292.
134. Tsutsumi, A., Sugiyama, T., Matsumura, R., Sueishi, M., Takabayashi, K., Koike, T., Tomioka, H., and Yoshida, S. (1991). Protein losing enteropathy associated with collagen diseases. *Ann. Rheum. Dis.* **50**, 178–181.
135. Cacoub, P., Benhamou, Y., Barbet, P., Piette, J. C., Le Cae, A., Chaussade, S., Cadranel, J. F., Callard, P., Opolon, P., and Godeau, P. (1993). Systemic lupus erythematosus and chronic intestinal pseudoobstruction. *J. Rheumatol.* **20**, 377–381.
136. Perlemuter, G., Chaussade, S., Wechsler, B., Cacoub, P., Dapoigny, M., Kahan, A., Godeau, P., and Couturier, D. (1998). Chronic intestinal pseudo-obstruction in systemic lupus erythematosus. *Gut* **43**, 117–122.
137. Munyard, P., and Jaswon, M. (1997). Systemic lupus erythematosus presenting as intestinal pseudo-obstruction. *J. R. Soc. Med.* **39**, 877–879.
138. Mok, M. Y., Wong, R. W. S., and Lau, C. S. (2000). Intestinal pseudo-obstruction in systemic lupus erythematosus *Lupus* **9**, 11–18.
139. Perlemuter, G., Cacoub, P., Chaussade, S., Wechsler, B., Couturier, D., and Piette, J. C. (1999). Octreotide treatment of chronic intestinal pseudoobstruction secondary to connective tissue diseases *Arthritis Rheum.* **42**, 1545–1549.
140. Al Hoqail, I., Naddaf, H., Al Rikabi, A., Al Arfaj, H., and Al Arfaj, A. (1997). Systemic lupus erythematosus and amyloidosis. *Clin. Rheumatol.* **16**, 422–424.
141. Betsuyaku, T., Adachi, T., Haneda, H., Suzuki, J., Nishimura, M., Abe, S., Ito, T., Fujioka, Y., and Kawakami, Y. (1993). A secondary amyloidosis associated with systemic lupus erythematosus. *Intern. Med.* **32**, 391–394.
142. Allais, J. M., Cavalieri, S. J., Bierman, M. H., and Clark, R. B. (1989). Listeria monocytogenes peritonitis in a patient on continuous ambulatory peritoneal dialysis. *Nebr. Med. J.* **74**, 303–305.
143. Zizic, T. M., Classen, J. N., and Stevens, M. B. (1982). Acute abdominal complications of systemic lupus erythematosus and polyarteritis nodosa. *Am. J. Med.* **73**, 525–531.
144. Low, V. H., Robins, P. D., and Sweeney, D. J. (1995). Systemic lupus erythematosus serositis. *Australas Radiolol.* **39**, 300.
145. Houtman, P. M., and Hofstra, S. S. (1992). Lupus peritonitis presented as vague abdominal complaints in a SLE patient. *Neth. J. Med.* **40**, 232–235.
146. Wakiyama, S., Yoshimura, K., Shimada, M., and Sugimachi, K. (1996). Lupus peritonitis mimicking acute surgical abdomen in a patient with systemic lupus erythematosus: Report of a case. *Surg. Today* **26**, 715–718.
147. Hammoudeh, M., and Siam, A. R. (1995). Recurrent peritonitis with ascites as the predominant manifestation of systemic lupus erythematosus. *Clin. Rheumatol.* **14**, 352–354.
148. Schocket, A. L., Lain, D., Kohler, P. F., and Steigerwald, J. (1978). Immune complex vasculitis as a cause of ascites and pleural effusions in systemic lupus erythematosus. *J. Rheumatol.* **5**, 33–38.
149. Biran, J., McShane, D., and Ellman, M. H. (1976). Ascites as the major manifestation of systemic lupus erythematosus. *Arthritis Rheum.* **19**, 782.
150. Jones, P. E., Rawcliffe, P., White, N., and Segal, A. W. (1977). Painless ascites in systemic lupus erythematosus. *Br. Med. J.* **1**, 1513.
151. Matolo, N. M., and Albo, D, J. r. (1971). Gastrointestinal complications of collgen vascular diseases: Surgical implications. *Am. J. Surg.* **122**, 678.
152. Mushner, D. R. (1972). Systemic lupus erythematosus: A cause of medical peritonitis. *Am. J. Surg.* **124**, 368.
153. Naylor, B. (1992). Cytological aspects of pleural, peritoneal and pericardial fluids from patients with systemic lupus erythematosus. *Cytopathology* **3**, 1–8.
154. Asherson, R. A., Thompson, R. P., MacLachlan, N., Baguley, E., Hicks, P., and Hughes, G. R. (1989). Budd Chiari syndrome, visceral arterial occlusions, recurrent fetal loss and the "lupus anticoagulant" in systemic lupus erythematosus. *J. Rheumatol.* **16**, 219–224.
155. Shesol, B. F., and Rosato, F. E. (1975). Concomitant acute lupus erythematosus and primary pneumococcal peritonitis. *Am. J. Gastroenterol.* **63**, 324.
156. Lipsky, P. E., Hardin, J. A., Shour, L., *et al*. (1975). Spontaneous peritonitis and systemic lupus erythematosus: Importance of accurate diagnosis of gram positive bacterial infections. *JAMA* **232**, 929.
157. Conn, H. O. (1987). Spontaneous bacterial peritonitis: Variant syndromes. *South. Med. J.* **80**, 1343–1346.
158. Yamaguchi, M., Kumada, K., Sugiyama, H., Okamoto, E., and Ozawa, K. (1990). Hemoperitoneum due to a ruptured gastroepiploic artery aneurysm in systemic lupus erythematosus. A case report and literature review. *J. Clin. Gastroenterol.* **12**, 344–346.

159. McCollum, C. N., Sloan, M. E., Davison, A. M., and Giles, G. R. (1979). Ruptured hepatic aneurysm in systemic lupus erythematosus. *Ann. Rheum. Dis.* **38**, 396–398.
160. Leong, K. P., and Boey, M. L. (1996). Systemic lupus erythematosus (SLE) presenting as acute pancreatitis—a case report. *Singapore Med. J.* **37**, 323–324.
161. Takasaki, M., Yorimitsu, Y., Takahashi, I., Miyake, S., and Horimi, T. (1995). Systemic lupus erythematosus presenting with drug-unrelated acute pancreatitis as an initial manifestation. *Am. J. Gastroenterol.* **90**, 1172–1173.
162. Reynolds, J. C., Inman, R. D., Kimberly, R. P., Chuong, J. H., Kovacs, J. E., and Walsh, M. B. (1982). Acute pancreatitis in systemic lupus erythematosus: Report of twenty cases and a review of the literature. *Medicine* **61**, 25–32.
163. Borum, M., Steinberg, W., Steer, M., Freedman, S., and White, P. (1993). Chronic pancreatitis: A complication of systemic lupus erythematosus. *Gastroenterology* **104**, 613–615.
164. Dujoune, C. A., and Azarnoff, D. L. (1973). Clinical complications of corticosteroid therapy. *Med. Clin. North. Am.* **57**, 1331.
165. Patterson, J. F., and Wierzbinski, S. J. (1962). Digestive system manifestations of collagen disease. *Med. Clin. North Am.* **46**, 1387.
166. Nelp, W. B. (1961). Acute pancreatitis associated with systemic lupus erythematosus. *Arch. Intern. Med.* **108**, 102.
167. Sparberg, M. (1967). Recurrent acute pancreatitis associated with systemic lupus erythematosus. Report of a case. *Am. J. Dig. Dis.* **12**, 522.
168. Lazarus, S. S., and Bencosme, S. A. (1956). Development and regression of cortisone induced lesions in rabbit pancreas. *Am. J. Clin. Pathol.* **26**, 1146.
169. Stumpf, H. H., Wilens, S. L., and Somoza, C. (1956). Pancreatic lesions and peripancreatic fat necrosis in cortisone-treated rabbits. *Lab. Invest.* **5**, 224.
100. Yeh, T. S., Wang, C. R., Lee, Y. T., Chuang, C. Y., and Chen, C. Y. (1993). Acute pancreatitis related to anticardiolipin antibodies in lupus patients visiting an emergency department. *Am. J. Emerg. Med.* **11**, 230–232.
171. Wang, C. R., Hsieh, H. C., Lee, G. L., Chuang, C. Y., and Chen, C. Y. (1992). Pancreatitis related to antiphospholipid antibody syndrome in a patient with systemic lupus erythematosus. *J. Rheumatol.* **19**, 1123–1125.
172. Warshaw, A. L., and Fuller, A. F., Jr. (1975). Specificity of increased renal clearance of amylase in diagnosis of acute pancreatitis. *N. Engl. J. Med.* **292**, 325–328.
173. Swanepoel, C. R., Floyd, A., Allison, H., Learmonth, G. M., Cassidy, M. J., and Pascoe, M. D. (1983). Acute acalculous cholecystitis complicating systemic lupus erythematosus: Case report and review. *Br. Med. J. (Clin. Res. Ed.)* **286**, 251–252.
174. Suwa, A., Hama, N., Kawai, S., Ishiyama, K., Tanabe, M., Yamada, T., Goto, M., Nakajima, A., Kashiwazaki, S., and Inada, S. (1995). [A case of Sjogren's syndrome and systemic lupus erythematosus complicated with necrotizing angiitis of the gallbladder]. *Ryumachi* **35**, 904–909.
175. Rhoton, A. J., Gilliam, J. H., and Geisinger, K. R. (1993). Hemobilia in systemic lupus erythematosus. *South. Med. J.* **86**, 1049–1051.
176. Papaioannou, C. C., Hunder, G. G., and Lie, J. T. (1979). Vasculitis of the gallbladder in a 70-year-old man with giant cell (temporal) arteritis. *J. Rheumatol.* **6**, 71–76.
177. Tolaymat, A., Al Mousily, F., Haafiz, A. B., Lammert, N., and Afshari, S. (1995). Spontaneous rupture of the spleen in a patient with systemic lupus erythematosus. *J. Rheumatol.* **22**, 2344–2345.
178. Liote, F., Angle, J., Gilmore, N., and Osterland, C. K. (1995). Asplenism and systemic lupus erythematosus. *Clin. Rheumatol.* **14**, 220–223.
179. Ostensen, M., and Villiger, P. M. (2001). Nonsteroidal anti-inflammatory drugs in systemic lupus erythematosus. *Lupus* **10**, 135–139.
180. Falk, J. M., and Thomas, F. B. (1975). A cute pancreatitis due to procaineamide induced lupus erythematosus. *Ann. Intern. Med.* **83**, 832.
181. Stratton, M. A. (1985). Drug-induced systemic lupus erythematosus. *Clin. Pharm.* **4**, 657–663.
182. Veale, D. J., Ho, M., and Morley, K. D. (1995). Sulphasalazine-induced lupus in psoriatic arthritis. *Br. J. Rheumatol.* **34**, 383–384.
183. Bray, V. J., West, S. G., Schultz, K. T., Boumpas, D. T., and Rubin, R. L. (1994). Antihistone antibody profile in sulfasalazine induced lupus. *J. Rheumatol.* **21**, 2157–2158.
184. Gunnarsson, I., Pettersson, E., Lindblad, S., and Ringertz, B. (1997). Olsalazine-induced lupus syndrome. *Scand. J. Rheumatol.* **26**, 65–66.
185. Al-Hakeem, M. S., and McMillen, M. A. (1998). Evaluation of abdominal pain in systemic lupus erythematosus. *Am. J. Surg.* **176**, 291–294.

35

HEPATIC DISEASE AND SYSTEMIC LUPUS ERYTHEMATOSUS: COINCIDENCE OR CONVERGENCE

Ian R. Mackay

ABSTRACT

The chapter is introduced with the problem nomenclature of diseases for which the nature is not fully understood, well exemplified by autoimmune hepatitis and systemic lupuns erythematosus. Autoimmune hepatitis was initially called chronic active hepatitis and lupoid hepatitis, and was seen in the 1960s as being akin to systemic lupus erythematosus (SLE) by reason of shared features indicative of an autoimmune pathogenesis. Later, promulgations of Criteria Committees on both diseases defined distinctions rather clearly. Estimated coexistences in cases primarily designated as SLE would be about 1%, and in cases primarily designated as autoimmune hepatitis about 5% but with some multisystem features in a higher proportion of cases. In autoimmune hepatitis, the clinical presentation can extend from organ (liver)-specific to multisystem features, but differences from SLE are seen in hepatic histopathology and autoimmune serologic reactions. Among the latter, distinguishing markers include the much higher frequency and persistence of anti-double-stranded (ds)DNA in SLE and the occurrence of particular autoantibodies, to F-actin (SMA), microsomal cytochrome P450 species (anti-LKM (liver–kidney microsomes)), and neutrophil cytoplasm (anti-neutrophil cytoplasmic antibody (ANCA)), in autoimmune hepatitis but not in SLE. The immunogenetic (including HLA-based) predisposition to autoimmune hepatitis and SLE has shared as well as disease specific components, consistent with degrees of clinical overlap sufficient in some cases for criteria for both diseases to be fulfilled.

INTRODUCTION

Liver Disease and Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder with varying major involvement of diverse organs and tissues. Hence definition and diagnosis raise striking challenges to nosologists, clinicians, epidemiologists, geneticists, and others. This challenge of defining SLE has been met twice by committees of the American Rheumatism Association, in 1971 [1] and 1982 [2], and useful criteria for SLE have emanated. However, given that lupus is by definition "systemic," there is a nosologic dilemma when disease affects only one organ or system (kidneys, skin, synovium, or central nervous system (CNS)), but there is a positive anti-nuclear antibody (ANA) reaction and autoimmune-type histopathology. A particular example is provided by the varied neuropsychiatric syndromes of

SLE, when present in isolation [3]. The liver is another example yet, despite the usual "organ-specific" nature of autoimmune hepatitis, some otherwise typical cases show multisystemic features akin to SLE; this in fact prompted the initial designation of autoimmune hepatitis as lupoid hepatitis [4].

Additionally, there have been two expectations raised by the striking advances in autoimmune serologic diagnosis over the past 30 years [5]. One, partly realized, is that serologic specificities would correlate sufficiently well with particular clinical features to provide for accurate classification of multisystem autoimmune diseases. The other, as yet unrealized, is that serologic specificities would provide key insights into etiology and pathogenesis, by elucidation of the origin and nature of the initial provocation of the disease. It will be evident that the liver is not a usual target organ for the "lupus process." The rationale for this chapter is that there is a definable type of chronic hepatitis that has in common with SLE an autoimmune background, some shared serologic features and, occasionally, overlapping clinical expressions. We therefore seek to analyze the various points of convergence and divergence for the two diseases.

The Logic of Naming and Diagnosis of Disease and Establishing Causality

We endorse a claim that scant attention has been paid to defining disease in clinical medicine [7]. Problems of nosology must arise frequently in a volume on a disease such as SLE, for which many of the various components of etiology and pathogenesis are still unknown; thus some comment is needed on the logic of diagnosis and disease terminology. Scadding [8] in 1996 and Temple *et al.* [7] in 2001 distinguished two schools, *essentialist* (reductionist) and *nominalist*, as providing alternative approaches to the definition or description of disease. Essentialists have the expectation of an ideal form behind every affect or concept, expressed in medicine by the hankering for a unified concept of disease that can be related to an identifiable class of agents causing illness. In other words, there is an underlying pathological etiology and the disease state should be defined by the essential lesion [7]. Thus essentialists, in the case of autoimmune hepatitis as well as SLE, would seek to define these in terms of the immunologic abnormalities that are demonstrable in the laboratory or, with advances in genetics and genomics, in terms of a genetic abnormality. Unfortunately, for most of the presumed autoimmune disorders, this is still not sustainable. Nominalists on the other hand hold that the purpose of definition is simply to state the features by which the members of the class might be recognized, and designate diseases by criteria which require no presumption of any particular underlying cause, autoimmune or other. In practice autoimmune hepatitis is now specified in terms of an aggregate of features, inclusive and exclusive, that have received a consensus endorsement by the International Autoimmune Hepatitis Group (IAIHG) [9, 10]. Hence the adjectival use of "autoimmune" is, in the present state of knowledge, simply a convenient way of emphasising particular characteristics that are lacking in other types of chronic hepatitis, and is at best only presumptive of pathogenetic (autoimmune) mechanisms unique to the disease. Thus for the present, for both autoimmune hepatitis and SLE, the nominalists hold sway.

CHRONIC ACTIVE AND LUPOID HEPATITIS: EARLY DESCRIPTIONS

Semantic problems started early on [11]. Thus the term *chronic active hepatitis* (CAH) was first introduced in the mid-1940s to describe a relatively benign albeit protracted type of epidemic (presumably viral) hepatitis affecting soldiers in World War II [12]. In the 1950s the term (or a variant, active chronic hepatitis) became applied, in fact more aptly, to a new and striking syndrome particularly affecting young women. This was recognized in several centers much at the same time, and marked by a progressive and often fatal hepatitis with superadded immunologic and endocrine abnormalities [13–16]. The reported occurrence in one patient with CAH of a positive lupus erythematosus (LE) cell test [17], and an association in several cases of CAH with features seen in SLE, including positive LE cell tests, prompted the term lupoid hepatitis [4]. Thereafter, the recognition of the immunologic basis of the LE cell test and evolving ideas on the autoimmune pathogenesis of SLE, together with the detection of anticytoplasmic antibodies [18], led to autoimmunity being nominated in the pathogenesis of CAH [19].

A review of the literature from the 1960s illustrates that authors frequently observed a "spill over" of features of CAH into those of SLE and, in more recent years, there has been a shift in the clinical presentation of CAH from younger-aged women with more severe and progressive disease to older-aged women with a milder disease [20, 21]. Our description in 1956 of seven subjects with lupoid hepatitis specified various features that are included among the revised criteria for SLE: malar rash, nonerosive arthritis, serositis, renal disorder, hematologic disorder with cytopenias, and immunologic disorder [4], and other contemporary reports on CAH in young women were in keeping with this experience. These included 26 cases of which a "surprising number" had either arthralgia or actual arthritis which, together with serositis, pneumonia, or erythema multiforme,

prompting a suspicion of collagen disease [16]; nine cases with liver cirrhosis in whom a diagnosis of a "lupus erythematosus-like syndrome" could be made, with five having Sjögren's syndrome [22]; two cases "with probable DLE with cirrhosis or severe hepatitis" [23]; and one case with chronic hepatitis and a classic syndrome of SLE with pericarditis, pleurisy, and joint pains [24]. In a later study [25], there were tabulated 81 cases of "active juvenile cirrhosis" with accompanying diseases that included rashes, arthralgia, "lupus kidney," autoimmune thyroid disease, and ulcerative colitis, although the authors considered that "this form of chronic liver disease cannot be equated with systemic lupus erythematosus." Another study [26] described four cases of "progressive hyperglobulinemic hepatitis" with extrahepatic features, but again, the syndrome differed from the authors' experience with typical SLE. Two case studies in the 1960s further illustrate the uncertainties. In one [27], there were 15 patients of whom 13 were considered to have lupoid hepatitis and two SLE with a coexisting liver disease, viral hepatitis and alcoholic cirrhosis; of the former 13, the systemic features were minor and not reflective of SLE (arthralgia, 8; fever, 1; a weakly reactive VDRL test, 3). In the other [28], there were 20 patients with CAH of whom most had a positive test for ANA and prominent systemic features (arthritis or arthralgia, 16; pleurisy, 4; rashes, 5; ulcerative colitis, 3; convulsions, 2; cytopenias, 11) and a high frequency of familial stigmata of autoimmunity such that the disease expressions "were remarkably similar to those encountered in systemic lupus erythematosus."

A large case experience with CAH has accrued at the Mayo Clinic, Rochester, MN since the 1960s. Of 88 cases reported in 1975 [29], and 126 cases in 1983 [30], that were seronegative for HBsAg and with known etiologies excluded, and hence deemed suitable for corticosteroid treatment, an associated autoimmune disease was recorded in 37 of 88 in the first series, and in 21 of 126 in the second. While the frequency of a positive test for ANA was 55%, the authors rejected a relationship of the disease to SLE, and also the notion that "lupoid" features defined a subset of cases distinguishable from others; the opinion was that "the autoimmune markers associated with CAH may reflect dysglobulinemia associated with the severity of hepatocellular inflammation and necrosis" [29].

DIAGNOSTIC CRITERIA: SYSTEMIC LUPUS ERYTHEMATOSUS AND AUTOIMMUNE HEPATITIS

This section examines the possible connection between SLE and autoimmune hepatitis from the standpoint of the clinical and laboratory criteria used to define these diseases.

Criteria for Systemic Lupus Erythematosus

Systemic lupus erythematasus is a disease with a pleomorphic expression and widely varying severity. In 1971 the American Rheumatism Association (ARA) selected a set of 14 major manifestations (criteria) that included 21 items considered to characterize SLE, and recommended that SLE could be diagnosed if four or more of these criteria were present, serially or simultaneously, during a period of observation [1]. These criteria were evaluated in 1974 for early SLE by Trimble *et al.* [31]. Application of the initial criteria was claimed to give a 90% sensitivity and 98% specificity, but the criteria were criticized. In 1982 an ARA subcommittee published revised criteria in which defining terms were clarified, some items were eliminated, and recently recognised serologic markers were included [2]. The 11 criteria, in abbreviated form, included the following. 1: malar rash; 2: discoid rash; 3: photosensitivity; 4: oral ulcers; 5: nonerosive arthritis; 6: serositis; 7: renal disorder; 8: neurologic disorder; 9: hematologic disorder, either hemolytic anemia or reduced numbers of cellular elements in the blood; 10: immunologic disorder on serologic testing, including LE cells, anti-DNA, anti-Sm; and 11: ANA to an abnormal titer at any point in time. A diagnosis of SLE required four of these criteria and, when fulfilled, provided a sensitivity and specificity of 96%. Notably, liver dysfunction of any type does not appear among these criteria.

Criteria for Autoimmune Hepatitis

IASL Criteria Committee, 1976

The first definition of "generic" CAH was presented by a Criteria Committee of the International Association for Study of the Liver (IASL) in 1976 [32], but the definition reflected the prevailing uncertainty over diseases that should be included under the rubric of CAH. However, even in 1971, heterogeneity of CAH was well recognized [33], since cases with autoimmune serologic markers seemed separable from cases positive for the recently recognized marker for hepatitis B virus (HBV) infection, the HB surface antigen (HBsAg). Subsequently "chronic active hepatitis" was seen as a spectrum of diseases [34] which could include, in addition to autoimmune and viral hepatitis, hepatic expressions that occur in drug sensitivity, alpha$_1$-antitrypsin deficiency, Wilson's disease, or ethanol abuse. At that time, posttransfusion non A, non B virus infection, now attributable to hepatitis C virus (HCV), was not the major

contributor to the chronic hepatitis case load that it is today.

Nominated Attributes, 1980s

The autoimmune type of chronic hepatitis became better defined during the 1980s with the specification of the following attributes that were characteristic albeit not exclusive markers for diagnosis [35]: 1: female gender; 2: age at onset 10 to 30 years or after 50 years; 3: Eurocaucasian racial background; 4: multisystem disease expression; 5: histologic features of periportal piecemeal necrosis; 6: seronegativity for HBsAg and anti-HCV, and lack of other evident etiologies; 7: hypergammaglobulinemia during disease relapses, more than 30 g/liter; 8: autoantibodies to nuclei, smooth muscle (SMA) or liver–kidney microsomes (LKM), to "acceptable" titer (see later); 9: HLA-B8, DR3 by histocompatability typing; 10: responsiveness to treatment with corticosteroid drugs; and 11: sparing from hepatocellular carcinoma that tends to supervene on most other types of chronic hepatitis or cirrhosis.

International Autoimmune Hepatitis Group (IAIHG): Brighton Report, 1992

The International Autoimmune Hepatitis Group [9] was convened in 1992 in Brighton, United Kingdom, to "review all the features of autoimmune hepatitis (AIH) and determine whether a consensus could be reached on criteria" The ensuing Brighton Report presented diagnostic criteria for definite and probable autoimmune hepatitis, based on features generally similar to those cited in other reports, but with the innovation that the inclusive or exclusive criteria, clinical, biochemical, histologic and serologic, could be given positive or negative scores or weightings according to their estimated contribution to the diagnosis. The prototype of this scoring system was developed from retrospective analysis of 145 patients attending one center with classic features of autoimmune hepatitis, and 250 with other liver disorders. The system was then modified through three subsequent revisions based on panellists' experience with their own case material.

IASL Criteria Committee, 1994

A Criteria Committee of the IASL [36] revised their 1976 Manual in 1994 in which autoimmune chronic hepatitis was distinguished from other types of chronic hepatitis according to "the presence of significant titers of circulating tissue antibodies and the absence of other causes of chronic hepatitis," namely viruses, drugs, toxins, and metabolic abnormalities. The required titer for antibodies to nuclei, and/or to smooth muscle with specificity for actin, was 40 or greater, but laboratory conditions including test substrates and appropriate standards for performance of tests were not nominated. This committee also presented indicative rather than diagnostic histologic criteria, including plasmacytosis, bridging necrosis, and pseudo-glandular rosettes of liver cells (see later). A quick response of the disease to low-dose corticosteroid treatment was accorded diagnostic significance.

World Congress of Gastroenterology (WCG) Working Party, 1994

An international working party [37] was organized and funded by the WCG of 1994 to report on the Terminology of Chronic Hepatitis. The term autoimmune hepatitis was recommended in preference to earlier synonyms including chronic active hepatitis, lupoid hepatitis, and plasma cell hepatitis. The concise definition of autoimmune hepatitis used by this group was "unresolving predominantly periportal hepatitis, usually with hypergammaglobulinemia and tissue autoantibodies, which is responsive to immunosuppressive therapy in most cases." The laboratory descriptors included a five-fold selective increase in serum immunoglobulin (Ig)G concentration, and titers of autoantibodies, either ANA, SMA, or anti-LKM, of 1:40 on tissues, or 1:160 for ANA on the HEp2 cell substrate. Almost all patients (Eurocaucasoid) would carry the HLA class II alleles DR3 or DR4. Patients with markers of virus infections should not be considered to have autoimmune hepatitis. An indicative significance was accorded to biopsy features of bridging necrosis, plasmacytosis, and formation of liver cell rosettes.

International Autoimmune Hepatitis Group, Revised Criteria, 1999

The IAIHG Brighton Report of 1992 (see International Autoimmune Hepatitis Group (IAIHG): Brighton Report, 1992, see earlier) has been assessed and reevaluated in several studies in recent years, with modifications to accommodate newer diagnostic methods, histopathologic appearances on liver biopsy, and ascertained imprecisions in the original scoring system. The revised criteria and scoring system [10], with scores for "definite" and "probable" autoimmune hepatitis provide a sensitivity for diagnosis of 97–100%. However, specificity is much lower, in the range of 45–92%, by reason of liver diseases other than autoimmune hepatitis, fulfilling criteria for a "probable" diagnosis. This shortfall should be overcome in the

future by improved and more stringent application of autoimmune serologic testing.

AUTOIMMUNE HEPATITIS: CONTEMPORARY CLINICAL APPRAISAL

Description of the Disease

Autoimmune hepatitis can present through a spectrum from asymptomatic disease recognized only by biochemical abnormalities of liver function to an acute hepatitis in some 25% of cases [38], and is progressively cirrhotogenic [39–41]. It is predominantly a disease of women (F : M, 8 : 1), and the age incidence is bimodal with peaks in early adult life and after 40. The disease usually has a long asymptomatic preclinical course which is a feature of many other autoimmune disorders, and may be disclosed by extrahepatic expressions including amenorrhoea in women, or by incidentally detected abnormalities in biochemical tests of liver function. Symptomatic cases have evidence of hepatocellular dysfunction, with symptoms of nausea, anorexia, and jaundice, and signs that include hepatosplenomegaly as well as features of multisystem immune disease. The subset referred to as type 2 autoimmune hepatitis (see later) is often expressed as severe hepatitis in early childhood. The biochemical features on serum analysis are high levels of transminase enzymes and modest elevations in levels of alkaline phosphatase and gamma glutamyl transpeptidase. A highly elevated level up to 100 g/liter of IgG of polyclonal type is seen resulting in a marked overall increase in serum globulins, with "marker status" accorded to a level above 30 g/liter; this greatly exceeds the minimal to modest increase in SLE. Other autoimmune diseases in which there is pronounced hypergammaglobulinemia are those that are localized to parenchymal tissues, primary Sjögren's syndrome and thyroiditis. Treatment with prednisolone induces such characteristic improvement in all clinical and biochemical indices and in histologic changes that this has diagnostic utility. As mentioned earlier, the diseases identified as CAH and lupoid hepatitis in the 1950–1960s that predominantly affected young women now appear less prevalent than the more indolent versions that affect middle-aged to elderly females [20, 21]. This has connotations for therapy which was based mainly on studies of the prototype disease of earlier years.

Extrahepatic Manifestations of Autoimmune Hepatitis

The earlier descriptions of clinical features of autoimmune hepatitis (see earlier) included features simulating those seen in SLE, facial rash, arthralgia, hemolytic anemia, thrombocytopenic purpura, and mild nephropathy. In a study of 108 older subjects with CAH [42] there were prominent frequencies (26–35%) of features of Sjögren's syndrome, renal tubular acidosis, or fibrosing alveolitis as well arthropathy, rashes, and thyroid disease. Coexistence with ulcerative colitis was cited in many of the earlier reports [19, 28, 39, 42]. However, while there is clear overlap between autoimmune hepatitis and other autoimmune disorders, in terms of clinical expression or morphological features such as lymphoid infiltrations, the clinical terrain of autoimmune hepatitis and SLE differs in several respects. These include the usual absence in autoimmune hepatitis of the renal lesions characteristic of lupus nephritis and cerebral involvement, and the presence of coexistences that are infrequent accompaniments of SLE, notably ulcerative colitis. In particular, the type 2 variant of autoimmune hepatitis has no co-associations with SLE.

Subtypes of Autoimmune Hepatitis

Serologic reactants are a cornerstone of diagnosis of autoimmune hepatitis. In addition to the earlier classic markers of CAH, ANA, and SMA (see later), there was described in 1973 a novel reactant enriched in microsomes of liver and kidney (LKM) [43]. Subsequent reports of anti-LKM-associated CAH suggested a predominance of cases in children presenting with a particularly severe type of hepatitis [44, 45]. In 1987, Homberg and colleagues [46] formalized the description of CAH with anti-LKM and considered that this was a sufficiently distinct variant to be identified as type 2. The utility of separating types 1 and 2 is debatable [9]. The rationale for this (Table 1) is based on the childhood predominance in type 2, disease associations that are more "multisystem" in type 1 and "organ-specific" in type 2, the greater and so far unexplained geographical prevalences for type 2 in middle and southern European countries vs those for type 1 the United States [47], Australia [48], Sweden [49], Japan [50], and the United Kingdom except among children [51], and the polarized serologic reactivities. Suggestions for subclassification of autoimmune hepatitis into yet further types based on other serologic reactivities have not been endorsed.

Autoimmune Overlap Syndromes Involving the Liver

It is well known that multiple autoimmune diseases may aggregate in one patient or related family members; in fact this clustering is a defining marker for

TABLE 1 Revised Scoring System for Diagnosis of Autoimmune Hepatitis [10]

Parameters/features[a]	Score	Notes[b]	Parameters/features[a]	Score	Notes[b]
Female sex	+2		Liver histology		
ALP : AST (or ALT) ratio			Interface hepatitis	+3	
<1.5	+2	1	Predominantly lymphoplasmacytic infiltrate	+1	
1.5–3.0	0		Roasting of liver cells	+1	
>3.0	–2		None of the above	–5	
Serum globulins *or* IgG above normal			Biliary changes	–3	5
>2.0	+3		Other changes	–3	6
1.5–2.0	+2		Other autoimmune disease(s)	+2	7
1.0–1.5	+1		Optional additional parameters:		8
<1.0	0		Seropositivity for other *defined* autoantibodies	+2	9
ANA, SMA, or LKM-1			HLA DR3 or DR4	+1	10
>1 : 80	+3	2	Response to therapy		
1 : 80	+2		Completes	+2	11
1 : 40	+1		Relapse	+3	
<1 : 40	0		Interpretation of aggregate scores		
AMA positive	–4		Pretreatment:		
Hepatitis viral markers			Definite AIH	>15	
Positive	–3	3	Probable AIH	10–15	
Negative	+3		Postreatment:		
Drug history			Definite AIH	>17	12
Positive	–4	4	Probable AIH	12–17	
Negative	+				
Average alcohol intake					
<25 g/day	+2				
>60 g/day	–2				

[a] ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ANA, antinuclear antibodies; SMA, smooth muscle antibodies; LKM-1, type 1 liver–kidney microsomal antibodies.

[b] Explanatory notes: 1, The ALP : AST (or ALT) ratio relates to the degree of *elevation above upper normal limits* (unl) of these enzymes, i.e. = (IU/I ALP ÷ unl ALP) ÷ (IU/I AST ÷ unl AST); 2, Titers determined by indirect immunofluorescence on rodent tissues or, for ANA, on HEp-2 cells. Lower titers (especially of LKM-1) are significant in children and should be scored at least +1; 3, Score for markers of hepatitis A, B, and C viruses (i.e., positive/negative for IgM anti-HAV, HAsAg, IgM anti-HBc, anti-HCV, and HCV-RNA). If a viral etiology is suspected despite seronegativity for these markers, tests for other potentially hepatotropic viruses such as CMV and EBV may be relevant; 4, History of recent or current use of known or suspected hepatotoxic drugs; 5, "Biliary changes" refers to bile duct changes typical of PBC or PSC (i.e., granulomatous cholangitis, or severe concentric periductal fibrosis, with ductopenia, established in an *adequate* biopsy specimen) and/or a substantial periportal ductular reaction (so-called marginal bile duct proliferation with a cholangiolitis) with copper/copper-associated protein accumulation; 6, Any other *prominent* feature or combination of features suggestive of a different etiology; 7, Score for history of any other autoimmune disorder(s) in patient or first-degree relatives; 8, The additional points for other defined autoantibodies and HLA DR3 or DR4 (if results for these parameters are available) should be allocated *only* in patients who are seronegative for ANA, SMA and LKM-1; 9, Other "defined" autoantibodies are those for which there are published data relating to methodology of detection and relevance to AIH. These include pANCA, anti-LC1, anti-SLA, anti-ASGPR, anti-LP and anti-sulfatide (see text); 10, HLA DR3 and DR4 are mainly of relevance to Northern European caucasoid and Japanese populations. One point may be allocated for other HLA Class II antigens for which there is published evidence of their association with AIH in other populations; 11, Assessment of response to therapy may be made at any time. Points should be added to those accrued for features *at initial presentation*.

an autoimmune basis for a given disease, and SLE is a particular example. A distinction can be made between "coexistence" when two distinct disease entities (albeit both autoimmune in nature) occur together in the one individual, and "overlap" when there is coexpression of two usually distinct entities within a single organ, for example, the liver, such that it is difficult to establish a primary diagnosis of one or the other.

Autoimmune Hepatitis–Systemic Lupus Erythematosus

The overlap between features of chronic active hepatitis and SLE was the initial prompt for the diagnosis of lupoid hepatitis (see Chronic Active and Lupoid Hepatitis: Early Descriptions). However, as experience with autoimmune hepatitis has accrued, the general

viewpoint would be this is a disease with characteristics independent of those of SLE and that when features of both are expressed in the one individual, the use of coexistence rather than overlap is preferable.

Autoimmune Hepatitis–Primary Biliary Cirrhosis

This overlap between two autoimmune diseases of the liver that are usually quite distinct histologically and serologically, autoimmune hepatitis and primary biliary cirrhosis (PBC), has received considerable attention in the more recent hepatological literature [52, 53]. Overlap specifies the coexistence in one individual of features specific for either disease, including clinical presentation, biochemical abnormalities of liver function, histologic lesions, and marker autoantibodies; the disease expression involves both periportal hepatocytes as in autoimmune hepatitis, and small intrahepatic bile ducts, as in PBC.

According to publications, the perception of overlap depends on the perspective of the observer. Thus, among 162 cases with the primary diagnosis as autoimmune hepatitis [54], there was PBC overlap in 8 (5%) whereas among 130 cases with a primary diagnoses of PBC [55], there was an autoimmune hepatitis overlap in 12 (9%); thus a "mid figure" for the frequency of overlap could be about 7%. However, the "basic" disease among such overlap cases may well be PBC according to observations by Lohse *et al.* [56], since their cases were intrinsic PBC on an immunogenetic background (permissive HLA alleles, B8 and DR3) of autoimmune hepatitis.

An aspect of interest is the high frequency, up to 50%, of antinuclear serologic reactions in PBC of particular specificities, anti-centromere, anti-Sp100 which is directed against a transcriptional regulatory protein that aggregates in nuclear dots, and anti-gp210 and anti-p62 that are components of the nuclear pore complex [57]. Anti-dsDNA and anti-chromatin are not expressed and hence the rarity of coexisting PBC and SLE is not surprising. We can, however, note the presence in PBC of anti-centromere reactivity which is often accompanied by the clinical features of the CREST variant of systemic sclerosis, which itself coexists in about 10% of cases of PBC [58]. This coexistence of disease and serologic reactivity is provocative, since mitochondrial autoantigens of PBC and the centromeric autoantigens of CREST exist in separate organelles of the cell, and there is no demonstrable cross-reactivity between them [59].

Autoimmune (Immuno) Cholangitis (AIC)

As originally described [60], AIC is a syndrome expressed clinically by a chronic cholestatic liver disease and histologically by periductular lymphoid aggregates in the liver, in these respects serologically simulating PBC, but tests for the antimitochondrial antibody are negative, whereas tests for ANA are positive. At one stage, AIC was aligned with autoimmune hepatitis but the ANA specificities are inconsistent with this. Since, opinion has veered toward the idea that AIC is a serologic variant of PBC [61]. Notably the specificity of the ANA reactions is identical with that of those seen in PBC, namely, anti-centromere (ACA), anti-Sp100, and antibodies to the nuclear pore complex [62]. The viewpoint of Czaja *et al.* [63], was that AIC cannot be assimilated into any single diagnostic category, but instead represented either a variant form of diverse conditions, a transition stage of another disease such as PBC, or a single entity with varying manifestations; it was recommended that its status be "protected" pending further knowledge.

THE LIVER IN SYSTEMIC LUPUS ERYTHEMATOSUS: CLINICAL ANALYSIS

The liver is not spared in SLE, as judged by the exemplary 570 cases reported by Pestina *et al*. [64]. There is hepatomegaly in 50% of cases attributed mainly to fatty liver or congestion, and an increase in aminotransferase levels or other biochemical functional abnormalities in 30% to 60% of cases, reflecting nonspecific reactive hepatitis, a term used to describe mild hepatic abnormalities accompanying inflammatory lesions elsewhere in the body [65]. Some case studies have been particularly directed to the question whether the "lupus process" can involve the liver in the same way as other vulnerable tissues. The study by Runyon *et al.* [66] comprised 238 cases in which the diagnosis of SLE was based on fulfilment of four or more of the initial ARA criteria; liver function tests were performed in 206. There was at least one abnormal liver function test result in 124 cases, and the authors' criteria for "liver disease" were met in 43 (21%), among which disease was drug-related in 7. Liver biopsy findings in 33 of the cases are described later. In another study on 81 cases of SLE, hepatomegaly was present in 42% and biochemical abnormalities of liver function in 44%, with 14 of the latter cases being drug-related [67]. In a third study on 264 cases of SLE surveyed prospectively, in which clinical and laboratory indices were derived over a 12-month period of observation, there was evidence of liver disease in 67 (25%), but this was drug-related in 29 and attributed to causes unrelated to SLE, including alcohol abuse in 13 [68].

There is an infrequent liver syndrome that has become recognized as a "lupus variant," this being an

SLE-like disease with antibodies against phospholipids (anti-phospholipid syndrome) associated with vascular occlusive disease of the liver and/or nodular regenerative hyperplasia [69–72]. The presence of the lupus anticoagulant, whether occurring in the primary anti-phospholipid syndrome, or that accompanying SLE, has been observed in several instances with hepatic venous thrombosis and Budd-Chiari syndrome [69], or with hepatic arterial occlusion. Notably, the presence of anti-phospholipid (anti-cardiolipin/lupus anticoagulant) is not a reported feature of autoimmune hepatitis [70]. Moreover the lupus anticoagulant has been associated with liver infarction due to hepatic arterial occlusion, without ensuing portal hypertension [71]. Hepatic veno-occlusive disease, as seen in cases of SLE, or subjects with anti-phospholipid antibody, is related to the Budd-Chiari syndrome but the occlusive lesion affects central or sublobular veins rather that larger hepatic veins. Nodular regenerative hyperplasia of the liver is now an acknowledged hepatic complication of SLE, when the latter is accompanied by anti-phospholipid antibody [65, 67]. A literature survey by Sekiya *et al.* [72] covered 46 patients with nodular regenerative hyperplasia of the liver with accompanying rheumatic disorders that included SLE in 15, Felty syndrome in 12, rheumatoid arthritis in 5, systemic sclerosis in 2, Sjögren's syndrome in 1, and primary anti-phospholipid syndrome in 2. On the other hand, the overall frequency of nodular regenerative hyperplasia among Japanese cases of SLE was quite low, 0.3% [72].

AUTOIMMUNE HEPATITIS: THE VISION OF THE HISTOPATHOLOGIST

Histopathology: The Deviation of Hepatitis and Lupus

Histopathology is the point at which autoimmune hepatitis moves closer to the realm of hepatology and organ-specific autoimmunity, and away from the multisystem autoimmune diseases. As indicated earlier (Chronic Active and Lupoid Hepatitis: Early Descriptions), the earlier clinical and histologic descriptions of "chronic active hepatitis" were virtually generic for all types of chronic hepatitis and only in the 1970s was it realized that quite different liver diseases were encompassed within the ICD9 rubric 570.71, chronic active hepatitis. The availability of liver biopsy samples led to numerous descriptions of the histopathology of chronic active hepatitis, and these became formalized by reports from an International Group of Pathologists, 1969 and 1977 [73–74]; their recommendations provide the basis for current morphological criteria.

The Histopathology of Autoimmune Hepatitis

The earliest descriptions of CAH emphasized the inflammatory cellular infiltrates in portal tracts of the liver and within lobules, with plasma cells particularly prominent [16, 75, 76], and with fibrosis, nodule formation, and cirrhosis as sequential lesions (Fig. 1). An early lesion to be recorded was bridging necrosis, denoting the confluence of areas of hepatocellular necrosis between adjacent portal tracts, considered to be a precursor to nodule formation and cirrhosis [77]. The most characteristic feature was that first described as "piecemeal necrosis" [76], and now as interface hepatitis, representing destruction of liver cells at an interface between the parenchyma and connective tissue in portal tracts, with accompanying dense lymphoid infiltrations including lymphocytes and plasma cells. Interface hepatitis is evident at the edge of portal tracts and septa from where lymphoid cells invade the limiting plate of the hepatic lobule, and where hepatocellular injury is most intense, shown by hydropic swelling or apoptosis of hepatocytes. Lymphocytes can be seen in close contact with hepatocytes—peripolesis—and within them—emperipolesis, as effectors of hepatocellular destruction. Other morphological features include intralobular aggregates of lymphocytes contiguous with damaged hepatocytes (lobular hepatitis) and formation by hepatocytes of pseudo-glandular structures (rosettes). Hepatocellular damage in autoimmune hepatitis is typically expressed as apoptosis, seen as shrunken acidophilic remnants of hepatocytes equivalent to the Councilman or acidophilic bodies long familiar to hepatic histopathologists [78], and now seen as indicative of immunologically mediated cellular damage.

There are particular aspects to the histopathology of autoimmune hepatitis. *First*, many of the changes described above are seen in all types of chronic hepatitis, whether attributable to virus infection, autoimmunity, or allergic drug reactions, and are generic to immune-mediated damage to hepatocytes. This type of tissue parenchymal lesion is not a noted feature of the pathology of SLE (*vide infra*). *Second*, histopathologists in their earlier descriptions distinguished lesions with interface hepatitis, chronic aggressive (active) hepatitis, from lesions marked only by hepatic portal infiltrates without interface hepatitis, chronic persistent hepatitis, in which progression toward cirrhosis was deemed unlikely [73, 74], but there is now less emphasis on this distinction [9]. *Third*, morphological appearances as described above do not inform on the immune mechanisms of hepatocellular injury, whether cytolytic T cells, cytokines, or antibody-dependent cellular cytotoxicity

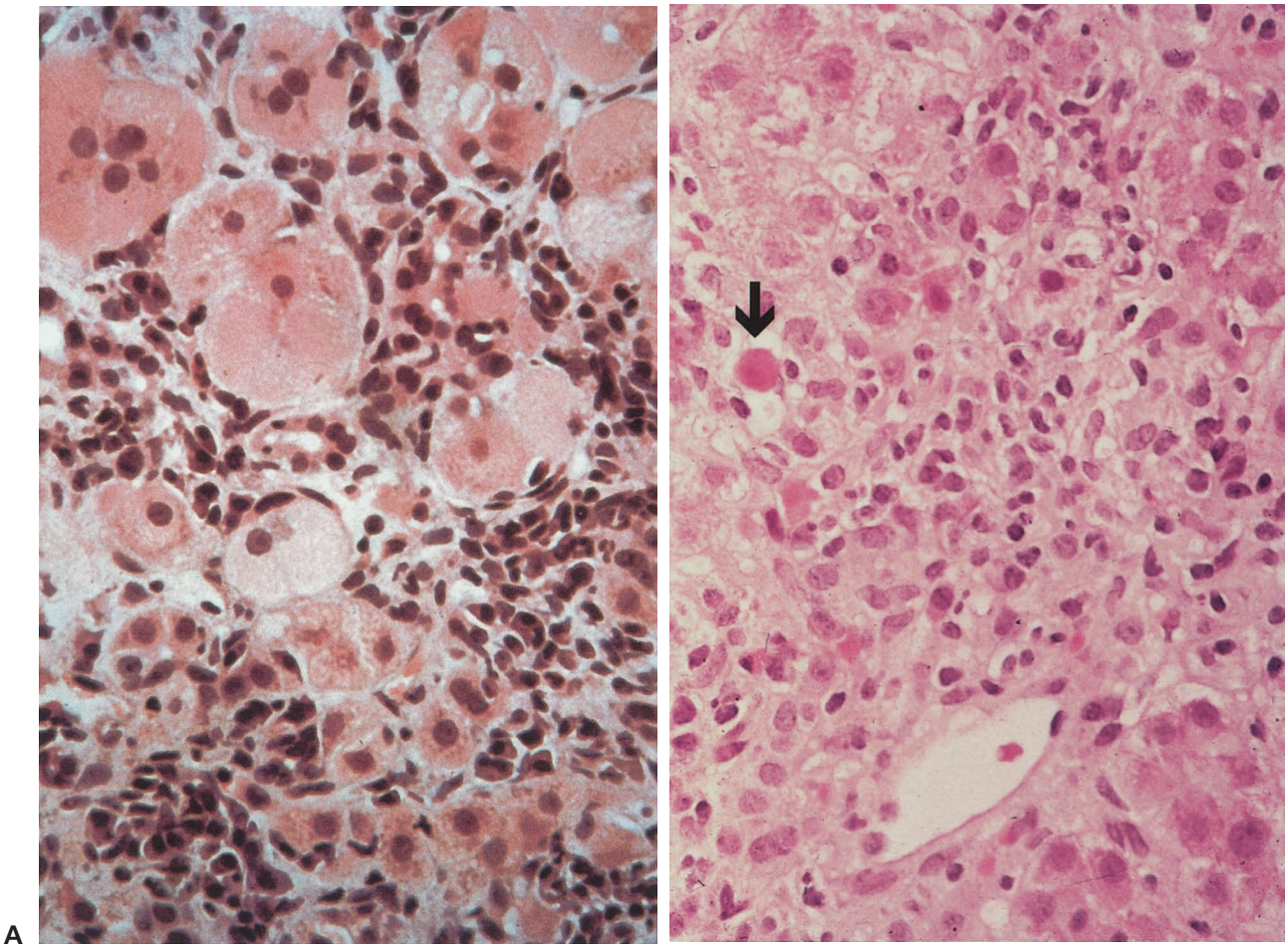


FIGURE 1 Histologic appearances in liver biopsy samples from autoimmune hepatitis. Intralobular lesions illustrating (**A**) cytolysis with ballooned hepatocytes with contiguous infiltrating lymphoid cells (left), and (**B**) apoptotic body, among damaged hepatocytes, arrowed (right) (hematoxylin eosin, ×550 and ×400, respectively). (Fig. 1A, kindly provided by Dr. Nigel Swanston.)

(ADCC). Plasmacytosis is a feature of autoimmune hepatitis that is widely recognized (although not by all [79]); intrahepatic plasmacytosis and local antibody formation would facilitate the occurrence of ADCC, but demonstration of a membrane-located autoantigen is needed to uphold ADCC as a basis for hepatocellular injury.

Histopathology of the Liver in SLE

The relevant studies are those in which there was an established diagnosis of SLE and histologic data on the liver were available. A report by the author in 1959 [80] included 19 cases with the following findings: normal liver or entirely nonspecific lesions including venous congestion, 6; portal fibrosis and/or minor lymphoid infiltrates interpreted as nonspecific reactive hepatitis, 11; inactive cirrhosis, 1; and hepatitis 1 with the diagnosis revised to chronic active hepatitis. In the study by Runyon *et al.* [66] on 238 cases a histologic diagnosis was available in 33 and included cirrhosis in 4, chronic hepatitis in 4, and miscellaneous entities in 25. They separated SLE and CAH by concluding that "patients who satisfy the ARA criteria for SLE and have clinical and histologic evidence of chronic active hepatitis as well should be considered as having both diseases." The study in 1981 by Gibson and Myers [67] included 81 cases with biopsies obtained in 7, showing a portal inflammatory infiltrate in 5 and CAH in 1. Finally the report of Miller *et al.* [68] in 1984 included liver biopsy data in 14 of 264 cases, with none showing any serious or unusual lesion. The rare association of nodular regeneration hyperplasia of the liver with SLE is discussed earlier. The upshot is that histologic examination of the liver in the "standard" case of SLE will show either normal findings, lesions attributable to drug toxicity, or

nonspecific changes, and that no lupus-specific pathology will be evident. Some cases that fulfill clinical and histologic criteria for autoimmune hepatitis may have sufficient multisystem features and serologic characteristics to justify a diagnosis of SLE. However, such cases appear to be minor "contaminants" in collected series of cases of SLE.

AUTOIMMUNE HEPATITIS: THE VISION OF THE SEROLOGIST

Refinements in serologic testing in autoimmune diseases have led to the reasonable expectation that profiles of autoantibody reactions would align with diagnostic specificity and even signify the pathogenetic origins of syndromes or diseases [6]. The recognition of positivity for anti-nuclear antibodies in chronic hepatitis initially suggested a pathogenetic relationship between autoimmune hepatitis and SLE, but the serologic profiles for these diseases were found to show clear distinctions, particularly for autoimmune hepatitis the much lower frequency of autoantibodies to dsDNA and RNPs and singular reactivities such as reactivity to F-actin in type 1 disease and to cytochrome P450 (LKM antigen) in type 2 disease. In this section, the serologic reactions in type 1 and type 2 autoimmune hepatitis will be considered separately, since these are nonoverlapping, notwithstanding the fact that the histologic evolution and outcome, and perhaps even the basic pathogenesis, are similar for the two types. In particular, there are no lupoid features in type 2 AIH, and serologic expressions tend to align more with the organ-specific than the multisystem autoimmune diseases (see later).

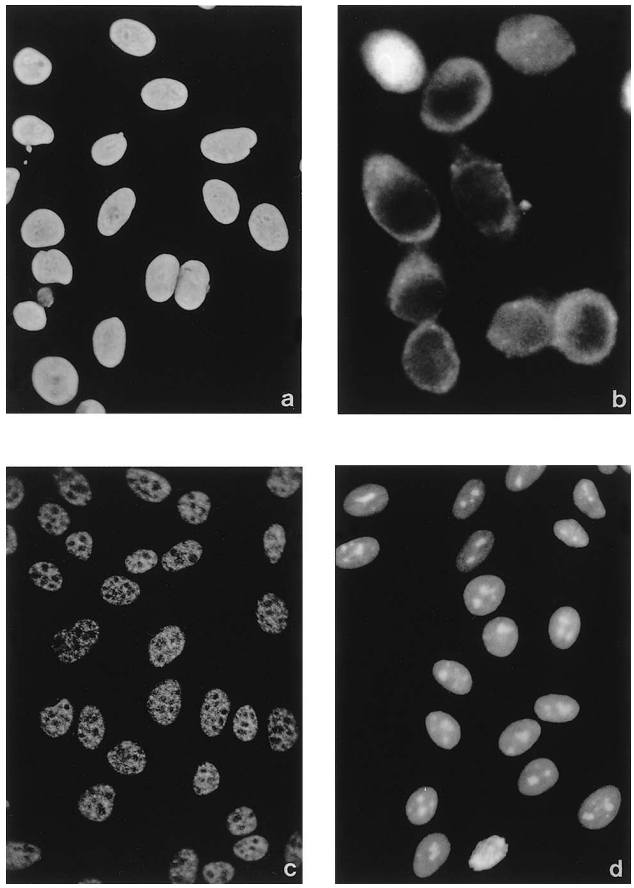


FIGURE 2 Antinuclear staining patterns by immunofluorescence displayed by autoimmune hepatitis sera. (**A**) diffuse homogeneous (anti-histone); (**B**) peripheral (anti-nuclear membrane); (**C**) speckled (anti-RNP); (**D**) anti-nucleolar and diffuse staining. (Fig. 2 kindly provided by Dr. Josefa Wesierska-Gadek)

Serologic Reactions in Type 1 Autoimmune Hepatitis

Anti-nuclear Autoantibodies

Entire Nucleus—Immunofluorescence ANA

Positive results for ANA in autoimmune hepatitis were initially recognized by the LE cell test and later by immunofluorescence on tissue substrates (Fig. 2). Specific patterns of immunofluorescence reactivity on nuclei were first described in 1961, [81] and these became better defined with the use of cultured cell substrates (HEp-2 cells), suggesting that a variety of nuclear antigens may contribute to ANA reactivity in autoimmune hepatitis. A representative frequency of a positive ANA test in autoimmune hepatitis is about 70% [19, 82]. A homogeneous pattern of staining appears more frequently in active disease but, overall, the frequencies of homogeneous (34%) and speckled patterns (38%) may be equivalent [82], reflecting multiple nuclear reactants. However, better data based on standardized techniques under international workshop conditions, as has pertained for diabetes-related autoantibodies [83], would be helpful. An informative multicenter study was conducted under the aegis of the IUIS Standardization committee in which frequencies were cited for ANA positivity on HEp-2 cells according to serum dilution [84]; the respective frequencies for normal subjects at 1:40, 1:80, 1:160, and 1:320 were 32, 13, 5, and 3, which clearly illustrates the sensitivity–specificity "trade-off." Interpretations on the frequency of ANA in autoimmune hepatitis should be made in the light of this study, together with the recognition that the level of ANA reactivity will depend on disease activity, and that any given serum can have multiple ANA specificities at different levels. Particular specificities of ANA

in autoimmune hepatitis do not appear to correlate with particular clinical features.

Nucleosome—Chromatin and Histones

The nucleosome comprises an octamer of histones enwrapped by two coils of DNA (146 base pairs). Histones are small conserved nuclear proteins that bind to DNA in chromatin, and exist as five main classes, H1, H2A, H2B, H3, and H4, which have particular structural features [85]. The octamer is constituted by two H2A–H2B dimers and an H3–H4 tetramer stabilized by noncovalent bonds, and completing the structure is an H1 linker molecule that connects separate nucleosomes; a linear array of nucleosomes forms a primary chromatin fiber of 100 Å diameter. The chromatin fiber supercoils into increasingly larger fibers based on histone-dependent internucleosomal interactions. The condensation of chromatin into a quaternary protein structure is believed to constitute an epitope, if not *the* epitope, for both autoimmune T and B cells that generates anti-nuclear antibodies, whether in SLE or autoimmune hepatitis [85]. Other nuclear structures including individual histones may then be drawn in due to intermolecular epitope spreading. A quaternary protein structure as a primary autoimmune B-cell epitope is exemplified by various other nuclear autoantigens including Ku, U1-RNP, and the proliferating cell nuclear antigen (PCNA), and perhaps also by other autoantigens such as the $\beta_2$glycoprotein–phospholipid complex of the anti-phospholipid syndrome [86].

Antibodies to histones occur typically in spontaneous and drug-induced SLE, with all subtypes recognized as autoantigens in differing configurations. It has been assumed that antihistone is the predominant antinuclear reactivity in autoimmune hepatitis, although a frequency by ELISA of only 25% of 20 sera has been cited [87], and in another study by ELISA on 65 cases previously screened by immunofluorescence for a range of ANA reactivities, the frequency was 23 of 65 (35%) [88]; 11 of these 23 sera were reactive also with other nuclear autoantigens and 12 only with histones.

dsDNA

Anti-double-stranded (ds) DNA is the hallmark reactivity in SLE and is seldom lacking in active disease. The numerous studies to ascertain the frequency of anti-dsDNA in autoimmune hepatitis have given divergent results attributable to technical aspects of the assays used. Studies in the 1970s using liquid or solid phase immunoassays and commercial sources of DNA reactant indicated a high frequency of positivity but similarly positive results were obtained in other types of chronic liver disease indicating low specificity [89, 90], likely attributable to contaminant single-stranded (ss)DNA to which there is nonspecific reactivity in various chronic inflammatory disorders. Smeenk *et al.* [91] in 1982 used a more specific immunoassay for anti-dsDNA based on precipitation of immune complexes by polyethylene glycol, and obtained a positivity rate of 10%. Gurian *et al.* [92] in 1985 reported on assays (in 8 cases of lupoid hepatitis) based on Farr-type immunoprecipitation, a nitrocellulose membrane filtration method, and *Crithidia luciliae* immunofluorescence; there were positive results by the Farr-type assay but negative results in all cases by the other more specific assays. Leggett *et al.* [93] in 1987 studied 25 cases by immunofluorescence with *Crithidia luciliae* and a reliable immunoprecipitation assay for anti-dsDNA with 4 patients giving positive tests, 2 transiently and 2 persistently. The report of Wood *et al.* [94] in 1986 on 91 cases of CAH using an ELISA of claimed high sensitivity and specificity for anti-dsDNA, according to calibration against *Crithidia luciliae* immunofluorescence, cited a positivity rate of over 50%, and with no significant differences in positivity according to either ANA positivity or other disease characteristics; since this is not in line with other studies, the ELISA may have been detecting low affinity anti-dsDNA.

A conspectus of the various studies on anti-dsDNA in autoimmune hepatitis prompts comment on assay format and disease activity. As mentioned earlier, rigorous standardization of most autoimmune serologic tests is still an unmet need, and minimal requirements include availability of standard reference antisera and serum-exchange to derive an optimal assay format, and the convening of international workshops for uniform results between laboratories. A study underscored the tendency of ELISAs to show greater variability than other assay formats [95]. In terms of the disease itself, good case definition is important and, whatever the reactivity is that is being tested for, the general experience is that the stage of disease has a marked influence on the degree of positivity, this being much greater in active than in inactive disease. In regard to anti-dsDNA, a reasonable consensus is that this frequency is low (about 10% of cases) and positivity is transient so that, in this respect, autoimmune hepatitis differs serologically from SLE.

Centromere Proteins

The anti-centromeric pattern of ANA by immunofluorescence represents reactivity to centromere proteins (Cenp) A, B, and C. These reactivities have been defined by immunoblotting, and ELISA using recombinant centromere proteins, and are characteristically associated with the CREST variant of scleroderma [95]. Autoantibodies to centromere (ACA) were identified by immunofluorescence (ACA) and/or ELISA in as

many as 42% of 65 cases of AIH [88]. However, in our experience (I. R. Mackay, unpublished) and that of others (J. Wesierska-Gadek and E. Penner, personal communication), the frequency and serum titer of ACA in autoimmune hepatitis are low and, notably, recorded coexistences of CREST and autoimmune hepatitis are scarce, in contrast to what pertains for PBC [58, 59].

Ribonucleoproteins

Ribonucleoproteins (RNP) include a family of proteins present in the nucleus and cytoplasm that are complexed with, and influence the processing of different low molecular weight ribonucleic acids. The various RNP antigens that are reactants for ANAs present in SLE and Sjögren's syndrome are recognized by a characteristic speckled pattern by immunofluorescence, and reactivities with specific epitopes have been studied using recombinant proteins. In SLE and related multisystem autoimmune diseases, antibodies to various RNP antigens tend to align with a particular clinical syndrome or disease subset [6].

In general, autoantibodies to the well-defined RNP molecules are reported at moderate frequency in autoimmune hepatitis sera [96–98]. In one study on 30 cases, there was reactivity to the 70-kDa U1-RNP in 8, and to the Ro/La (SS-A/SS-B) antigens in 3 (97), and in another report on cases from North America and Asia, 95 and 47, respectively, the frequencies for the North American cases were 43% for snRNPs and 38% for Ro/La, and frequencies for the Asian cases were generally higher [98]. One reactivity described was to an unidentified 52-kDa RNP, in 23% of 65 cases of AIH [82], but there has been no follow-up on this.

Nucleoli

Anti-nucleolar antibodies (ANoA) occur in SLE and related multisystem diseases, and particularly in primary systemic sclerosis (PSS). ANoA were identified by immunofluorescence on monolayers of human fibroblasts in autoimmune hepatitis, as well as in SLE and PSS (99). Major reactants for ANoA are fibrillarin [6] and a 113-kDa molecule with three functional domains characterized as poly (ADP-ribosyl) transferase (pADPRT) [86]. Anti-pADRPT were detected in 7 of 50 cases of autoimmune hepatitis, but reactive sera differed from those in other rheumatic diseases in not inhibiting the catalytic activity of the enzyme, indicating differences in the nature of the autoepitope(s) [86].

Nuclear Envelope

The nuclear envelope is a complex porous assembly of proteins that separates the nucleus from the cytoplasm. The three structural components are (a) the inner and outer nuclear membranes, (b) the nuclear pore complex that serves as a conduit for proteins between nucleus and cytoplasm, and (c) the nuclear lamina that provides part of the architectural framework of the nucleus [86]. Each of these components has autoantigenic reactivity for sera in various of the multisystem rheumatic diseases and in autoimmune liver diseases. Autoantibodies to the lamina occur more particularly in autoimmune hepatitis [86], and to the nuclear membrane and pore complex more particularly in PBC [57]. The nuclear lamina, depending on the cell type and differentiation state, contains 1–6 immunologically related polypeptides, the lamins. The three major members are lamins A, B, C present in equimolar amounts, and three minor members are B2, D, and E. The lamin polypeptides have similar molecular masses of 60–75 kDa, but are readily separated by two-dimensional gel electrophoresis, so allowing for identification of different reactants in disease sera. Wesierska-Gadek *et al.* [100] detected and characterized antibodies to lamins by Western blotting in 12 (24%) of 51 autoimmune hepatitis sera, with zero reactivity for normal control sera. Lamins A and C were reactants at greatest frequency; all 12 of the reactive sera were from the 16 "active" cases in the series, whereas the 35 remission sera were nonreactive. This high frequency (75%) of antilamin reactivity in active autoimmune hepatitis is in contrast with quite low frequencies in SLE and other rheumatic diseases, and in PBC.

Anti-cytoplasmic Autoantibodies

Entire Cytoplasm

Numerous cytoplasmic structures act as autoantigens in SLE and other multisystem autoimmune (rheumatic) disease, and also in autoimmune hepatitis as illustrated by the complement-fixing autoantibodies to cell homogenates described in the 1950s [18]. Certain of these cytoplasmic reactions that have been characterized at the molecular level in autoimmune hepatitis polarize uniquely with either type 1 or type 2 disease, and do not occur in SLE (see later).

Neutrophil Specific Cytoplasmic Antibodies (ANCA)

Considerable interest developed in the 1960s in what were termed granulocyte-specific anti-nuclear antibodies. These were seen particularly in rheumatoid arthritis and in autoimmune hepatitis in which titers by immunofluorescence were considerably greater, by several logs, than those for conventional ANA [101]. The likelihood is that a good proportion at least of these granulocyte-specific ANA described earlier represented what is now recognized as anti-neutrophil cytoplasmic antibody

(ANCA) in which the cytoplasmic reactants clump in a perinuclear distribution. There are two discrete species of ANCA, cytoplasmic (c)ANCA and perinuclear (p)ANCA that have different disease associations and different substrate reactivities. cANCA occur in Wegener's disease and vasculitides in which the major antigens are found in azurophilic granules, particularly a 29-kDa protein triplet, proteinase 3. There are different species of pANCA, with that detected in systemic necrotizing vasculitis being identified as myeloperoxidase. In a study from Italy, the frequency of pANCA in type 1 autoimmune hepatitis was 65% (but 0% in type 2), and in chronic hepatitis C infection with autoimmune features, 13% [102]. ANCA of various specificities are reported in SLE but their presence does not correspond to any particular subtype and their frequency and titer are low relative to type 1 autoimmune hepatitis [103]. The reactant for pANCA in autoimmune hepatitis and other gastrointestinal diseases is not the usual myeloperoxidase. One study reported this as lactoferrin or elastase, another as a 50-kDa nuclear envelope protein that is restricted to neutrophils and myeloid cell lines, and yet another as high mobility group (HMG) proteins 1 and 2 [104].

LP Antigen, SLA

A liver pancreas (LP) antigen was described in 1981 by Berg *et al.* [105] by complement fixation, and this has been coidentified with the reactant described in 1987 as soluble liver antigen (SLA) by Manns *et al.* [106] by radioimmunoassay; SLA sometimes occurred in type 1 autoimmune hepatitis in the absence of all other serologic markers. The reactant for SLA has been successively attributed to cytokeratins 8, 18, [107] glutathione *S*-transferase [108], and most recently as the UGA-serine transfer RNA–protein complex or tRNA-associated protein (tRAP) [109, 110]. Structural considerations placed this protein in the family of serine hydroxymethyl transferases [111]. Reactivity with SLA is not reported in SLE.

Ribosomal P Proteins

Ribosomal P proteins are a set of three ribosomal phosphoproteins of 38 kDa (P_O), 19 kDa, (P_1), and 17 kDa, (P_2) that contain 22 carboxy terminal residues constituting a main immunodominant region or autoepitope [112]. Autoantibodies to ribosomal P proteins occur in some 16% of patients with SLE, and are a marker of active disease and, possibly, cerebral lupus (see Chapters 26 and 27). Autoantibodies to ribosomal P proteins have been described in type 1 autoimmune hepatitis, and this is a reactivity that could link autoimmune hepatitis with SLE; thus there is described a patient with SLE who, after 4 years follow-up, lost ANA with the appearance of anti-Po followed by anti-P_1 and anti-P_2, and this coincided with the development of an autoimmune hepatitis [113]. The temporal relationship of anti-P and liver dysfunction suggested a pathogenic (hepatocytotoxic) effect of anti-P, noting that an epitope similar to that at the carboxy terminus of ribosomal P proteins is present on the surface of cultured hepatoma cells [114]. Moreover, antibody to ribosomal P (anti-38kD, P_O) was shown to penetrate living hepatoma cells and induce cellular dysfunction [115]. This same protein is demonstrable on the surface of intact T lymphocytes [116]. The significance of anti-P for liver disease specifically associated with SLE was assessed in 131 patients with SLE among whom 4 (3%) had liver disease with histologic features similar to those of classic CAH [117]; in all 4, together with 2 additional referred cases, antiribosomal P was demonstrable whereas in cases of autoimmune hepatitis without evidence of SLE, antiribosomal P was not detectable. Moreover, in a case-control study on 20 subjects with SLE in whom anti-ribosomal P was, or was not detectable, the former group had significantly greater liver involvement [118]. Perhaps there is a subset of cases of autoimmune hepatitis in which there are lupus features characterized by and possibly even caused by circulating anti-ribosomal P; however, this possibility does not appear to have received recent attention.

Asialoglycoprotein Receptor

The asialoglycoprotein receptor (ASGPR) was identified in 1984 as a component of liver membrane protein preparations used as antigenic reactants with autoimmune hepatitis sera [119, 120]. The ASGPR, itself a glycoprotein, effects the binding and endocytosis of serum glycoproteins for disposal by the liver. It is a well-conserved protein but with differing numbers of subunits according to species; there are two subunits in humans. The properties and isolation of ASGPR and detection of antibodies by radioimmunoassay are described by McFarlane [120], subsequent studies have been limited because ASGPR is cumbersome to purify biochemically and difficult to express to high yield as a recombinant protein from a cDNA. A multinational study on reactivity of ASGPR, showed a 76% positively rate in autoimmune hepatitis, but positive results in 7–19% of cases in various other categories of liver disease indicated limited specificity [121]. ASGPR is not cited as an autoantigen in SLE.

Anti-cytoskeletal Autoantibodies

The cytoskeleton comprises structural proteins of the cell that support subcellular organization, cellular contractility, and locomotion [122]. The three types of

filaments and fibers include actin microfilaments (6 nm), intermediate sized filaments (15 nm), and microtubules (30 nm). Autoantibodies are revealed by immunofluorescence on substrates such as smooth muscle rich in these components [123]; the smooth muscle layer in mouse gastric mucosal preparations has proved to be a convenient substrate to demonstrate these [124].

Smooth Muscle Antibody (SMA)

It was found in the 1960s that SMA was a reliable marker for autoimmune hepatitis, with numerous studies affirming the high frequency of reactivity, about 70% [125]. While the diagnostic specificity of SMA is tempered by its presence in other destructive liver diseases including PBC, chronic viral hepatitis, and even alcoholic hepatitis, frequencies and titers are substantially lower than in autoimmune hepatitis [122]. It is relevant to the theme of this chapter that the presence of SMA, more than any other reactivity, segregates autoimmune hepatitis from SLE, meaning that SMA is in some way associated with hepatocellular destruction of a type that does not occur in SLE. Technical procedures for optimal testing for SMA are described by Fusconi *et al.* [126], and by Cançado *et al.* [127, 128] who emphasize the need for prior heat inactivation of sera. Ascertaining the subspecificity of the SMA reaction is critically important, as described later.

F-Actin

Actin is a globular protein of 46 kDa that is present in all cells as a constituent of microfilaments, and it is this cytoskeletal reactant that has diagnostic specificity for autoimmune hepatitis. Actin can exist as G-actin which is a monomer, or as F-actin which is polymerized into filaments; the antigenic reactivity depends on the conformation in the native molecule of epitopes which can be destroyed under conditions such as immunoblotting. Unfixed or lightly fixed tissues, for example, vinblastin-treated cultured cells prepared as monolayers for immunofluorescence, are a favored substrate [125]. Antibody to F-actin to high titer is demonstrable in some 70–90% of cases of autoimmune hepatitis in acute or active phases, but frequencies and titers are reduced in remission phases [125]. A report on 99 patients with type 1 autoimmune hepatitis cited a positivity rate of 74%, with this correlating with younger age, progressive disease, and the HLA alleles B8 and DR3 [129]. Reactivity to F-actin has a much higher diagnostic specificity than does generic SMA, a point deemed worthy of reemphasis in a more recent article [130], since SMA reactions in viral infections and other miscellaneous diseases are mostly attributable to antibodies to other cytoskeletal elements, often intermediate filaments (vimentin) [123]. Anti-F actin autoantibodies give additional staining of the mesangium of renal glomeruli, brush borders and peri-tubular fibrils of renal tubules (this used to be called SMA-T) and, with strong reactions, a "polygonal" pattern of staining on liver sections due to the abundant submembranous distribution of actin in liver cells [104, 130, 131] (Fig. 3).

Antibody to F-actin may well have immunopathogenic significance in autoimmune hepatitis, since liver destruction from any cause could result in release of actin that would then behave as an autoantigen in individuals genetically predisposed (see later), and the copious submembranous actin of hepatocytes could serve as a target for an ongoing damaging immune response. However, despite the possible importance of F-actin as autoantigen for diagnosis and pathogenesis, studies on epitope analysis and T-cell responses have not been pursued to the same degree as for many other autoantigens.

Non-actin Cytoskeletal Filaments

Cytoskeletal filaments other than actin (vimentin, tubulin) account for SMA reactions, particularly low titer reactions in nonautoimmune liver diseases and viral infections [104, 123]. Autoantibodies to the intermediate filament desmin are reported in myocarditis, and to tubulin in infectious mononucleosis [125].

Unidentified Anti-liver Membrane Antibodies

The possibility of a specific reactant located in the liver cell membrane has prompted several immunoblotting studies using purified liver membrane preparations. While these consistently show multiple reactants at differing molecular weights, none has yet been identified as a true liver membrane-specific autoantigen. Many of these reactants decrease markedly in intensity of signal after increases in serum dilution beyond 1:1000 and also during immunosuppression-induced remission of disease, and may simply be consequential to hepatocellular damage [131].

Serologic Reactions in Type 2 Autoimmune Hepatitis

Anti-microsomal Autoantibodies

LKM-1 (P450 2D6)

Autoantibodies were detected in CAH in 1973 that reacted by immunofluorescence with cytoplasm of liver cells and the P3 segment of proximal renal tubular cells [45], and their microsomal location led to the designation of liver–kidney microsomal (LKM) antigen. Anti-LKM associated-CAH occurred predominantly in children with rapid progression to cirrhosis. A

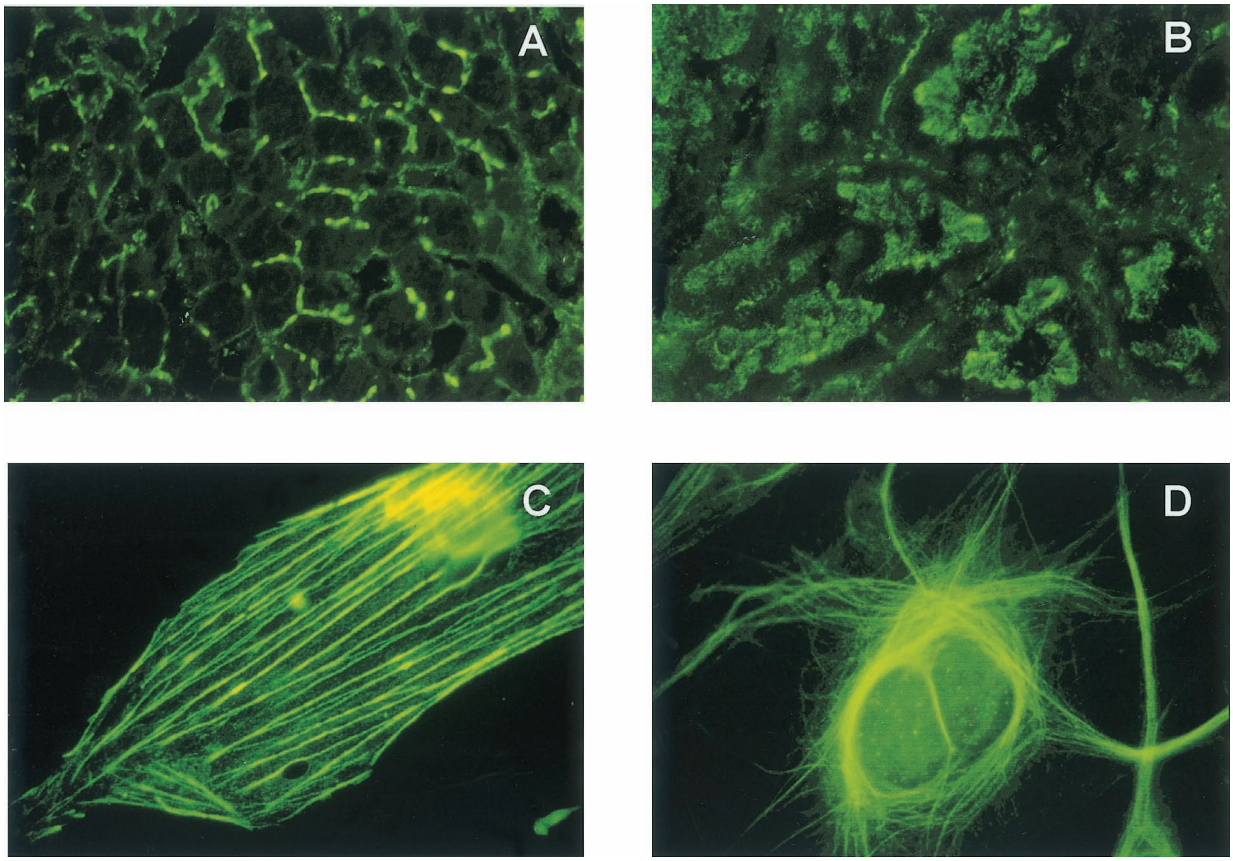


FIGURE 3 Anti-actin specific patterns by immunofluorescence of smooth muscle antibody that differentiate autoimmune hepatitis (**A**–**C**) from other disease sera (**D**). (**A**) Liver showing polygonal staining pattern of submembranous actin; (**B**) kidney showing actin-specific staining of brush borders of renal tubules and peritubular fibrils; (**C** and **D**) cultured fibroblasts showing actin microfilaments ("cables") (**C**), and vimentin intermediate filaments (**D**). (Reproduced by kind permission of publishers of *Autoimmunity* [104].)

dichotomy between the lupoid and anti-LKM types of chronic hepatitis seemed evident from the earliest descriptions [45], and was substantiated in 1987 by analysis of 87 cases [46]. In particular there is a serologic polarization, in that there was no overlap among cases of autoimmune hepatitis of ANA/SMA and anti-LKM reactions. Accordingly, despite similar histopathology and outcome (cirrhosis), there is general recognition of two distinct types, 1 and 2, of autoimmune hepatitis [46, 132] (Table 2). Subsequently different types of anti-LKM were nominated according to the disease background, with the LKM autoantigen associated with spontaneous type 2 disease being LKM-1, and other microsomal reactants being LKM-2 and LKM-3 (see later).

Molecular identification of LKM antigens was achieved during the 1980s, based initially on immunoblotting [133], and later by screening with sera of gene expression libraries [134]. The LKM-1 reactant was revealed as an isoform of the cytochrome oxidase P450 enzyme family, P450 2D6. The P450 2D6 autoantigen contains three linear epitopes of which the most reactive has the sequence DPAQPPRD within the region of residues 257–269 [135]. The molecular identification of the specific reactant for type 2 autoimmune hepatitis is in contrast to the still uncharacterized specific reactant(s) for type 1.

LKM-2 (P450 2C9) and LM (P450 1A2)

An anti-microsomal reactivity of serum, for which the reactant was termed LKM-2, was recognized in 1984 in cases of acute and chronic hepatitis induced occasionally by exposure to medicinal drugs, particularly tienilic acid [136]; the reactant was subsequently identified as another CYP450 isoform, 2C9 [137]. In cases of drug-induced hepatitis provoked by dihydralazine, sera react by immunofluorescence with microsomes only of liver cells; this liver microsomal (LM) reactant was identified

TABLE 2 Comparisons and Contrasts for Type 1 and Type 2 Autoimmune Hepatitis

	Type 1	Type 2[a]
Female	8:1	8:1
Age	Bimodal	Bimodal, peak in early childhood
Course	Progressive, but steroid-responsive	Progressive, but steroid-responsive
Associated clinical features[b]	Arthritis, serositis, cytopenias, colitis, thyroiditis	Thyroiditis, gastritis, diabetes, vitiligo
Genetics, HLA	DR4,B8,DR3,C4AQO haplotype	More data needed
Trigger factors	EBV? HAV? drugs, rarely	None described[c]
Models	See Ref. 104	None described
Autoantibodies to	Various nuclear antigens, esp. chromatin; smooth muscle (actin)	Microsomal cytochrome P450 2D6 (LKM-1), thyrogastric antigens

[a] Data on type 2 from Homberg *et al.* [46].
[b] Type 1 with systemic features can resemble SLE; type 2 does not.
[c] Chronic hepatitis with anti-LKM occurs with HCV infection or hepatic drug reactions, but these cases are diffentiated from spontaneous type 2.

as yet another isoform, CYP450 1A2 [138]. Further still, other drugs can induce immune-mediated hepatitis, each associated with antibodies to a particular CYP450 isoform involved in its hydroxylation [104,139].

A theoretically interesting disease is the LKM-associated type 2 autoimmune hepatitis that occurs in some 10–20% of cases of juveniles with the autoimmune polyendocrine syndrome type 1 (APS-1). This syndrome results from defective function of the nuclear transcriptional protein encoded by the autoimmune regulator (*AIRE*) gene. APS-1 is a complex autoimmune–immunodeficiency syndrome resulting from homozygous inheritance of a mutant *AIRE* gene, and the autoantibody expressions include reactivity to two CYP450 isoforms 1A2 and 2A6 among others [139]. The *AIRE* protein expressed in dendritic cells in various sites is in some way involved in the establishment of immune tolerance, but clinical expressions are limited to organ-specific rather than multisystem disease type autoantigens.

LKM3 (Anti-UDP Glucuronosyl Transferase)

An anti-LKM reactivity was recognized in 1983 in cases of hepatitis associated with delta (D) hepatitis virus infection and became known as anti-LKM-3 [140]. This microsomal autoantigen has been identified not as a CYP450 isoform but instead as the bilirubin conjugating enzyme uridine 5′-diphosphate glucuronosyl transferase (UGT) 1.6 [141]. A rare spontaneously occurring type 2 autoimmune hepatitis has been identified with anti-UGT (LKM-3) reactivity of serum [142]. More recently autoantibodies reacting with various UGT isoforms were detected in 3 of 18 cases of type 2 autoimmune hepatitis, most strongly against UGT1A1 which is the main isoform involved in the glucuronidation and disposal of bilirubin [143].

Liver Cytosol Type 1 (LC-1)

Autoantibodies were recognized in 1988 to a soluble liver cytosolic antigen in children with type 2 autoimmune hepatitis [144], and this reactivity was further characterized in 1992 [145]. Anti-LC1 reactivity is of interest. because it is organ (liver) specific, and also disease-specific for type 2 autoimmune hepatitis in that, unlike LKM-1, it is not demonstrable in chronic hepatitis C virus infection (see later); however, anti-LC1 may occur in children with type 1 autoimmune hepatitis [146]. The LC-1 antigen can be eluted from liver cytosol as a protein of 240–290 kDa and, since the signal by immunoblot under reducing conditions is at 62 kDa, it may exist as a tetramer [147]. By immunofluorescence, anti-LC1 shows a characteristically decreased homogeneous staining of hepatocytes toward the central vein, usually obscured by accompanying anti-LKM-1 reactivity, and is demonstrable also by immunodiffusion, immunoblot, or counterimmunoelectrophoresis [147]. Anti-LC-1 has more recently been identified as formiminotransferase cyclodeaminase (FTCA) or argininosuccinate lysase [131], but FTCA is more likely according to molecular weight [148]. The FTCA molecule has two domains, a globular FT domain joined by a short linker to the CA domain; autoantibodies mainly recognize a conformational structure associated with the FT domain [149].

Hepatitis C Virus and Autoimmune Serologic Reactions

There are many literature citations on the association between HCV infection of the liver and serum reactivities characteristic of autoimmune hepatitis, particularly anti-LKM-1. These raise provocative questions on

etiopathogenesis and therapy, but there are no indications that HCV is an initiator of multisystem autoimmunity [150]. In a study from France on 83 patients with HCV infection, the frequency of anti-LKM (anti CYP450 2D6) was as high as 77% [151]; in common with other studies on HCV–associated anti-LKM-1, titers were relatively low and males were overrepresented. Also, compared with spontaneous type 2 autoimmune hepatitis, there is a selection for an antibody epitope other than that within residues 257–269 of CYP450 2D6 [152]. In regard to autoantibodies associated with type 1 autoimmune hepatitis, HCV infection was reported to be associated with rheumatoid factor, with SMA although actin-specificity was not stated, and with ANA at low frequency, but with AMA very rarely [153]. The predominance of type 1 autoimmune hepatitis in geographic areas of the world where HCV is least endemic, and the absence of markers of HCV infection in typical type 2 in children, argues against HCV being an important antecedent of autoimmune hepatitis. However, the association of LKM-1 reactions with HCV infection and (weak) sequence homologies between envelope proteins of HCV and CYP450 molecules have led to speculation that HCV might, in some instances at least, provide a molecular mimic that could initiate autoimmune hepatitis [154].

How Close Is the Serologic Overlap for Autoimmune Hepatitis and SLE?

With multiple variable disease expressions in autoimmune hepatitis and SLE, some degree of serologic overlap is to be expected. The major overlaps are the presence in both diseases of antibodies to chromatin-histones as the major ANA reactant, anti-DNA albeit weakly expressed in autoimmune hepatitis, and anti-ribosomal P proteins as a low frequency contributor. On the other hand, there are serologic reactions in autoimmune hepatitis that do not occur in SLE, including, in type 1, antibodies to F-actin, to the molecule originally described as soluble liver antigen and now identified as tRNA-associated protein (see earlier), and to various microsomal (CYP450) enzyme antigens and the liver-specified molecule FTCA (LC-1) in type 2 disease. Thus the contrasts outweigh the similarities.

T-Cell Reactivity in Autoimmune Hepatitis

T cells are important in the induction and effector phases of all autoimmune responses, and currently functional assays are based either on cell proliferation or lymphokine release on exposure of T cells to autoantigen. A new technique with future potential based on binding of antigenic peptides (when indentified) to MHC tetramers, should indicate the level of representation in blood or tissues of autoantigen-specific CD8 or CD4 T cells [104]. However, more knowledge is needed on the moieties of autoantigens that are relevant to autoimmune induction, and effector activities of T cells in autoimmune hepatitis as well as in SLE.

IMMUNOGENETICS PREDISPOSITION

General Comments

In Chapter 5 of the previous edition, Reveille optimistically commented "by the time the next edition of this book is published the genetic contribution to SLE will be almost completely defined." The actual progress in the genetics of SLE is discussed herein in Chapter 4, and a 2001 benchmark is struck by the review of Wakeland *et al.* [155]. The latter reviews the genetic contributions of alleles of HLA, Fcγ receptors, and null alleles at complement loci, and results of linkage studies based on genome-wide screens in SLE families that reveal several chromosomal regions of interest. Of note, some regions associated with susceptibility in humans are syntenic with genome segments identified in one or another of the various mouse strains with susceptibility to lupus. Emerging technologies for identification of loci of interest are expected to advance knowledge of SLE very rapidly, but less so for autoimmune hepatitis by reason of the lower population prevalence and level of clinical interest, and absence of a spontaneous disease in animal models [131]. However, when data from genome wide screens do become available from autoimmune hepatitis families, the forecast is for some sharing with SLE of susceptibility loci.

MHC (HLA) Alleles

An association was first recognized in 1971 between type 1 autoimmune hepatitis and the class 1 alleles A1, B8, with B8 as the primary risk allele [156]. Later, a DR3 association was defined, and family studies identified association with the HLA A1-B8-DR3 haplotype with DR3 as the likely risk allele, with linkage disequilibrium explaining the earlier associations [157]. The association with HLA DR3 was widely confirmed and this was calculated to confer a relative risk for disease of 6 to 7 [158]. The DR3 allele was shown to be part of an extended haplotype which included C4A gene deletions [159, 160], providing a genetic basis for low levels of the C4 component of complement in autoimmune hepatitis. An association with DR4 was subsequently recognized [161]. There may exist two nonexclusive immunogenetic subsets of type 1 autoimmune hepatitis, the HLA DR3 subset with a younger age of onset, more

progressive disease, a greater tendency to relapse despite corticosteroid therapy, and a higher requirement for liver transplantation, and an HLA DR4 subset with an older age of onset and more benign disease [162]. Advances in molecular genotyping have further characterized the immunogenetic background of type 1 disease [163, 164]. There is a high representation of the HLA DRB1*0301-DRB3*0101-DQA1*0501-DQB1*0201 haplotype, 52 vs 19% of controls, and a strong secondary association with the DR4 allele DRB1*0401, 54% of DRB3*0101 negative patients compared with 23% of DRB3*0101 negative controls. Among cases from North America, a lysine residue at position 71 of the HLA DRβ polypeptide ($lysine_{71}$) is central to a proposed "disease susceptibility motif" that may bind the still unknown disease initiating autoantigen for type 1 autoimmune hepatitis. There are different HLA risk alleles among populations of non Eurocaucasian background, but these mostly, but not exclusively, express the DRβ1 $lysine_{71}$ disease susceptibility motif [164]. Notably, type 2 autoimmune hepatitis is serologically distinct from type 1, and immunogenetically distinct as well, although susceptibility alleles are yet to be determined [164].

Comparing autoimmune hepatitis with SLE, HLA DR3 is a risk allele for both diseases although the level of risk is lower for SLE (2–3 vs 6–7), whereas DR2 is a risk allele only for SLE, and DR4 only for type 1 autoimmune hepatitis [164, 165]. Complement loci, particularly C4QA null, confer risk for both diseases.

Non-MHC Alleles

Non-MHC (low) risk alleles for type 1 autoimmune hepatitis include the TNG-A gene that results in high constitutive and induced levels of TNF-α, and the G allele of the CTLA-4 molecule that mediates down regulation of the T-cell responses [164]. Inheritance of two copies of mutant autoimmune regulator (*AIRE*) gene leads to a remarkable phenotype of disordered immunity (autoimmune polyendocrine syndrome type 1) that can include a type 2-like autoimmune hepatitis with antibodies to CYP450 isoforms [104], but not SLE. The *sle*1 gene region identified in murine models of SLE, that has a likely ortholog (1q 22–24) in humans according to genome wide scans [155], includes genes that determine nuclear (chromatin) antigen targeting; this region could be implicated as well in type 1 autoimmune hepatitis. Other than the *sle*1 region there are various other suspected loci involved in the pathogenesis of SLE [155, 166] (and see Chapters 3 and 14), with functional effects via clearance of antigen/immune complexes, lymphoid signaling, apoptosis, and epitope modification. The degree of sharing of such genes in autoimmune hepatitis and SLE will be an intriguing question for the future.

In broad outline, genetic determinants of autoimmunity can be collected loosely into four groups. The first group, probably shared among most autoimmune diseases, comprises genes that confer global immunologic hyperresponsiveness, with genes particular to female sex a clear example, or genes that interfere with processes of tolerance including apoptosis, since there is characteristic clustering of similar or different autoimmune expressions within affected patients and related family members. Curiously, however, reported coexistences within autoimmune hepatitis families of cases of SLE [167], are less frequent than would be expected. The second group comprises genes responsible for immunogenetic targeting of immune responses to particular autoantigens, and/or diseases to particular tissues, and includes MHC alleles that encode products with a high binding capacity for a tissue-specific molecule, genes in the *sle*1 region (see earlier), and perhaps genes that influence the assembly and structure for T- and B-cell receptors. The third group of genes includes those that influence events after immune activation, for example, polymorphisms of cytotoxic lymphocyte antigen (CTLA)-4, or genes that encode inflammatory cytokines including tumor necrosis factor. The fourth group, "end organ" genes, influences susceptibility to immune attack of a particular target tissue, for example, the genetic defect in thyroid cells that predisposes to thyroiditis of obese chickens [167]; such genes could confer predisposition to autoimmune damage to the kidney in lupus [155], or in some way confer end-organ susceptibility in autoimmune hepatitis.

ENVIRONMENTAL AGENTS

The understanding of the genetic–environmental interactions that precipitate or perpetuate autoimmune diseases remains sketchy, even for the model diseases in inbred mice that are susceptible to reductionist analysis. Environment contributes 50% of the risk or usually more for autoimmunity, with the simplest explanation being that a source of injury, usually an infectious agent, causes release of intracellular antigens to which tolerance has not been established, with induction of self-reactive lymphocytes that maintains an autoimmune reaction. Additionally, the infectious agent may contain sequences which sufficiently resemble a critical host molecule such that autoimmune induction is potentiated; this molecular mimicry hypothesis is the subject of much contemporary discussion in relation to autoimmunity [168].

Suspected environmental determinants of SLE, including the Epstein-Barr virus (EBV), are discussed in Chapters 3, 44, 45. In autoimmune hepatitis, suspected viruses have included EBV [169] and measles (now considered unlikely) and the hepatitis viruses, particularly A and C (HAV, HCV). Type 1 autoimmune hepatitis is occasionally preceded by HAV infection [40] and the frequent reporting of cases in young women in the 1960s coincided at that time with a wave of endemicity of HAV infection. Type 2 autoimmune hepatitis in adults, but not in children, is associated with markers of HCV infection in some geographical regions [132], and mimicry sequences have been identified between the HCV polyprotein and sequences of cytochrome P450 autoantigen [170]. At present, however, there appears to be insufficient evidence to incriminate virus infection as a trigger for either autoimmune hepatitis or SLE. Therapeutic drugs, although quite infrequent instigators of either autoimmune hepatitis or SLE, raise interesting considerations. A drug-related hepatitis with antibodies to CYP450 can occur after use of drugs disposed of by this pathway [171], but without concomitant expressions of SLE. However, there are some drugs, particularly α-methyl dopa and minocycline, that can induce by unknown means a clinical replica of autoimmune hepatitis, or a syndrome resembling SLE; these conditions are essentially drug-dependant and, unlike the spontaneously occurring counterparts, recede when the offending drug is withdrawn [171].

CONCLUSIONS

This chapter has considered issues raised by the nosologic problems of autoimmune (organ-specific) hepatitis and lupus affecting the liver. Accordingly the chapter included brief comment on the nomenclature of diseases, particularly those for which causes are either unknown or multifactorial and/or which have retained traditional and sometimes illogical names. Three propositions provided the infrastructure to the chapter. First, there is a disease (autoimmune hepatitis, previously CAH) intrinsic to the liver and in the domain of hepatology, that, by reason of its autoimmune causation, can on occasions exhibit sufficient criteria for SLE for it to become coidentified with that disease. Second, SLE itself is a disease with such widespread inflammatory expressions, including "toxic" effects from tissue breakdown, that there will be secondary damage to the liver equivalent to that seen in conditions such as severe infections. Third, there could be a very broad recruitment of immune effector processes, the "lupus process" that might cause damage to the liver of a type similar to that occurring in other tissues, kidney, brain, skin, etc. that are usually affected in lupus. The arguments for convergence include the autoimmune basis for both diseases, the shared serologic feature of anti-nuclear (anti-chromatin) antibody, and some sharing of immunogenetic determinants.

Acknowledgments

Appreciation is expressed to Drs. Josefa Wesierska-Gadek and Nigel Swanston for illustrative material, and Ms. Elaine Pearson for her expert preparation of the manuscript.

References

1. Cohen, A. S., Reynolds, W. E., Franklin, E. C., Kulka, J. P., Ropes, M. W., Shulman, L. E., and Wallace, S. L. (1971). Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21**, 643–648.
2. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N., and Winchester, R. J. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 1271–1277.
3. Anonymous (1999). The American College of Rheumatology nomenclature and case definitions for neuropsychiatric lupus syndromes. *Arthritis Rheum*. **42**, 599–608.
4. Mackay, I. R., Taft, L. I., and Cowling, D. C. (1956). Lupoid hepatitis. *Lancet* **2**, 1323–1326.
5. Mackay, I. R. (1994). Diagnosis of autoimmune disease: Past and present. In "Autoimmunity, State of the Art" (P. Lydyard and J. Brostoff, Eds.), pp. 137–154. Blackwell, London.
6. Tan, E. M. (1989). Antinuclear antibodies: Diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* **44**, 93–151.
7. Temple, L. K. F., McLeod, R. S., Gallinger, S., and Wright, V. G. (2001). Defining disease in the genomics era. *Science* **293**, 807–808.
8. Scadding, J. G. (1996). Essentialism and nominalism in medicine: Logic of diagnosis in disease terminology. *Lancet* **348**, 594–596.
9. Johnson, P. J., and McFarlane, I. G. (1993). Meeting report: International Autoimmune Hepatitis Group. *Hepatology* **18**, 998–1005.
10. Alvarez, F., Berg, P. A., Bianchi, F. B., *et al.* (1999). International Autoimmune Hepatitis Group report: Review of criteria for diagnosis of autoimmune hepatitis. *J. Hepatol.* **31**, 929–938.
11. Mackay, I. R., and Tait, B. D. (1994). The history of autoimmune hepatitis. In "Autoimmune Hepatitis" (M. Nishioka, G. Toda, M. Zeniya, Eds.), pp. 3–23. Elsevier, Amsterdam.
12. Barker, M. H., Capps, R. B., and Allen, F. W. (1945). Chronic hepatitis in the Mediterranean theatre. *JAMA* **129**, 653–659.
13. Wood, I. J., King, W. E., Parsons, P. J., Perry, J. W., Freeman, M., and Limbrick, L. (1948). Non-suppurative

hepatitis: A study of acute and chronic forms with special reference to biochemical and histological changes. *Med. J. Aust.* **11**, 249–261.
14. Waldenstrom, J. (1950). Leber, Blutproteine und Nahrungseiweiss. *Dtsch. Gesellschaft Verdauungs-und Stoffwechselkrankheiten.* **15**, 113–119.
15. Kunkel, H. G., Ahrens, J. R., Eisenmenger, W. J., Bongiovanni, A. M., and Slater, R. J. (1951). Extreme hypergammaglobulinemia in young women with liver disease of unknown etiology. [Abstract]. *J. Clin. Invest.* **30**, 654.
16. Bearn, A. G., Kunkel, H. G., and Slater, R. J. (1956). The problem of chronic liver disease in young women. *Am. J. Med.* **21**, 3–15.
17. Joske, R. A., and King, W. E. (1995). The L.E. cell phenomenon in active chronic viral hepatitis. *Lancet* **2**, 477–480.
18. Mackay, I. R., and Gajdusek, D. C. (1958). An "autoimmune" reaction against human tissue antigens in certain acute and chronic diseases: II. Clinical correlations. *Arch. Intern. Med.* **101**, 30–46.
19. Mackay, I. R., Weiden, S., and Hasker, J. (1965). Autoimmune hepatitis. *Ann. N. Y. Acad. Sci.* **124**, 767–780.
20. Bradbear, R. A., Robinson, W. N., Cooksley, W. G. E., Halliday, J. W., Harris, O. D., and Powell, L. W. (1984). Are the causes and presentation of chronic hepatitis changing? An analysis of 104 cases over 15 years. *Q. J. Med.* **LIII**, 279–288.
21. Parker, D. R., and Kingham, I. G. C. (1997). Type 1 autoimmune hepatitis is primarily a disease of later life. *Q. J. Med.* **90**, 289–296.
22. Krook, H. (1961). Liver cirrhosis in patients with a lupus erythematosus-like syndrome. *Acta Med. Scand.* **169**, 713–726.
23. Applebaum, J. J., Job, H., and Kern, F. (1961). Hepatitis associated with disseminated lupus erythematosus. *Gastroenterology* **40**, 766–771.
24. Robson, M. D. (1959). Systemic lupus erythematosus complicating chronic liver disease. *Guy's Hosp. Rep.* **108**, 438–443.
25. Read, A. E., Sherlock, S., and Harrison, C. V. (1963). Active "juvenile" cirrhosis as part of a systemic disease and the effect of corticosteroid therapy. *Gut* **4**, 378–393.
26. Miescher, P. A., Braverman, A., and Amorosi, E. (1966). Progressive hypergammaglobulinaemic hepatitis. *Dtsch. Med. Wschr.* **91**, 1525–1532.
27. Reynolds, T. B., Edmondson, H. A., Peters, R. L., and Redeker, A. (1964). Lupoid hepatitis. *Ann. Intern. Med.* **61**, 650–666.
28. MacLachlan, M. J., Rodnan, G. P., and Cooper, W. M., and Fennell R. H., Jr. (1965). Chronic active ("lupoid") hepatitis: A clinical, serological and pathological study of 20 patients. *Ann. Intern. Med.* **62**, 425–462.
29. Soloway, R. D., Summerskill, D. M., Baggenstoss, A. H., and Shoenfield, L. J. (1975). "Lupoid" hepatitis, a nonentity in the spectrum of chronic active liver disease. *Gastroenterology* **63**, 458–465.
30. Czaja, A. J., Davis, G. L., Ludwig, J., Baggenstoss, A. H., and Taswell, H. F. (1983). Autoimmune features as determinants of prognosis in steroid-treated chronic active hepatitis of uncertain etiology. *Gastroenterology* **85**, 713–717.
31. Trimble, R. B., Townes, A. S., Robinson, H., Kaplan, S. B., Chandler, R. W., Hanissian, A. S., and Masi, A. T. (1974). Preliminary criteria for the classification of systemic lupus erythematosus (SLE). Evaluation in early diagnosed SLE and rheumatoid arthritis. *Arthritis Rheum.* **17**, 184–188.
32. Leevy, C. M., Popper, H., and Sherlock, S. (1976). Diseases of the liver and biliary tract. Standardization of nomenclature, diagnostic criteria and diagnostic methodology. "Fogarty International Centre Proceedings No. 22," DHEW Publication No. (NIH) 76-725. U.S. Government Printing Office, Washington, D.C.
33. Mackay, I. R. (1972). The prognoses of chronic hepatitis. *Ann. Intern. Med.* **77**, 649–651.
34. Hodges, J. R., Millward-Sadler, G. H., and Wright, R. (1982). Chronic active hepatitis: The spectrum of disease. *Lancet* **1**, 550–552.
35. Mackay, I. R. (1991). Pathogenesis of autoimmune chronic hepatitis. In "Autoimmune Liver Diseases" (E. L. Krawitt and R. Wiesner, Eds.), pp. 21–42 Raven, New York.
36. International Hepatology Informatics Group. (1994). Diseases of the liver and biliary tract. In "Standardisation of Nomenclature, Diagnostic Criteria and Prognosis" (C. M. Leevy, S. Sherlock, N. Tygstrup, and R. Zetterman, Eds.), Raven, New York.
37. International Working Party. (1995). Terminology of chronic hepatitis. *Am. J. Gastroenterol.* **90**, 181–189.
38. Mackay, I. R. (1985). Autoimmune diseases of the liver. In "The Autoimmune Diseases" (N. R. Rose and I. R. Mackay, Eds.), pp. 291–337. Academic Press, Orlando, Florida.
39. Czaja, A. J. (1986). Autoimmune chronic active hepatitis. In "Chronic Active Hepatitis. The Mayo Clinic Experience" (A. J. Czaja, and E. R. Dickson, Eds.), pp. 105–126. Dekker, New York.
40. Krawitt, E. L. (1996). Autoimmune hepatitis. *N. Engl. J. Med.* **334**, 897–903.
41. Crapper, R. M., Bhathal, P. S., Mackay, I. R., and Frazer, I. H. (1986). "Acute" autoimmune hepatitis. *Digestion* **34**, 216–225.
42. Golding, P. L., Smith, M., and Williams, R. (1973). Multisystem involvement in chronic liver disease. Studies on the incidence and pathogenesis. *Am. J. Med.* **55**, 772–782.
43. Rizzeto, M., Swana, G., and Doniach, D. (1973). Microsomal antibodies in active chronic hepatitis and other disorders. *Clin. Exp. Immunol.* **15**, 331–344.
44. Smith, M. G. M., Williams, R., Walker, G., Rizzetto, M., and Doniach, D. (1974). Hepatic disorders associated with liver kidney microsomal antibodies. *Br. Med. J.* **2**, 80–84.
45. Rizzeto, M., Bianchi, F. B., and Doniach, D. (1974). Characterization of the microsomal antigen related to a subclass of active chronic hepatitis. *Immunology* **26**, 589–601.

46. Homberg, J. C., Abuaf, N., Bernard, O., *et al.* (1987). Chronic active hepatitis with antiliver/kidney microsome antibody Type 1: A second type of autoimmune hepatitis. *Hepatology* **7**, 1333–1339.
47. Czaja, A. J., Carpenter, H. A., Santrach, P. J., and Moore, S. B. (1995). Immunologic features and HLA associations in chronic viral hepatitis. *Gastroenterology* **108**, 157–164.
48. Mackay, I. R. (1993). Towards diagnostic criteria for autoimmune hepatitis. *Hepatology* **18**, 1006–1008.
49. Lindgren, S., Braun, H.-B., Michel, G., Nemeth, A., Nilsson, S., Thome-Kromer, B., and Ericksson, S. (1997). Absence of LKM antibody reactivity in autoimmune and hepatitis C-related chronic liver disease in Sweden. *Scand. J. Gastroenterol.* **32**, 175–178.
50. Tanaka, E., Kiyosawa, K., Seki, T., Matsumoto, A., Sodeyama, T., Furuta, S., Kumagai, T., and Kohara, M. (1993). Low prevalence of hepatitis C infection in patients with autoimmune hepatitis type 1. *J. Gastroenterol. Hepatol.* **8**, 442–447.
51. Gregorio, G. V., Portman, B., Reid, F., Donaldson, P. T., Doherty, D. G., McCartney, M., Mowat, A., Vergani, D., and Mieli-Vergani, G. (1997). Autoimmune hepatitis in childhood: A 20 year experience. *Hepatology* **25**, 541–547.
52. Mackay, I. R. (2000). Autoimmune disease overlaps and the liver: Two for the price of one? *J. Gastroenterol. Hepatol.* **15**, 3–8.
53. Woodward, J., and Neuberger J. (2001). Autoimmune overlap syndromes. *Hepatology* **33**, 994–1002.
54. Kenny, R. P., Czaja, A. J., Ludwig, J., and Dickson, E. R. (1986). Frequency and significance of antimitochondrial antibodies in severe chronic hepatitis. *Dig. Dis. Sci.* **31**, 705–711.
55. Chazouillères, O., Wendiem, D., Serfaty, L., *et al.* (1998). Primary biliary cirrhosis: Autoimmune hepatitis overlap: Clinical features and response to therapy. *Hepatology*, **28**, 296–301.
56. Lohse, A. W., Meyer zum Büschenfelde, K.-H., Franz, B., *et al.* (1999). Characterization of the overlap syndrome of primary biliary cirrhosis and autoimmune hepatitis: Evidence for it being a hepatitic form of PBC in genetically susceptible individuals. *Hepatology* **29**, 1078–1084.
57. Mackay, I. R., Whittingham, S., Fida, S., Myers, M., Ikuno, N., Gershwin, M. E., and Rowley, M. J. (2000). The peculiar immunity of primary biliary cirrhosis. *Immunol. Rev.* **174**, 226–237.
58. Powell, F. C., Schroeter, A. L., and Dickson, E. R. (1987). Primary biliary cirrhosis and the CREST syndrome: A report of 22 cases. *Q. J. Med* **62**, 75–82.
59. Mackay, I. R., Rowley, M. J., and Whittingham, S. F. (1993). Nuclear autoantibodies in primary biliary cirrhosis. In "Immunology and Liver: Falk Symposium Number 70, Immunology and Liver" (K.-H. Meyer zum Buschenfelde, J. Hoofnagle, and M. Manns, Eds.), pp. 397–409. Kluwer, Dordrecht.
60. Brünner, G., and Klinge, O. (1987). A cholangitis with antinuclear antibodies (immuno-cholangitis) resembling chronic destructive non-suppurative cholangitis. *Dtsch. Med. Wschr.* **112**, 1454–1458.
61. Goodman, Z. D., McNally, P. R., Davis, D. R., and Ishak, K. G. (1995). Autoimmune cholangitis: A variant of primary biliary cirrhosis. Clinicopathologic and serologic correlation in 200 cases. *Dig. Dis. Sci.* **40**, 1232–1242.
62. Kinoshita, H., Omagari, K., Matsuo, I., *et al.* (1999). Autoimmune cholangitis: Serological features in 21 Japanese cases compared with those in primary biliary cirrhosis and autoimmune hepatitis. *Liver* **14**, 122–128.
63. Czaja, A. J., Carpenter, H. A., Santrack, P. J., and Moore, S. B. (2000). Autoimmune cholangitis within the spectrum of autoimmune liver disease. *Hepatology* **31**, 1236–1238.
64. Pistina, M., Wallace, D. J., Nessim, S., Metzger, A. L., and Klinenberg, J. R. (1991). Lupus erythematosus in the 1980s: A survey of 570 patients. *Semin. Arthritis Rheum.* **21**, 55–64.
65. Schaffner, F., and Popper, H. (1959). Non-specific reactive hepatitis in aged and infirm people. *Am. J. Dig. Dis.* **4**, 399.
66. Runyon, B. A., LaBreque, D. R., and Anuras, S. (1980). The spectrum of liver disease in systemic lupus erythematosus. *Am. J. Med.* **69**, 187–194.
67. Gibson, T., and Myers, A. R. (1981). Subclinical liver disease in systemic lupus erythematosus. *J. Rheumatol.* **8**, 752–759.
68. Miller, M. H., Urouitz, M. B., Gladman, D. D., and Blendis, L. M. (1984). The liver in systemic lupus erythematosus. *Q. J. Med.* **LIII**, 401–409.
69. Van Steenbergen, W., Beyls, J., Vermylen, J., Fevery, J., Marchal, G., Desmet, V., and De Groote, J. (1986). Lupus anticoagulant and thrombosis of the hepatic veins (Budd-Chiari syndrome). Report of three patients and review of the literature. *J. Hepatol.* **3**, 87–94.
70. Leggett, B. A. (1993). The liver in systemic lupus erythematosus. *J. Gastrolenterol. Hepatol.* **8**, 84–88.
71. Ruiz, F. P., Martinez, J. O., Mendoza, A. C. Z., Del Arbol, L. R., and Caparros, A. M. (1991). Nodular regenerative hyperplasia of the liver in rheumatic diseases: Report of seven cases and review of the literature. *Semin. Arthritis Rheum.* **21**, 47–54.
72. Sekiya, M., Sekigawa, I., Hishikawa, T., Iida, N., Hashimoto, H., and Hirose, S. (1997). Nodular regenerative hyperplasia of the liver in systemic lupus erythematosus. *Scand. J. Rheumatol.* **26**, 215–217.
73. de Groote, J., Desmet, V. J., Gedigk, P., *et al.* (1968). A classification of chronic hepatitis. *Lancet* **2**, 626–628.
74. International Group of Pathologists (1977). Acute and chronic hepatitis revisited. *Lancet* **2**, 914–919.
75. Page, A. R., and Good, R. A. (1960). Plasma cell hepatitis with special attention to steroid therapy. *J. Dis. Child.* **99**, 288–314.
76. Popper, H., Paronetto, F., and Schaffner, F. (1965). Immune processes in the pathogenesis of liver disease. *Ann. N. Y. Acad. Sci.* **124**, 781–799.
77. Cooksley, W. C. E., Bradbear, R. A., Robinson, W., Harrison, H., Halliday, J. W., Powell, L. W., Ng, H.-S., Seah, C.-S., Okuda, K., Scheuer, J., and Sherlock, S. (1986). The prognosis of chronic active hepatitis without cirrhosis in relation to bridging necrosis. *Hepatology* **6**, 345–348.

78. Searle, J., Harmon, B. V., Bishop, C. S., and Kerr, J. F. R. (1987). The significance of cell death by apoptosis in hepatobiliary disease. *J. Gastroenterol. Hepatol.* **2**, 77–96.
79. Dienes, H. P. (1989). "Viral and Autoimmune Hepatitis," pp. 47–51. Fischer Verlag, Stuttgart.
80. Mackay, I. R., Taft, L. I., and Cowling, D. C. (1959). Lupoid hepatitis and the hepatic lesions of systemic lupus erythematosus. *Lancet* **1**, 65–69.
81. Beck, J. S. (1961). Variations in the morphological patterns of "autoimmune" nuclear fluorescence. *Lancet* **1**, 1203–1205.
82. Czaja, A. J., Nishioka, M., Morshed, S., and Hachiya, T. (1994). Patterns of nuclear immunofluorescence and reactivities to recombinant nuclear antigens in autoimmune hepatitis. *Gastroenterology* **107**, 200–207.
83. Mire-Sluis, A. R., Gaines Das, R. Lernmark, Å., *et al.* (2000). The World Health Organization International Collaborative Study for islet cell antibodies. *Diabetologia* **43**, 1282–1292.
84. Tan, E. M., Feltkamp, T. E. W., Smolen, J. S., *et al.* (1997). Range of antinuclear antibodies in "healthy" individuals. *Arthritis Rheum.* **40**, 1601–1611.
85. Mackay, I. R. (2001). Antinuclear (chromatin) autoantibodies in autoimmune hepatitis. *J. Gastroenterol. Hepatol.* **16**, 245–247.
86. Wesierska-Gadek, J., and Penner, E., (1996). Nuclear antigens. In "Molecular Basis of Autoimmune Hepatitis" (I. G. McFarlane and R. Williams, Eds.), pp. 23–44. Landes, Austin.
87. Konikoff, F., Swissa, M., and Schoenfeld, Y. (1989). Autoantibodies to histones and their subfractions in chronic liver diseases. *Clin. Immunol. Immunopathol.* **51**, 77–82.
88. Czaja, A. J., Ming, C., Shirai, M., and Nishioka, M. (1995). Frequency and significance of antibodies to histones in autoimmune hepatitis. *J. Hepatol.* **23**, 32–38.
89. Jain, S., Markham, T., and Thomas, H. C. (1976). Double-stranded DNA-binding capacity of serum in acute and chronic liver disease. *Clin. Exp. Immunol.* **26**, 35–41.
90. Peretz, A., Mascart-Lemone, F., Nuttin, G., and Famaey, J. P. (1982). Chronic active hepatitis with a high level of anti-ds-DNA detected by a solid phase radioimmunoassay. *Clin. Rheumatol.* **1**, 208–211.
91. Smeenk, R., van der Lelij, G., and Swaak, T. (1982). Specificity in systemic lupus erythematosus of antibodies to double-stranded DNA measured with the polyethylene glycol precipitation assay. *Arthritis Rheum.* **25**, 631–638.
92. Gurian, L. E., Rogoff, T. M., Ware, A. J., Jordan, R. E., Combes, B., and Gilliam, J. N. (1985). The immunological diagnosis of chronic active "autoimmune" hepatitis: Distinction from systemic lupus erythematosus. *Hepatology* **5**, 397–402.
93. Leggett, B. A., Collins, R. V., Cooksley, W. G. E., Prentice, D. I., and Powell, L. W. (1987). Evaluation of the crithidia assay to distinguish between chronic active hepatitis and systemic lupus erythematosus. *J. Gastroenterol. Hepatol.* **2**, 202–211.
94. Wood, J. R., Czaja, A. J., Beaver, S. J., Hall, S. J., Ginsburg, W. W., Kaufman, D. K., and Markowitz, H. (1986). Frequency and significance of antibody to double-stranded DNA in chronic active hepatitis. *Hepatology* **6**, 976–980.
95. Emlen, W., and O'Neill, L. (1997). Clinical significance of antinuclear antibodies. Comparison of detection with immunofluorescence and enzyme-linked immunosorbent assays. *Arthritis Rheum.* **40**, 1612–1618.
96. Konikoff, F., Schoenfeld, Y., Isenberg, D. A., Barrison, I., Sobe, T., Theodor, E., and Slor, H. (1987). Anti-Rnp antibodies in chronic liver diseases. *Clin. Exp. Rheumatol.* **5**, 359–361.
97. Penner, E., Kindas-Mugge, I., Hitchman, E., and Sauermann, G. (1986). Nuclear antigens recognized by autoantibodies present in liver disease sera. *Clin. Exp. Immunol.* **63**, 428–433.
98. Nishioka, M., Morshed, S. A., Parveen, S., and Ming-C. (1998). Heterogeneity of antinuclear antibodies in autoimmune liver diseases. In "Autoimmune Liver Diseases" (E. L. Krawitt, R. H. Wiesner, and M. Nishioka, Eds.), 2nd ed., pp. 179–216. Elsevier, Amsterdam.
99. Kenneally, D., Mackay, I. R., and Toh, B. H. (1984). Antinucleolar autoantibodies demonstrated by monolayers of human fibroblasts in sera from patients with systemic lupus erythematosus, progressive systemic sclerosis and chronic active hepatitis. *J. Clin. Lab. Immunol.* **14**, 13–16.
100. Wesierska-Gadek, J., Penner, E., Hitchman, E., and Sauermann, G. (1988). Antibodies to nuclear lamins in autoimmune liver disease. *Clin. Immunol. Immunopathol.* **49**, 107–115.
101. Smalley, M. J., Mackay, I. R., and Whittingham, S. (1968). Antinuclear factors and human leucocytes: Reaction with granulocytes and lymphocytes. *Aust. Ann. Med.* **17**, 28–32.
102. Zauli, D., Ghetti, S., Grassi, A., Descovich, C., Cassam, F., Ballardini, G., Muratori, L., and Bianchi, F. B. (1997). Neutrophil cytoplasmic antibodies in type 1 and type 2 autoimmune hepatitis. *Hepatology* **25**, 1005–1007.
103. Spronk, P. E., Bootsma, H., Horst, G., Huitema, M. G., Limburg, P. C., Cohen Tervaert, J. W., and Kallenberg, C. G. (1996). Antineutrophilcytoplasmic antibodies in systemic lupus erythematosus. *Br. J. Rheumatol.* **35**, 625–631.
104. Mackay, I. R., and Toh, B.-H. (2002). Autoimmune hepatitis. The way we were, the way we are today, and the way we hope to be. *Autoimmunity* **35**, 293–305.
105. Berg, P. A., Stechemesser, E., Strienz, J., *et al.* (1981). Hypergammaglobulinämische chronisch aktive Hepatitis mit Nachweis von leber-pankreas-spezifischen komplemtbindenden Autoantiköpern. *Verh. Dtsch. Ges. Inn. Med.* **87**, 921–927.
106. Manns, M., Gerken, G., Kyriatsoulis, A., *et al.* (1987). Characterization of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. *Lancet* **1**, 292–294.
107. Wächter, B., Kyriatsoulis, A., Lohse, A. W., Gerken, G., Meyer zum Büschenfelde, K.-H., and Manns, M. P. (1990). Characterisation of liver cytokeratin as a major target antigen of anti-SLA antibodies. *J. Hepatol.* **11**, 232–239.
108. Wesierska-Gadek, J., Grimm, R., Hitchman, E., and Penner, E. (1998). Members of the glutathione *S*-

transferase gene family are antigens in autoimmune hepatitis. *Gastroenterology* **114**, 329–335.
109. Wies, I., Brunner, S., Henninger, J., *et al.* (2000). Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. *Lancet* **355**, 1510–1515.
110. Costa, M., Rodriguez-Sanchez, J. L., Czaja, A. J., and Gelpi, C. (2000). Isolation and characterization of cDNA encoding the antigenic protein of the human tRNP (ser) sec complex recognized by autoantibodies from patients with type-1 autoimmune hepatitis. *Clin. Exp. Immunol.* **121**, 364–374.
111. Kevenbeck, T., Lohse, A. W., and Grötzinger, J. A. (2001). A coinformational approach suggests the function of the autoimmune hepatitis target antigen soluble liver antigen/liver pancreas. *Hepatology* **34**, 230–233.
112. Elkon, K., Skelly, S., Parnassa, A., Moller, A. P., Danho, W., Weissbach, H., and Brot, N. (1986). Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7419–7423.
113. Koren, E., Schnitz, W., and Reichlin, M. (1993). Concomitant development of chronic active hepatitis and antibodies to ribosomal P proteins in a patient with systemic lupus erythematosus. *Arthritis Rheum.* **36**, 1325–1328.
114. Koren, E., Reichlin, M. W., Koscec, M., Fugate, R. D., and Reichlin, M. (1992). Autoantibodies to the ribosomal P proteins react with a plasma membrane related target on human cells. *J. Clin. Invest.* **89**, 1236–1241.
115. Koscec, M., Koren, E., Wolfson-Reichlin, M., Fugate, R. D., Trieu, E., Targoff, I. N., and Reichlin, M. (1997). Autoantibodies to ribosomal P proteins penetrate into live hepatocytes and cause cellular dysfunction in culture. *J. Immunol.* **159**, 2033–2041.
116. Winfield, J. B. (1997). Are anti-ribosomal P protein antibodies a type of anti-lymphocyte antibody? *Clin. Exp. Immunol.* **109**, 1–3.
117. Arnett, F. C., and Reichlin, M. (1995). Lupus hepatitis: An under-recognized feature associated with autoantibodies to ribosomal P. *Am. J. Med.* **99**, 465–472.
118. Hulsey, M., Coldstein, R., Scully, L., Surbeck, W., and Reichlin, M. (1995). Anti-ribosomal P antibodies in systemic lupus erythematosus: A case control study correlating hepatic and renal disease. *Clin. Immunol. Immunopathol.* **74**, 252–256.
119. McFarlane, I. G., McFarlane, B. M., Major, G. N., Tolley, P., and Williams, R. (1984). Identification of the hepatic asialoglycoprotein receptor (hepatic lectin) as a component of liver specific membrane lipoprotein (LSP). *Clin. Exp. Immunol.* **55**, 347–354.
120. McFarlane, B. (1996). Hepatocellular membrane antigens. In "Molecular Basis of Autoimmune Hepatitis" (I. G. McFarlane and R. Williams, Eds.), pp. 75–104. Landes, Austin.
121. Treichel, U., McFarlane, B., Seki, T., *et al.* (1994). Demographics of anti-asialoglycoprotein receptor autoantibodies in autoimmune hepatitis. *Gastroenterology* **107**, 799–804.
122. Kurki, P. (1994). Cytoskeleton antibodies. In "Autoimmune Hepatitis" (M. Nishioka, G. Toda, M. Zeniya, Eds.), pp. 185–198. Elsevier, Amsterdam.
123. Toh, B.-H. (1979). Smooth muscle autoantibodies and autoantigens. *Clin. Exp. Immunol.* **38**, 621–628.
124. Whittingham, S., Irwin, J., Mackay, I. R., and Smalley, M. (1966). Smooth muscle autoantibody in "autoimmune" hepatitis. *Gastroenterology* **51**, 499–505.
125. Whittingham, S. F., and Mackay, I. R. (1996). Smooth muscle autoantibodies. In "Autoantibodies" (J. B. Peter and Y. Schoenfeld, Eds.), pp. 767–773. Elsevier, Amsterdam.
126. Fusconi, M., Cassani, F., Lenzi, M., Ballardini, G., Volta, U., and Bianchi, F. B. (1990). Anti-actin antibodies: A new test for an old problem. *J. Immunol. Methods* **130**, 1–8.
127. Cançado, E. L. R., Vilas-Boas, L., Abrantes-Lemos, C. P., Novo, N. F., Porta, G., Da Silva, L. C., and Laudanna, A. A. (1996). Heat serum inactivation as a mandatory procedure for anti-actin antibody detection in cell culture. *Hepatology* **23**, 1098–1104.
128. Cançado, E. L. R., Abrantes-Lemos, C. P., Vilas-Boas, L. S., Novo, N. F., Carrilho, F. J., and Laudanna, A. A. (2001). Thermolabile and calcium-dependent serum factor interferes with polymerized actin, and impairs anti-actin antibody detection. *J. Autoimmun.* **17**, 223–228.
129. Czaja, A. J., Cassani, F., Cataleta, M., Valentine, P., and Bianchi, F. B. (1996). Frequency and significance of antibodies to actin in type 1 autoimmune hepatitis. *Hepatology* **24**, 1068–1073.
130. Silvestrini, R. A., and Benson, E. M. (2001). Whither smooth muscle antibodies in the third millenium. *J. Clin. Pathol.* **54**, 677–678.
131. Yasuura, S., Veno, T., Watanabe, S., Hirose, M., and Namahisa, T. (1989). Immunocytochemical localization of myosin in normal and phalloidin-treated rat hepatocytes. *Gastroenterology* **97**, 982–989.
132. Mackay, I. R. (1999). Immunological perspectives on chronic hepatitis: Virus infection, autoimmunity and xenobiotics. *Hepatogastroenterology* **46**, 3021–3033.
133. Waxman, D. J., Lapenson, D. P., Krishnan, M., Bernard, O., Kreibich, G., and Alvarez, F. (1988). Antibodies to liver/kidney microsomes in chronic active hepatitis recognize specific forms of hepatic cytochrome P450. *Gastroenterology* **95**, 1326–1331.
134. Manns, M. P., Johnson, E. F., Griffin, K. J., Tan, E. M., and Sullivan, K. F. (1989). Major antigen of liver kidney microsomal autoantibodies in idiopathic autoimmune hepatitis in cytochrome P450db. *J. Clin. Invest.* **83**, 1066–1072.
135. Manns, M. P., Griffin, K. J., Sullivan, K. F., and Johnson, E. F. (1991). LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P450 monooxygenase. *J. Clin. Invest.* **88**, 1370–1378.
136. Homberg, J.-C., André, C., and Abuaf, N. (1984). A new anti-liver/kidney microsome antibody (anti-LKM2) in tienilic induced hepatitis. *Clin. Exp. Immunol.* **55**, 561–570.
137. Beaune, P. H., Dansette, P. M., Mansuy, D., Kiffel, L., Finck, M., Amar, C., Leroux, J. P., and Homberg, J.-C.

(1987). Human anti-endoplasmic reticulum autoantibodies appearing in a drug-induced hepatitis directed against a human liver cytochrome P450 that hydroxylates the drug. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 551–555.

138. Bourdi, M., Larrey, D., Nataf, J., Bernuau, J., Pessayre, D., Iwasaki, M., Guengerich, P., and Beaune, P. H. (1990). Anti-liver endoplasmatic reticulum auto-antibodies are directed against human cytochrome P450 IA2: A specific marker of dihydralazine-induced hepatitis. *J. Clin. Invest.* **85**, 1967–1973.
139. Manns, M. P., and Obermayer-Straub, P. (1997). Cytochromes P450 and UDP-glucuronyl transferases: Model antigens to study drug-induced, virus-induced and autoimmune liver diseases. *Hepatology* **26**, 1054–1066.
140. Crivelli, O., Lavarini, C., Chiaberge, E. A., Amoroso, A., Farci, P., Negro, F., and Rizzetto, M. (1983). Microsomal auto-antibodies in chronic infection with the HBsAg associated delta (δ) agent. *Clin. Exp. Immunol.* **54**,232–238.
141. Durazzo, M., Philipp, T., van Pelt, F. N. A. M., Lüttig, B., Borghesio, E., Michel, G., Schmidt, E., Loges, S., Rizzetto, M., and Manns, M. (1995). Heterogeneity of microsomal auto-antibodies (LKM) in chronic hepatitis C and D virus infection. *Gastroenterology* **108**, 455–462.
142. Strassburg, C. P., Obermayer-Straub, P., Alex, B., Durazzo, M., Rizzetto, M., Tukey, R. H., and Manns, M. P. (1996). Autoantibodies against glucuronosyltransferases differ between viral hepatitis and autoimmune hepatitis. *Gastroenterology* **111**, 1582–1592.
143. Bachrich, T., Thalhammer, T., Jäger, W., Haslmayer, P., Alihodzic, B., Bakos, S., Hitchman, E., Senderowicz, A. M., and Penner, E. (2001). Characterization of autoantibodies against uridine-diphosphate glucuronosyltransferase in patients with inflammatory liver diseases. *Hepatology* **33**, 1053–1059.
144. Martin, E., Abauf, N., Cavalli, F., Durand, V., Johanet, C., and Homberg, J.-C. (1988). Antibody to liver cytosol (anti-LC1) in patients with autoimmune chronic active hepatitis type 2. *Hepatology* **8**, 1662–1666.
145. Abauf, N., Johanet, C., Chretien, P., Martini, E., Soulier, E., Laperche, S., and Homberg, J.-C. (1992). Characterization of the liver cytosol antigen type 1 reacting with autoantibodies in chronic active hepatitis. *Hepatology* **16**, 892–898.
146. Han, S., Tredger, M., Gregorio, G. V., Mieli-Vergani, G., and Vergani, D. (1995). Anti-liver cytosolic antigen type 1 (LC1) antibodies in childhood autoimmune liver disease. *Hepatology* **21**, 58–62.
147. Muratori, L., Cataleta, M., Muratori, P., Manotti, P., Lenzi, M., Cassani, F., and Bianchi, F. B. (1995). Detection of anti-liver cytosol antibody type 1 (anti-LC1) by immunodiffusion, counterimmunoelectrophoresis and immunoblotting: Comparison of different techniques. *J. Immunol. Methods* **187**, 259–264.
148. Lapierre, P., Hajoui, O., Homberg, J.-C., and Alvarez, F. (1999). Formaminotransferase cyclodeaminase is an organ-specific autoantigen recognised by sera of patients with autoimmune hepatitis. *Gastroenterology* **116**, 643–649.
149. Muratori, L., Sztul, E., Muratori, P., Gao, Y.-S., Ripalti, A., Ponte, C., Lenzi, M., Landini, M. P., and Bianchi, F. B. (2001). Distinct epitopes on formaminotransferase cyclodeaminase induce liver cytosol antibody type 1. *Hepatology* **34**, 494–501.
150. McMurray, R. W., and Elbourne, K. (1997). Hepatitis C virus infection and autoimmunity. *Semin. Arthritis Rheum.* **26**, 689–701.
151. Lunel, F., Abauf, N., Frangeul, L., *et al.* (1992). Liver/kidney microsome antibody type 1 and hepatitis C virus infection. *Hepatology* **16**, 630–636.
152. Choudhuri, K., Miele-Vergani, G., and Vergani, D. (1997). Cytochrome P4502D6: Understanding an autoantigen. *Clin. Exp. Immunol.* **108**, 381–383.
153. Clifford, B. D., Donahue, D., Sonith, L., Cable, E., Lüttig, B., Manns, M., and Bonkovsky, H. L. (1995). Prevalence of serological markers of autoimmunity in patients with hepatitis C. *Hepatology* **21**, 613–619.
154. Kammer, A. R., Van der Burg, S. H., Grabscheid, B., Hunziger, I. P., Kwappenberg, K. M., Reichen, J., Meleif, C. J., and Cerny, A. (1999). Molecular mimicry of human cytochrome P450 by hepatitis C virus at the level of cytotoxic T cell recognition. *J. Exp. Med.* **190**, 169–176.
155. Wakeland, E. K., Liu, K., Graham, R. R., and Behrens, T. W. (2001). Delineating the genetic basis of systemic lupus erythematosus. *Immunity* **15**, 397–408.
156. Mackay, I. R., and Morris, P. J. (1972). Association of autoimmune chronic hepatitis with HLA-A1-B8. *Lancet* **2**, 793–795.
157. Mackay, I. R., and Tait, B. D. (1980). HLA associations with autoimmune-type chronic active hepatitis: Identification of B8-DRw3 haplotype by family studies. *Gastroenterology* **79**, 95–98.
158. Mackay, I. R., O'Brien, R. M., Whittingham, S., and Tait, B. D. (1991). Autoimmune hepatitis and other diseases of the liver: Immunogenetic aspects. In "The Immunogenetics of Autoimmune Disease Volume 2" (N. Farid, Ed.), pp. 119–213. CRC Press, Boca Raton, Florida.
159. Tait, B., Mackay, I. R., Board, P. H., Coggan, M., Emery, P., and Eckardt, G. (1989). HLA A1, B8, DR3 extended haplotypes in autoimmune chronic hepatitis. *Gastroenterology* **97**, 479–481.
160. Doherty, D. G., Underhill, J. A., Donaldson, P. T., Manabe, K., Mieli-Vergani, G., Eddleston, A. L. F. W., Vergani, D., Demaine, A. G., and Williams, R. (1994). Polymorphism in the human complement C4 genes and genetic susceptibility to autoimmune hepatitis. *Autoimmunity* **18**, 243–249.
161. Donaldson, P. T., Doherty, D. G., Hayllar, K. M., McFarlane, I. G., Johnson, P. J. and Williams, R. (1991). Susceptibility to autoimmune chronic active hepatitis: Human leukocyte antigens DR4 and A1-B8-DR3 are independent risk factors. *Hepatology* **13**, 701–706.
162. Czaja, A. J., Strettell, M. D., Thomson, L. J., Santrachi, P. J., Moore, S. B., Donaldson, P. T., and Williams, R. (1997). Associations between alleles of the major histocompatibility complex and type 1 autoimmune hepatitis. *Hepatology* **25**, 317–323.

163. Doherty, D. G., Donaldson, P. T., Underhill, J. A., *et al.* (1994). Allelic sequence variation in the HLA class II genes and proteins in patients with autoimmune hepatitis. *Hepatology* **19**, 609–615.
164. Czaja, A. J., and Donaldson, P. T. (2000). Genetic susceptibilities for immune expression and liver cell injury in autoimmune hepatitis. *Immunol. Rev.* **174**, 250–259.
165. Fronek, Z., Timmerman, L. A., Alper, C. A., Hahn, B. H., Kalunian, K., Peterlin, B. M. and McDevitt, H. O. (1990). Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus. *Arthritis. Rheum.* **33**, 1542–1553.
166. Vyse, T. J., Todd, J. A., and Kotzin, B. L. (1998). Non-MHC genetic contributions to autoimmune disease. In "The Autoimmune Diseases" (N. R. Rose and I. R. Mackay, Eds.), 3rd ed., pp. 85–118. Academic Press, San Diego.
167. Wick, G., Hála, K., Wolf, H., Ziemiecki, A., Sundick, R. S., Stöffler-Meilicke, M., and DeBaets, M. (1986). The role of genetically-determined primary alterations of the target organ in the development of spontaneous autoimmune thyroiditis in obese strain (OS) chickens. *Immunol. Rev.* **94**, 113–136.
168. Multiauthor Review (Co-ordinator: J. M. Davies) (2000). Molecular mimicry. *Cell Mol. Life Sci.* **57**, 523–578.
169. Vento, S., Guella, L., Mirandola, F., Cainelli, F., Di Perri, G., Solbiati, M., Ferraro, T., and Concia, E. (1995). Epstein-Barr virus as a trigger for autoimmune hepatitis in susceptible individuals. *Lancet* **346**, 608–609.
170. Kita, H., Mackay, I. R., Van de Water, J., and Gershwin, M. E. (2001) The lymphoid liver: considerations on pathways to autoimmune injury. *Gastroenterology* **120**, 1485–14501.
171. Mackay, I. R. (1996). Immune mechanisms and liver toxicity. In "Handbook of Experimental Pharmacology Series: Drug-Induced Hepatotoxicity" (R. G. Cameron, G. Feuer, and F. A. de la Inglesia, Eds.), Chapter 10, pp. 221–247. Springer, Berlin.

36

THE CELLULAR HEMATOLOGY OF SYSTEMIC LUPUS ERYTHEMATOSUS

Ronit Simantov
Jeffrey Laurence
Ralph L. Nachman

Hematologic abnormalities are common in systemic lupus erythematosus (SLE); nearly every patient will manifest some hematologic disturbance at one point in the disease. If this association is not recognized, such changes can lead to the spurious diagnosis of a primary blood disorder.

The American College of Rheumatology revised criteria for the classification of SLE established hematologic parameters for the diagnosis of SLE (Table 1) [1, 2]. The four attributes emphasized (hemolytic anemia, leukopenia, lymphopenia, and thrombocytopenia) are the most common hematologic abnormalities seen in SLE patients, and are discussed in this chapter, as are some other aspects of cellular hematologic disturbances in SLE.

Hematologic abnormalities are often an initial manifestation of SLE, and are among a variety of important prognostic variables which predict the course of the disease. In a study of 464 patients with SLE, autoimmune hemolytic anemia (AIHA) and immune thrombocytopenic purpura (ITP) represented a serious or life-threatening complication in 8 and 16% of cases, respectively [3]. Similarly, in a prospective study of 126 patients in the Netherlands, hemolytic anemia was found in 13%, neutropenia in 47%, lymphopenia in 20%, and thrombocytopenia, which was also found to be associated with decreased survival, was found in 27% [4]. In a study of familial lupus in French Caucasian patients, thrombocytopenia was found in 21% and hemolytic anemia in 6.6%, a rate similar to that seen in a matched group of patients with sporadic SLE. Although other manifestations of SLE such as renal disease and discoid rash were significantly different in sporadic and familial SLE, the rate of hematologic disase was constant [5].

In general, hematologic abnormalities represent a marker for disease severity in SLE, reflecting the effects of the inflammatory disease on bone marrow function and on peripheral blood cell survival [6]. Characteristics of hematologic abnormalities may predict the course of disease. Therefore, recognition and proper management of hematologic complications in patients with SLE is critical because of differences with respect to prognosis, therapy, and survival.

ANEMIA

Anemia, defined as a hemoglobin <12 g/dl for women and 13.5 g/dl for men, is common in patients with SLE. Multiple pathogenic mechanisms have been recognized, including impaired erythropoieisis, metabolic anemias, and immune-mediated peripheral destruction of red cells. The causes of anemia in SLE are outlined in Table 2.

Anemias Due to Decreased Red Cell Production

The majority of SLE patients with moderate decrements in hemoglobin concentration (8–11 g/dl) and a

TABLE 1 Hematologic Criteria for the Diagnosis of SLE[a]

Hemolytic anemia: hematocrit <35% with an increased reticulocyte index
Leukopenia: <4000/mm^3 on two or more determinations
Lymphopenia: <1500/mm^3 on two or more determinations
Thrombocytopenia: <100,000/mm^3, in the absence of drug therapy known to be associated with a decreased platelet count

[a] Data derived from Tan *et al*. [1].

TABLE 2 Pathophysiology of Anemia in SLE

Nonimmune mechanisms
Anemia of chronic disease
Renal disease
Drug-induced
Iron deficiency
Bone marrow hypoplasia
Immune-mediated mechanisms
Autoimmune hemolytic anemia
Cryoglobulinemia
Drug-induced (penicillin, quinine, α-methyldopa)
Pure red cell aplasia
Anti-erythropoietin antibodies

normal peripheral blood smear are diagnosed as having anemia of chronic disease (ACD). By definition, ACD excludes anemia due to bleeding, hemolysis, or bone marrow infiltration, and is characterized by hypoferremia with normal or increased serum ferritin; normal or elevated serum total iron binding capacity; increased red cell protoporphyrins; reduced or absent marrow sideroblasts in the presence of adequate iron stores; and a normal bone marrow myeloid to erythroid ratio. The pathogenesis of ACD is related to ineffective iron incorporation into the erythron despite normal iron stores. This may be due to trapping of iron in cytokine-activated macrophages, effectively reducing the amount of circulating iron available to developing erythrons [7].

In patients with ACD, the plasma level of erythropoietin is often low in relation to hemoglobin concentration. Patients with SLE have been found to have high levels of inflammatory cytokines which may be related to the pathogenesis of ACD [8]. *In vitro*, IL-1 and TNF-α, as well as interferon α and interferon β, inhibit erythropoietin mRNA levels and erythropoietin synthesis in cell cultures and perfused rat kidney models [9]. Inflammation-associated cytokines, such as TNF-α, IL-1, and TGF-β, have an inhibitory effect on erythroid colony formation *in vitro* [10]. It is likely, therefore, that the pathophysiology of ACD in patients with SLE involves the inhibition of erythropoietin production by inflammatory cytokines, and impaired response of the bone marrow to erythropoietin.

The ACD in SLE complicated by renal failure represents a distinct subset of patients with normochromic, normocytic anemias. The most important pathophysiologic mechanism in the anemia of renal failure is bone marrow hypoproliferation due to a decrease in erythropoietin production by the diseased kidney. Recombinant erythropoietin therapy has been shown to be safe and effective in the treatment of anemia of chronic renal disease [11, 12]. In addition, it is essential that iron stores be adequate in all SLE patients with anemia.

There is evidence that recombinant erythropoietin can improve the hematocrit in patients with impaired erythropoiesis due to inflammatory disease. Erythropoietin therapy has been used in patients with ACD related to rheumatoid arthritis, malignancies, AIDS, and inflammatory bowel disease, with improvement in hematocrit by several percentage points, a decrease in red cell transfusion requirements, and an improvement in quality of life parameters [13]. A randomized, placebo-controlled trial of erythropoietin therapy in rheumatoid arthritis patients showed a significant improvement in hemoglobin concentration and an overall decrease in disease activity [14]. In a study of recombinant erythropoietin therapy in five patients with SLE without renal failure, at doses of 400 units once a week to 3000 units three times a week for 3–7 months, all patients experienced a rise in the hematocrit (mean increase from 32 ± 2 to 42.2 ± 3), with decrease to baseline values 1 to 2 months after erythropoietin was discontinued. SLE activity measured by serum C3, creatinine, and urinalysis was not affected, and therapy was tolerated well, although one patient with anticardiolipin antibodies had an episode of thrombophlebitis while on erythropoietin therapy [15].

Although recombinant erythropoietin therapy may be appropriate for certain subsets of patients, including those who have severe anemia requiring transfusion [16], more recent studies have shown resistance to erythropoietin activity in SLE due to autoantibodies directed against erythropoietin [17]. In a series of 100 SLE patients with anemia, anti-erythropoietin antibodies were detected in 21, although the presence of these antibodies did not correlate directly with the level of anemia [18]. Anti-erythropoietin antibodies have also been demonstrated in pure red cell aplasia (PRCA), an immune-mediated anemia characterized by normochromic, normocytic anemia, reticulocytopenia, and marrow erythroid hypoplasia [19]. Antibodies in PRCA, a rare cause of anemia in SLE [20], have been reported to inhibit proliferation of erythroid precursors as demonstrated by decreased colony-forming units of

erythropoiesis (CFU-E) in bone marrow cultures [21, 22].

Erythropoietin therapy has been used with some success in patients with PRCA [23], even in patients with high endogenous erythropoietin levels [24, 25]. It has been proposed that pharmacologic doses of erythropoietin may overcome the interaction of autoantibodies with erythropoietin receptors on red cell precursors.

Other treatment modalities for presumed immune causes of PRCA have included high-dose cyclophosphamide, cyclosporin A, danazol, azathioprine, antilymphocyte globulin, and intravenous gamma globulin [26, 27]. An important etiology to exclude as a cause of aplasia is infection with human parvovirus B19, which may clinically mimic SLE, and is generally treated with intravenous gamma globulin [28, 29].

Iron deficiency anemia due to blood loss or malabsorption may occur in SLE, alone or in combination with other anemias. In a series of 132 SLE patients with anemia, iron deficiency anemia was as common as ACD (35.6 and 37.1%, respectively) [21]. The peripheral blood smear in iron deficiency reveals microcytic, hypochromic erythrocytes with anisocytosis and poikylocytosis. Thrombocytosis is a common finding. Iron studies reveal hypoferremia, elevated total iron binding capacity, and low transferrin saturation. Serum ferritin generally correlates well with storage iron levels but may appear normal in the setting of inflammatory processes. Bone marrow examination shows low or absent stainable iron.

Menstrual losses and/or pregnancy may explain iron deficiency in younger women with SLE, however, the finding of iron deficiency warrants a search for an underlying cause if there is no obvious source of bleeding. Patients with SLE may be particularly susceptible to gastrointestinal loss of iron due to the use of non-steroidal anti-inflammatory drugs, aspirin, and steroids. Therapy includes oral or parenteral iron supplementation.

Autoimmune Hemolytic Anemia

Increased erythrocyte destruction by autoantibodies, known as autoimmune hemolytic anemia (AIHA), can be an initial manifestation of SLE and occurs in approximately 10% of patients with SLE [4, 30]. Laboratory findings include anemia with reticulocytosis and a positive direct antiglobulin test (DAT, direct Coombs test), which detects IgG or C3b bound to the red blood cell membrane. A positive DAT may be found in SLE without concomitant hemolysis. Occasionally, the indirect antiglobulin test is also positive, indicating circulating autoantibodies to erythrocytes [31].

Additional laboratory testing includes indirect bilirubin and serum lactate dehydrogenase, which are elevated in hemolytic anemia, serum haptoglobin, which may be decreased. Review of the peripheral blood smear may reveal anisocytosis, spherocytosis, polychromasia, and nucleated red blood cells.

Erythrocyte autoantibodies are classified into two main groups based on their thermal requirements: "warm" antibodies of IgG or IgA isotype that associate with red cells optimally at 37°C; and IgM "cold" antibodies, which characteristically bind at 20°–25°C and dissociate when the temperature is raised to 35°C but coat the cell with complement. The most common form of AIHA is "warm" antibody AIHA, mediated by the binding of IgG (most often IgG1 or IgG3) and/or complement to antigens on the erythrocyte. The autoantibody is often directed toward an antigen in the Rh system. After binding to the erythrocyte, the Fc portion of the antibody then mediates binding to Fc receptors on splenic macrophages, resulting in destruction of the erythrocyte and clearance from the circulation.

Detailed serologic examination is indicated in actively hemolyzing patients with AIHA. Detection of antibodies may occasionally be complicated by the presence of circulating autoantibodies in patients with SLE. Serologic testing initially involves the DAT and ABO/Rh phenotyping. If the DAT is positive, a red cell eluate is made and tested for antibody reactivity, then titrated for quantitation and tested for specificity against a panel of red blood cells. If the DAT is negative but AIHA is strongly suspected, the more sensitive polybrene method is used. The serum is initially screened at 20° and 37°C, and tested for cold agglutinin. Further testing is done to determine thermal range and specificity, and antibodies are titrated against standard red cell panels for quantitation and specificity determination. In recently transfused patients, testing for alloantibodies is also done.

The cellular basis for AIHA in SLE can be divided into three areas: (1) cells responsible for autoantibody formation; (2) cells responsible for regulation of antibody-producing cells including T lymphocytes, monocytes, and idiotypic networks; and (3) cells which mediate the destruction of antibody-coated erythrocytes. The ultimate resolution of AIHA lies in the capacity to reestablish normal immunologic control mechanisms.

Several experimental models have helped to elucidate the mechanism of AIHA. The New Zealand Black (NZB) strain of mice spontaneously develops AIHA, and has been an important tool in studying the disease. Specific autoantibodies generated from these mice have been useful in characterizing red blood cell epitopes recognized by autoantibodies in AIHA [32, 33]. Studies

have shown the importance of genes regulating apoptosis in the development of autoreactive B cell clones. In particular, the expression of the bcl-2 gene can inhibit the autoantigen-induced apoptosis of B cells in the periphery, but not of immature B cells in the bone marrow, suggesting that regulation of autoreactive B cells occurs by a different mechanism in the bone marrow [34]. Transgenic models of autoimmune disease have also been useful in the study of AIHA. The anti-red blood cell Ig H + L (HL) mice were generated by introducing the heavy and light chain genes from an NZB-derived anti-red blood cell autoantibody producing hybridoma into fertilized mouse ova. The HL mice demonstrate hemolytic anemia despite clonal deletion of autoreactive B cells in the bone marrow and peripheral lymph nodes. In these mice, peritoneal cavity lymphocytes are sequestered without exposure to "self" red blood cell antigens and expand, subsequently undergoing activation by environmental antigens resulting in AIHA [35].

The importance of T helper cells in the expansion and activation of autoreactive B cells in the development of AIHA was supported by studies of RAG-2–/– mice, which lack mature B and T cells, crossed with HL mice. The RAG-2–/– × HL mice did not develop AIHA, indicating that the presence of T helper cells was required for the manifestations of the disease [36]. When the bcl-2 gene was introduced into HL mice, autoreactive B cells in the peritoneal cavity showed prolonged survival due to decreased apoptosis, and AIHA was potentiated. The cytokine network and genetic control leading to the generation and survival of B cells with specificity to erythrocytes remains to be elucidated.

Other mechanisms may also be involved in the pathophysiology of SLE-linked AIHA. In a prospective study of 500 consecutive patients with SLE, Alarcon-Segovia *et al.* showed that there was a significant association between the presence of hemolytic anemia and antiphospholipid antibodies (APLA) [37]. This relationship has been confirmed in other studies and suggests that the generation of APLA is not secondary to the hemolytic process, but rather may have some pathogenic significance in certain cases of AIHA [38, 39].

Therapy of AIHA is generally indicated in the presence of progressive hemolytic anemia with hemoglobin falling below 7 g/dl. Corticosteroids are the treatment of choice in IgG-mediated AIHA, with an initial daily dose of 1 mg/kg prednisone or its equivalent. Supplementary folic acid should be given to all patients with hemolytic anemia, due to increased requirements in the setting of rapid cell turnover. Response to therapy, indicated by increasing hematocrit and decreased reticulocyte count, is usually seen within 2 to 3 weeks. For responders, the prednisone is slowly tapered by about 5 mg daily every week. Approximately 75% of SLE patients have a serologic and clinical response to this therapy, although a positive DAT may persist following treatment.

The mechanism of corticosteroid activity is thought to be through the inhibition of binding of opsonized erythrocytes by Fc-receptors on mononuclear phagocytes. Corticosteroids decrease the number of Fc receptors expressed on monocytes, and can block the Fc receptor directly by competitive binding. In addition, corticosteroids inhibit the synthesis of autoantibodies by B cells [40].

Red blood cell transfusions may be indicated in the presence life-threatening anemia. Alternate forms of immunosuppressive therapy, including cyclosporin, cyclophosphamide, and azathioprine, are used to treat steroid-resistant AIHA. Because the spleen is the site of destruction of opsonized erythrocytes, splenectomy has long been used as a treatment of AIHA. Remission in response to splenectomy has been reported to be 50–75% [41]. Attempts have been made to predict response to splenectomy, which has been reported to be less successful in SLE patients than in non-SLE related AIHA. A valid predictor of response to splenectomy includes antibody type, as "warm" AIHA is more responsive than cold agglutinin–related hemolysis. Patients showing an initial prompt response to steroids are often reported to respond favorably to splenectomy compared to patients who require prolonged treatment; however, satisfactory remission rates in response to splenectomy have also been reported in patients who failed corticosteroid therapy [42]. Some patients will continue to require steroids to maintain a remission even after splenectomy [43].

Danazol, 600–800 mg per day, also appears a useful adjunct to glucocorticoid therapy in severe AIHA or in cases of steroid treatment failure [44]. Other therapeutic modalities include immunosuppressive therapy such as azathioprine and cyclophosphamide, which have been shown in uncontrolled trials to benefit patients who were unresponsive to steroids, splenectomy, or both. Antilymphocyte sera has been employed with variable and usually short-lived benefit. Plasmapheresis and plasma exchange has also been used, followed by maintenance therapy with an immunosuppressive agent [45]. Intravenous gamma globulin has also had positive results in patients with refractory AIHA [46]. Alternative therapy directed against autoantibody-producing cells includes Rituximab, an anti-CD20 monoclonal antibody which specifically depletes B lymphocyte and is approved for the treatment of lymphomas. Rituximab should be considered for treatment of refractory, life-threatening AIHA given several positive reports in patients with and without SLE [47].

Autoimmune hemolytic anemia occurring together with immune thrombocytopenia (ITP) is known as

Evans' syndrome. Patients usually have isolated episodes of thrombocytopenia and hemolytic anemia, which may also be accompanied by neutropenia. Autoantibodies against erythrocytes, platelets, and neutrophils have been demonstrated, and are directed against distinct, non-cross-reactive epitopes on red cells and platelets [48]. Evans' syndrome is seen in lupus patients and may be associated with a syndrome of immune dysregulation characterized by generalized lymphadenopathy and abnormal serum immunoglobulins, as well as with primary immunodeficiencies, lymphoproliferative disorders, and autoimmune lymphoproliferative syndrome [49, 50]. Treatment includes corticosteroids, cyclosporin, and intravenous immune globulin, although patients typically have a chronic and relapsing course with significant morbidity and mortality [51].

Other Causes of Anemia

Porphyria

Subsequent to the association of SLE with porphyria, first recognized in 1952, there have been numerous case reports of SLE associated with porphyria cutanea tarda, acute intermittent porphyria, and porphyria variegata [52].

Systemic lupus erythematosus and the hepatic porphyrias share a number of features, including rash, fever, photosensitivity, mucous membrane lesions, gastrointestinal symptoms, neuropathy, and psychosis. Both diseases may also be precipitated by sun exposure, fatigue, and drugs. Shared pathophysiologic mechanisms are suggested by findings of similar patterns of immunoglobulin and complement deposition in the epidermal basement membrane and dermal blood vessels. Case reports have emphasized the similarities in dermatologic and pathophysiologic features of SLE and porphyria cutanea tarda, other clinical SLE manifestations are often absent [53, 54]. Similarly, in a series of 15 patients with acute intermittent porphyria, 8 (53%) had a positive ANA, although only 1 had clinical evidence of SLE [55]. Indeed, the clinical and serologic data suggestive of a connective tissue disorder, when arising in an individual with porphyria cutanea tarda, may be secondary to hepatic dysfunction or to hepatitis C infection [56, 57].

THROMBOCYTOPENIA

Platelets are essential for normal hemostasis, acting to form an occlusive platelet plug at the site of injury and to accelerate the localized activation of circulating plasma procoagulants. Normal platelet counts range from 150,000–450,000/μl and the normal life span of circulating platelets is 7–10 days. Thrombocytopenia has been found to be present in 15–25% of SLE patients and is associated with increased mortality. In a single-institution study of 665 patients with SLE followed prospectively for 20 years, thrombocytopenia ($<100{,}000/mm^3$) was found to be an independent predictor for mortality, along with renal disease, lung involvement, disease activity, and age [58]. In a study of 389 patients with SLE, thrombocytopenia was found to be the only independent risk factor of poor prognosis [59]. Thrombocytopenia was also found to be one of several independent predictors of 6-month mortality in a series of 806 patients followed at the University of Toronto [60]. Interestingly, however, hemorrhagic complications of thrombocytopenia were a rare cause of death in these series. In a cohort of 300 lupus patients in the United Kingdom, 5% had hemolytic anemia and 17% had thrombocytopenia. In the 41 patients who died, the most common cause of death was malignancy (20%), and thrombocytopenia was not associated with an increased risk of death [61]. Acute thrombocytopenia occurring during SLE flares is particularly associated with complications and increased mortality [62].

The major causes of thrombocytopenia in SLE patients with platelet counts below $100{,}000/mm^3$ include (1) hypoproliferative thrombocytopenias; (2) peripheral destruction of platelets; and (3) abnormal platelet distribution due to congestive splenomegaly. The most common cause of thrombocytopenias is likely drug effect, and hundreds of different medications have been implicated. Medications causing peripheral destruction of platelets include quinine, quinidine, aspirin, heparin, sulfonamides, and non-steroidal anti-inflammatory drugs. Other agents, including thiazides, ethanol, and chemotherapeutic agents lead to depressed megakaryopoiesis. In this context, all drugs or chemicals should be suspected in patients with thrombocytopenia.

A study to determine the strength of clinical evidence for individual drugs as a cause of thrombocytopenia reviewed 515 patient case reports on patients with drug-induced thrombocytopenia (excluding heparin). In 247 patient case reports (48%), the drug appeared to be the causal agent of thrombocytopenia. Although a number of the reports reviewed in this study did not provide direct evidence for drug-related thrombocytopenia, over 98 drugs were implicated in thrombocytopenia, most frequently quinidine, trimethoprim-sulfamethoxazole, and gold [63].

An important, often underrecognized cause of thrombocytopenia in hospitalized patients is heparin-induced thrombocytopenia. This syndrome is characterized by a decrease in the platelet count typically 4 or more days after the onset of heparin exposure, and may

be accompanied by serious venous or arterial thrombosis. It is important to consider this diagnosis in SLE patients who are being treated with heparin, as the syndrome may mimic catastrophic antiphospholipid syndrome or vasculitis flare. The etiology involves the production of heparin-dependent anti-platelet factor 4 (PF4) antibodies which bind to platelets and endothelial cells and lead to immunologic destruction of platelets and a high risk of thromboembolic disease. Heparin is absolutely contraindicated in this condition and treatment should be initiated with a direct thrombin inhibitor such as lepirudin or argatroban [64].

In immune-mediated thrombocytopenias, the mechanism of platelet destruction involves the coating of platelets with IgG autoantibodies and clearance from the circulation by the phagocytic cells of the reticuloendothelial system. The platelet autoantibodies frequently are directed toward glycoprotein IIb/IIIa or GPIb/IX, the major adhesive protein receptors on the platelet surface. Circulating immune complexes are observed in some patients. In patients with chronic ITP, T cells reactive with altered GPIIb/IIIa complexes or with peptide fragments of GPIIb/IIIa have been detected. These reactive T cells were $CD4^+$ and HLA-DR restricted, and were involved in production of anti-GPIIb/IIIa antibodies in peripheral blood mixed lymphocyte cultures from ITP patients [65]. Attempts have been made to differentiate immune-mediated thrombocytopenia in SLE patients from "classic" ITP. Similar levels of platelet-associated IgG have been observed, but differences in T-cell responses have been noted [66].

Several studies have documented an association between the development of thrombocytopenia in patient with SLE and the presence of anti-phospholipid antibodies. Patients with a circulating lupus anti-coagulant or anti-cardiolipin antibody were three times more likely to have a history of a moderate to severe thrombocytopenia than were antibody-negative patients [67]. The presence of anti-phospholipid antibodies is associated with more severe thrombocytopenia in patients with SLE, but is also associated with endothelial cell activation and incurs a greater risk of thrombosis rather than clinical bleeding [68, 69]. The frequency of thrombocytopenia in SLE patients with anti-phospholipid antibodies has been shown to be 13–40% according to several series [70]. Conversely, a study of 109 patients with ITP showed that 69 (46%) had a positive lupus anti-coagulant or anti-cardiolipin antibody [71].

The diagnosis of ITP is made by excluding secondary causes of thrombocytopenia due to medications or associated with other illnesses [72]. Patients generally present with purpura, petechiae, gingival bleeding, and, in more severe cases, hemorrhagic bullae. Splenomegaly is evidence against ITP [73]. Typical characteristics of the history in ITP patients and findings on physical examination are described in Table 3.

TABLE 3 Diagnosis of ITP in SLE Patients[a]

History
Bleeding symptoms
Type of bleeding
Severity of bleeding
Systemic symptoms
Weight loss
Fevers
SLE disease activity
HIV risk factors
Pregnancy status
Medications
Alcohol use
Transfusion history
Comorbid conditions
Liver disease—may cause thrombocytopenia
Acute renal or neurologic dysfunction—suggest thrombotic thrombocytopenic purpura (TTP)
Physical examination
Skin: petechiae, ecchymoses
Mucosal signs of bleeding
Spleen—lack of splenomegaly
Evidence for thrombosis—consistent with anti-phospholipid antibody syndrome
Lymph nodes—enlargement consistent with SLE, or may suggest lymphoproliferative disease

[a] Adapted from Practice Guidelines in ITP developed by the American Society of Hematology [75].

The peripheral blood smear in ITP reveals a decrease in the number of platelets and an increase in megathrombocytes, and the bone marrow contains an increased number of megakaryocytes [74]. Laboratory studies for the diagnosis of ITP in SLE patients are outlined in Table 4. In order to standardize diagnosis and management of ITP, the American Society of Hematology convened a panel to establish specific practice guidelines for ITP. This guideline, which was published in 1996, is based on review of scientific evidence where available, and, for areas in diagnosis and management of ITP where only case series or no evidence was available, based on the consensus opinion of the expert panel [75].

The strategy of treatment of ITP associated with SLE is the same as for the idiopathic cases, with consideration of immediate need for intervention, long-term benefits, and long-term hazards of each therapeutic option. The benefits of steroid therapy for ITP have been known since the introduction of glucocorticoids into clinical practice in the early 1950s, and corticosteroids remain mainstay of therapy. Prednisone 1–2 mg/kg per day is the initial therapy of choice in ITP patients with

TABLE 4 Laboratory Studies in SLE Patients with Suspected ITP

Complete blood count
Peripheral blood smear
Findings consistent with ITP
Thrombocytopenia, some large platelets
Normal red blood cell morphology
Normal white blood cell morphology
Lupus anti-coagulant/anti-cardiolipin antibody
Bone marrow aspirate/biopsy
Findings consistent with ITP
Increased number of megakaryocyte
Normal myeloid and erythroid maturation
Consider
Biochemistry profile: BUN/creatinine, LDH
Direct antiglobulin test
HIV test
Thyroid function tests
Coagulation studies

[a] Adapted from Practice Guidelines in ITP developed by the American Society of Hematology [75].

platelet counts <30,000/mm^3 and/or purpura or other bleeding. If there is a response with a normalization of the platelet count over 1 to 2 weeks, prednisone is tapered by 5–10 mg/week. Although there are no randomized controlled trials comparing glucocorticoids to no treatment, or any studies showing an effect of glucocorticoids on overall morbidity or mortality, retrospective studies have reported complete response rates to steroids in adults to be 30–38%, overall response rates of platelet counts greater than 50,000/mm^3 of 65–80%, and prolonged remissions to steroids of 15–20% [76]. The proposed mechanism of corticosteroid action is decreased Fc-mediated clearance by antibody-coated platelets, and decreased production of autoantibodies. Corticosteroids may also reverse the bleeding diathesis prior to a rise in platelet count, by inhibition of vascular endothelial cell activation with resultant decrease in vascular permeability and vasoconstriction of the capillary bed.

Splenectomy is the oldest form of therapy for ITP. Platelets reside in the intravascular compartment and are destroyed by macrophages in the splenic sinusoids. The spleen provides an optimal microenvironment for the interaction of antibody- or immune-complex-coated platelets with phagocytes. Splenectomy is recommended for patients with persistent platelet counts <10,000/mm^3 for 6 weeks and little or no response to therapy. Splenectomy has been shown to lead to increase in platelet counts; however, a number of patients will relapse years after splenectomy. Response to splenectomy has been reported to be 50–80% in different case series, with 60–80% of patients achieving durable responses; half of relapses occur in the first 6 months [77, 78]. Laparoscopic splenectomy has been demonstrated to be effective in patients with ITP [79]. It is important to note that splenectomy is associated with a risk of infection with bacterial organisms, and patients should be vaccinated with pneumococcal, meningococcal, and *Haemophilus influenzae* vaccines prior to splenectomy.

IVIG, 1–2 gm/kg over 2 to 5 days, may be used in patients who are refractory to treatment with glucocorticoids, preoperatively prior to splenectomy, or in initial treatment for patients with severe bleeding. Anti-Rho (D), 50 µg/kg, should be considered in patients who are blood group Rh (+) and have not been splenectomized or for those in whom splenectomy is not feasible. In a study of 272 patients with ITP, a hemostatic platelet increase was found in greater than 70% of the Rh+ non-splenectomized patients. Response in splenectomized patients was minimal [80]. The proposed mechanism of action is blockade of the reticuloendothelial system with antibody–red cell antigen complexes. An advantage over intravenous immunoglobulin is the lower cost. Hemolysis may be seen, although it is generally moderate and self-limited.

Patients who relapse after splenectomy may be treated with prednisone at the lowest doses possible to maintain a safe platelet count. However, some patients will not respond to corticosteroids or will need doses higher than 10–15 mg/day, which might lead to unacceptable side effects and necessitate treatment with other agents. There is no single established algorithm for treatment of refractory ITP and pharmacologic treatment should be based on individual patient needs and the side effect profiles for the medications. If corticosteroids have failed, the next recommended agents are danazol 200 mg four times a day, vincristine 1–2 mg/week, colchicine, 0.6 mg three times per day, dapsone 75–100 mg per day, or vincristine 1–2 mg per week (IV). More potent immunosuppressive agents, including azathioprine 150 mg per day, oral cyclophosphamide (2 mg/kg per day), or high-dose intravenous cyclophosphamide (1–1.5 mg/m^2 every 4 weeks), may also be considered. Cyclosporin A is associated with a 40–50% response rate [81]. Pulsed high-dose dexamethasone (40 mg per day for 4 days) and high-dose intravenous methylprednisolone (15 mg/kg/day for 3 days) therapy [82] have also been used with some success in patients with refractory ITP [83]. Vincristine- and vinblastine-loaded allogeneic platelets have been used with some success in patients with refractory ITP (as well as refractory AIHA) as a method of delivering targeted vinca alkaloid to the reticuloendothelial system [84]. Some patients who do not respond to other pharmacologic

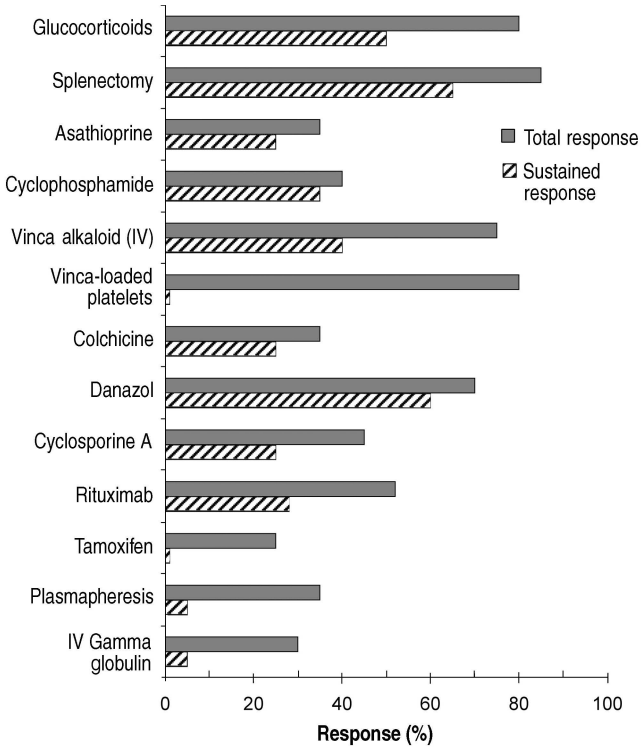


FIGURE 1 Rates of response (total or sustained) achieved with various therapeutic modalities for ITP are shown.

therapy continue to respond to intravenous gamma globulin at doses of 0.5–1.0 g/kg, although responses are always transient, lasting a few weeks, and the therapy is expensive. Reports have demonstrated the effectiveness of Rituximab, a chimeric monoclonal antibody directed against the CD20 antigen on B lymphocytes leading to B-cell depletion, in treatment of patients with chronic ITP [85]. Rates of response to various therapies for ITP are shown in Fig. 1.

A retrospective analysis of 59 patients with SLE and ITP showed that 80% of the 50 patients who were treated with prednisone alone responded initially but only 12% of these patients had a sustained remission. More favorable sustained responses were seen in patients treated with a combination of prednisone and either danazol or hydroxychloroquine (50 and 64%, respectively). Splenectomy was performed in 17 patients, 10 of whom had a sustained long-term response (65%) [86].

Thrombotic thrombocytopenic purpura (TTP) is comprised of a pentad of symptoms: thrombocytopenia, microangiopathic hemolytic anemia, fever, renal dysfunction, and neurologic manifestations. TTP has been described in patients with SLE, but strict criteria for an association are difficult to define as manifestations of SLE mimic many of the features of TTP [87]. In children, unlike in adults, TTP is associated with the development of SLE within the subsequent 4 years [88]. More recent discoveries have shed light on the pathogenesis of TTP. Plasma from patients with TTP has been found to contain unusually high molecular weight multimers of von Willebrand's factor (VWF). Levy *et al.* showed that patients with a rare familial form of TTP have inherited mutations in ADAMTS13, a member of the ADAMTS (a disintegrin-like domain, a metalloproteinase domain, and thrombospondin motifs) family of protein-cleaving proteases [89]. Simultaneously, three other groups reported the identification of ADAMTS13 as a protease that can cleave plasma VWF [90, 91]. Patients with TTP have been shown to contain an antibody against the protease [92]. SLE patients have been shown to contain an antibody against the von Willebrand factor protease and demonstrate reduced protease activity even in the absence of TTP. The significance of these findings in SLE remains to be explored [93].

The pathophysiology of TTP may also be related to the effect of plasma factors in TTP patients on microvascular endothelial cells (EC). Exposure of microvascular EC to diluted TTP plasma leads to the induction of apoptosis of EC [94]. Untreated TTP is fatal in over 80% of cases. Treatment of TTP includes emergent plasma infusion, plasmapheresis, steroids, and vincristine [95, 96].

Qualitative Platelet Defects

At sites of vascular injury, platelets adhere to the endothelium, display receptors for binding of fibrinogen and other coagulant factors, aggregate to form a hemostatic plug, and catalyze the rapid, localized production of thrombin. Within seconds, activated platelets release preformed vasoactive substances that contract vessels and accelerate blood clotting. They rapidly synthesize and release lipid mediators such as thromboxane A2 (TXA2), a potent vasoconstrictor and platelet activator.

Drug-induced platelet dysfunctions are the most frequent platelet disorders, and impaired platelet aggregation due to aspirin is by far the most common platelet dysfunction. Aspirin specifically blocks TXA2 formation in platelets by irreversibly inactivating platelet cyclooxygenase, a key enzyme in synthesis of TXA2. Platelets do not contain the inducible enzyme, cyclooxygenase-2 (COX-2), but only the constitutive form, cyclooxygenase-1, so that the new specific inhibitors of COX-2 developed for anti-inflammatory effects do not inhibit platelet function and thus do not induce a bleeding tendency. The thienopyridines such as clopidogrel block ADP binding to its receptors on platelets and therefore decrease platelet activation by

ADP but do not block the TXA2 pathway. Clopidogrel is used in prevention of thrombosis in vascular stents and for prevention of stroke and myocardial infarction.

Systemic lupus erythematosus patients also manifest autoimmune-mediated qualitative platelet defects. Antibodies directed against GPIIb/IIIa, the glycoprotein membrane receptor for fibrinogen, have been reported in SLE and can induce a thrombasthenia-like syndrome if they interact with or block functional sites on the GPIIb/IIIa complex. SLE patients with acquired storage pool deficiencies have also been reported.

Platelet adhesion requires interaction of membrane GPIb receptors with collagen or von Willebrand's factor (vWF) Acquired von Willebrand's disease is a rare complication of SLE [97]. In acquired von Willebrand's disease, the pathogenic mechanisms responsible include binding of anti-vWF IgG antibodies to vWF on endothelial cells and platelets leading to early removal of the vWF and a diminished level of large multimeric vWF, as well as the inactivation of vWF function by circulating anti-vWF antibodies [98]. Treatment of acquired VWD is similar to that of congenital disease, with replacement of factor or use of desmopressin to augment release of vWF, in conjunction with immunosuppressive agents [99].

LEUKOPENIA

Leukopenia, defined as a total peripheral blood count of less than 4500/mm^3 is common in SLE patients. Occasionally the leukocyte count will transiently fall to as low as 1000/mm^3; however, such a profound decrement should not be ascribed to SLE without a thorough search for other etiologies including drugs (e.g., antimalarials and immunosuppressive agents).

Although it might be anticipated that the leukopenia would be associated with an increased incidence of infection, in the absence of other factors this appears not to be the case. This was established by a prospective study of the clinical course of 223 SLE patients followed for 655 patient-years [100]. In this study, the frequency of infection increased progressively with increasing corticosteroid dose; however, leukopenia did not predispose to infection. These observations were confirmed by other studies which showed that bacterial infections were more frequent in SLE patients than in matched controls; however, no correlation was seen with levels of leukopenia [101]. The potential causes of leukopenia, related to peripheral destruction by immune- and non-immune-mediated mechanisms, sequestration, and drugs, are listed in Table 5 and discussed later.

Leukocytosis, in contrast, is rare in SLE in the absence of infection or corticosteroid therapy, though elevations in the leukocyte count may be seen during an acute exacerbation of SLE. During corticosteroid administration, leukocytosis with a relative lymphopenia is common because of granulocytosis.

TABLE 5 Mechanisms of Leukopenia in SLE

Antibody mediated
Anti-neutrophil antibodies
Anti-lymphocyte antibodies
Cross-reactive antibodies (e.g., anti-Ro)
Drug-induced
Bone marrow dysfunction
Decreased progenitor pool
Inadequate mobilization response
Peripheral destruction
Hypersplenism
Immune complexes
Circulating apoptotic neutrophils

Neutropenia

In a study of 126 patients with SLE, neutropenia occurred in 47%; however, there was no association of neutropenia with infections or overall mortality. The mechanisms of neutropenia in SLE include neutrophil-reactive autoantibodies, bone marrow suppression, and increased neutrophil apoptosis. Qualitative abnormalities of a range of granulocyte functions (adherence to vascular endothelium, chemotaxis, phagocytosis, and microbial killing) have been reported in SLE as well.

Anti-neutrophil antibodies have been described in patients with SLE in several series [102, 103]. Circulating neutrophils in SLE commonly bear increased amounts of membrane immunoglobulin, related to both immune-complex deposition and anti-neutrophil antibody. Evidence for antibody-mediated peripheral destruction in the autoimmune neutropenias is well documented [104]. Antigen specificity has been described but its significance is not known; anti-neutrophil cytoplasmic antibodies from a series of pediatric patients with SLE were directed toward a number of neutrophil antigens, including myeloperoxidase, cathepsin G, and elastase. These specificities, however, did not correlate with disease activity or with target organ involvement [105]. Specific autoantibodies to ribonucleoprotein particles (anti-Ro) have been shown to bind to a cross-reactive neutrophil membrane protein [106].

Arenas *et al.* found that the number of myeloid CFU-C is lower in SLE patients with severe neutropenia, suggesting an inhibition of myeloid development in patients with SLE [107]. Whether this inhibition is medi-

ated by cellular immunity, autoantibody effects, or bone marrow microenvironment is not known, although IgG autoantibodies from patients with SLE have been shown to bind to CD34 positive hematopoietic precursor cells [108]. Finally, SLE patients have been found to have increased numbers of apoptotic neutrophils, which correlate with increased disease activity [109]. One proposed mechanism is decreased expression of monocyte CD44 in SLE, leading to decreased binding and clearance of apoptotic neutrophils by monocyte-derived macrophages [110].

Recombinant human granulocyte colony stimulating factor (GCSF) has been used in combination with antibiotics to treat SLE patients with impaired wound healing, with a subsequent rise in their neutrophil counts [111]. Neutrophil counts have been shown to increase, with a decrease in the number of serious infections [112, 113]. Patients with SLE and neutropenia have also been treated with GCSF. GCSF has also been combined with high-dose corticosteroids in neutropenic patients with SLE [114]. GCSF and GM-CSF are generally well-tolerated, with the primary side effect being bone pain, although arthritis flares and leukocytoplasmic vasculitis have been reported in patients receiving GCSF [115]. The use of colony-stimulating factors remains a reasonable treatment option in selected patients with SLE whose neutrophil counts are below 1000/mm^3; however, studies of optimal use and cost–benefit analyses have yet to be performed [116].

Lymphocytes

Lymphopenia in SLE patients generally occurs during acute phases of the disease, with lymphocyte counts rising following effective therapy. Of 361 patients examined for 24 clinical and laboratory variables, lymphopenia was the only factor that occurred more frequently with increased age [117]. Lymphocytopenia is strongly associated with the presence of anti-Ro (SSA0 antibodies and with anti-ssDNA [118]. There is considerable evidence that anti-lymphocyte antibodies (ALA) may have a significant influence on the number and function of lymphocytes in SLE. These antibodies have been shown to react with T cells and disrupt T-cell functions such as interferon production. Another suggested mechanism of ALA-induced lymphopenia is the induction of cytokines which may in turn lead to reduced lymphocyte number [119]. Various specificities of SLE ALA have been related to lymphocyte membrane antigens as well as neural, nuclear, spermatozoan, and trophoblatic determinants. ALA have also been implicated in the pathogenesis of neuropsychiatric manifestations of SLE [120].

A proposed mechanism of lymphopenia associated with SLE is the regulation of apoptosis, involving Fas antigen and Fas ligand interactions. Fas antigen, a cell-surface molecule found in lymphocytes, mediates the transduction of apoptotic signals induced by activation [121, 122]. Fas antigen is also expressed on hematopoietic progenitor cells and may be involved in the regulation of hematopoiesis [123]. Lymphopoiesis has been explored in two murine models of SLE, the MRL-lpr/lpr mouse, which is defective in Fas antigen, and the C3H-gld/gld mouse, which has a defective Fas ligand. These studies showed that Fas/Fas ligand interactions caused apoptosis of T lymphocytes. In addition, overexpression of Fas antigen directly suppressed lymphopoiesis [124].

Monocytes

The percentage of monocytes in SLE is often greater than in normal controls, although absolute monocytosis (>800/mm^3) is unusual. Monocyte function such as generation of respiratory burst induced by phagocytosis has been shown to be decreased in monocytes isolated from patients with SLE [125]. Dysregulation of monocyte differentiation has also been observed in patients with SLE. Monocytes isolated from SLE patients were found to function as antigen-presenting cells *in vitro*, and serum from SLE patients induced normal monocytes to differentiate into antigen-presenting dendritic cells, which can induce T-cell activation and B-cell growth and differentiation, and may be involved in the induction of autoimmune responses [126]. Dysregulation of the production of cytokines such as IL-1 receptor antagonist [127], IL-10 [128], and IL-6 [129] by monocytes in SLE patients, may also play a role in the immunologic processes in this disease. These interactions are discussed in greater detail in other chapters.

Natural Killer Cells

Natural killer cells (NK) express the antigen CD56 and represent approximately 5% of circulating and splenic leukocytes. Natural killer cytotoxicity is a distinct arm of the host immune surveillance system and may be important in the elimination of tumors and virus-infected cells. NK bind to susceptible targets and effect rapid lysis, a function which has been reported to be impaired in SLE patients. NK cell cytolytic activity was shown to be greatly reduced in children with SLE, and defective function persisted even during inactive phases of the disease [130]. NK cells are also decreased in number in SLE patients, particularly during active disease [131].

BONE MARROW DISEASE IN SYSTEMIC LUPUS ERYTHEMATOSUS

The role of the bone marrow microenvironment in the maintenance of normal hematopoiesis is a subject of intense study. Cytokines produced by bone marrow stromal cells are essential for the differentiation and development of hematopoietic cells. Emerging characterization of bone marrow in patients with SLE has revealed defects in some of these processes, some of which may be important in the etiology of red cell hypoplastic syndromes in SLE [132]. Similarly, findings such as dyserythropoiesis, megaloblastic changes, or marrow hypoplasia, which suggest impaired erythroid production, may help to explain an impaired reticulocyte response in some SLE patients with AIHA [133]. Bone marrows of patients with lupus and cytopenias have also been reported to have storage and hemophagocytic histiocytes [134].

Myelofibrosis is a myeloproliferative disease characterized by the abnormal growth of erythroid, myeloid, and other hematopoietic precursors; varying degrees of marrow fibrosis; and extramedullary myeloid metaplasia in the spleen and liver. The pathogenesis of the prominent fibrosis is controversial, both in idiopathic cases and in those associated with infection, malignancy, and SLE, but may relate to the release of cytokines which stimulate fibroblast proliferation and collagen and fibronectin production. The onset is usually insidious, with symptoms of anemia or splenomegaly. Several cases have been recorded in association with SLE, and have been generally characterized by anemia and a leukoerythroblastic appearance on peripheral blood smear [135]. In addition, there have been a number of case reports of myelofibrosis as the presenting manifestation of SLE [136, 137]. Treatments that have been used include high-dose steroids as well as intravenous immunoglobulin, which may improve cytopenias but may not have a profound effect on the extent of bone marrow fibrosis [138].

Antimetabolite drugs such as azathioprine are often employed as immunosuppressive agents in SLE. The main hematopoietic toxicity of azathioprine is bone marrow depression with anemia, leukopenia, and thrombocytopenia. Long-term use of alkylating agents such as cyclophosphamide is also associated with an increased risk of myelodysplastic syndrome and secondary acute leukemia, which has been reported in SLE [139]. Close monitoring of hematologic parameters is important in patients receiving myelotoxic therapy.

Primary bone marrow failure, presenting with pancytopenia and a hypocellular bone marrow, also occurs in SLE [140]. Although the pathophysiologic mechanism of the bone marrow suppression is unknown, there is evidence to suggest an immune-mediated suppression of hematopoietic progenitor cell growth and differentiation. Therefore, management consists of immunosuppressive therapy in addition to replacement of blood products and management of infection.

Primary therapy may include withdrawal of myelotoxic drugs, high-dose corticosteroids, and possibly growth factors. Some patients may benefit from antithymocyte globulin (ATG), horse or rabbit antibody raised against human lymphocytes, a mainstay of therapy for aplastic anemia in patients without SLE. Prednisone 1 mg/kg is administered concomitantly to ameliorate the serum sickness associated with ATG. Several centers have incorporated methylprednisolone into combination treatment with ATG, cyclosporin, and/or androgenic steroids [141]. Allogeneic bone marrow transplantation is the treatment of choice for young patients with severe aplastic anemia who have an HLA-identical sibling donor [142].

Lymphadenopathy

Lymph node enlargement occurs in 50% of all SLE patients at some point in their clinical course. The most common nodal groups involved are cervical (43%), mesenteric (21%), axillary (18%), and inguinal (17%). Palpable nodes are typically nontender, soft, discrete, and vary from one to several centimeters. Pathology of lymph nodes is characterized by paracortical foci of necrosis and infiltration by histiocytes, lymphocytes, plasma cells, and immunoblasts [143]. The "hematoxylin bodies" seen in necrotic loci, which may contain aggregates of DNA, immunoglobulin, and polysaccharide, are considered pathognomonic of lupus lymphadenitis, but are not always present [144]. The histiocytic necrotizing lymphadenitis of SLE is pathologically similar to Kikuchi-Fujimoto disease, a self-limited systemic illness seen in young patients and characterized by lymphadenopathy [145].

Myeloid and Lymphoid Malignancies in SLE

Considerable attention has been directed toward the possible association of hematologic or other malignancies with SLE. While several studies have reported an association between SLE and lymphoproliferative malignancies, especially non-Hodgkin's lymphoma, the relationship between SLE and solid tumor malignancies remains controversial [146, 147].

A study of 724 SLE patients in a single center in Toronto identified 23 cancers (3.2%) over 7233 patient-years of follow-up. Three of these patients had non-

Hodgkin's lymphoma, and 3 others had other hematologic malignancies, implying a relative risk of 4.2 for hematologic malignancies. There was no increase in the risk for solid tumors in SLE patients in this study [148]. A Danish study of 1585 patients with SLE followed for an average of 6.8 years revealed 8 cases of non-Hodgkin's lymphoma (relative risk 5.2, 95% confidence interval 2.2–10.3). A significantly increased relative risk of cancer of the lung (15 cases), liver (5 cases), and vulva (3 cases) was also found [149]. Cervical cancer risk was found to be increased in a cohort of 297 SLE patients followed at the University of Saskatchewan [150]. In a British cohort of 276 SLE patients followed between 1978 and 1999, only the incidence of Hodgkin's lymphoma was increased as compared with the general population, and there was no correlation between with cytotoxic therapy and malignancy [151]. A proposed mechanism of the etiology of B-cell malignancies in SLE patients is that clones of self-reacting B cells undergo dramatic expansion in lymph nodes and are sensitive to DNA damage. Other possibilities include infectious etiologies such as Epstein-Barr virus (EBV) or common apoptotic defects in lupus and B-cell malignancies [152]. Further studies will be needed to address the potential pathogenic mechanisms of this association, which may involve immunosuppressive therapy, a common etiologic agent, immunoregulatory phenomena, or genetic factors.

References

1. Tan, E. M., Cohen, A. S., Fries, J. F., *et al.* (1982). Revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. **25**, 1271.
2. Hochberg, M. C. (1997). Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. **40**, 1725.
3. Pistiner, M., Wallace, D. J., Nessim, S., *et al.* (1991). Lupus erythematosus in the 1980s: A survey of 570 patients. *Semin. Arthritis Rheum*. **21**, 55.
4. Nossent, J. C., and Swaak, A. J. (1991). Prevalence and significance of hematologic abnormalities in patients with systemic lupus erythematosus. *Q. J. Med.* **80**, 605.
5. Michel, M., Johanet, C., Meyer, O., *et al.* (2001). Familial lupus erythematosus: Clinical and immunologic features of 125 multiplex families. *Medicine* **80**, 153.
6. Pisetsky, D. S., Gilkeson, G., and St. Clair, E. W. (1997). Systemic lupus erythematosus. Diagnosis and treatment. *Med. Clin. North Am.* **81**, 113.
7. Krantz, S. B. (1994). Pathogenesis and treatment of the anemia of chronic disease. *Am. J. Med. Sci.* **307**, 353.
8. Steinberg, A. D. (1991). Systemic lupus erythematosus. *Ann. Intern. Med.* **115**, 548.
9. Jelkmann, W. E., Fandrey, J., Frede, S., and Pagel, H. (1994). Inhibiton of erythropoietin production by cytokines. Implications for the anemia in inflammatory states. *Ann. N. Y. Acad. Sci.* **718**, 300.
10. Bertero, M. T., and Caligaris-Cappio, F. (1997). Anemia of chronic disorders in systemic autoimmune diseases. *Haemotologica* **82**, 375.
11. Winearls, C. G., Pippard, M. J., Reid, C. D., *et al.* (1989). Characterization of the anemia of chronic renal disease and the mode of its correction by r-HuEPO. *Q. J. Med.* **70**, 113.
12. Esbach, J. W., Egrie, J. L., Downing, M. R., *et al.* (1987). Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. *N. Engl. J. Med.* **316**, 73.
13. Krantz, S. B. (1995). Erythropoietin and the anemia of chronic disease. *Nephrol. Dial. Transplant.* **10**(Suppl 2), 10.
14. Peeters, H. R., Jongen-Lavrencic, M., Vreugdenhil, G., and Swaak, A. J. (1996). Effect of recombinant erythropoietin on anaemia and disease activity in patients with rheumatoid arthritis and anaemia of chronic disease: A randomised placebo controlled double blind 52 weeks clinical trial. *Ann. Rheum. Dis.* **55**, 739.
15. Hebert, L. A., Birmingham, D. J., Shen, X. P., Brandt, J. T., Sedmak, D. D., and Dillon, J. J. (1994). Effect of recombinant erythropoietin therapy on autoimmunity in systemic lupus erythematosus. *Am. J. Kidney Dis*. **24**, 25.
16. Means, R. T. Jr. (1994). Clinical applications of recombinant erythropoietin in the anemia of chronic disease. *Hematol. Oncol. Clin. North Am.* **8**, 933.
17. Schett, G., Firbas, U., and Fureder, W. (2001). Decreased serum erythropoietin levels and its relation to anti-erythropoietin antibodies in anaemia of systemic lupus erythematosus. *Rheumatology* **40**, 424.
18. Voulgarelis, M., Kokori, S. I., Ioannidis, J. P., Tzioufas, A. G., Kyriaki, D., and Moutsopoulos, H. M. (2000). Anaemia in systemic lupus erythematosus: Aetiological profile and the role of erythropoietin. *Ann. Rheum. Dis*. **59**, 217.
19. Finelli, C., Visan, G., Gamberi, B., *et al.* (1993). Steroid resistant acquired pure red cell aplasia. A partial remission induced by recombinant human erythropoietin. *Br. J. Haemotol.* **83**, 28.
20. Habib, G. S., Saliba, W. R., and Froom, P. (2002). Pure red cell aplasia and lupus. *Semin. Arthritis Rheum.* **31**, 279.
21. Kiely, P. D., McGuckin, C. P., Collinns, D. A., Bevan, D. H., and Marsh, J. C. (1995). Erythrocyte aplasia and systemic lupus erythematosus. *Lupus* **4**, 407.
22. Casadevall, N., Dupuy, E., Mohlo-Sbatier, P., *et al.* (1996). Autoantibodies against human erythropoietin in a patient with pure red-cell aplasia. *N. Engl. J. Med.* **7**, 630.
23. Finelli, C., Visan, G., Gamberi, B., *et al.* (1993). Steroid resistant acquired pure red cell aplasia. A partial remission induced by recombinant human erythropoietin. *Br. J. Haematol.* **83**, 28.
24. Zeigler, Z. R., Rosenfeld, C. S., Shadduck. (1993). Resolution of transfusion dependence by recombinant human erythropoietin (rHuEPO) in acquired pure red cell aplasia (PRCA) associated with myeloid metaplasia. *Br. J. Haematol.* **83**, 28.

25. Orbach, H., Ben-Yehuda, A., Ben-Yehuda, D., *et al.* (1995). Successful treatment of pure red cell aplasia in systemic lupus erythematosus with erythropoietin. *J. Rheumatol.* **22**, 2166.
26. Marmont, A. M. (1991). Therapy of pure red cell aplasia. *Semin. Hematol.* **26**, 285.
27. Ilan, Y., and Naparstek, Y. (1993). Pure red cell aplasia associated with systemic lupus erythematosus: Remission after a single course of intravenous immunoglobulin. *Acta Haematol.* **89**, 152.
28. Koch, W. C., Massay, G., Russell, C. E., *et al.* (1990). Manifestations and treatment of human parvovirus B19 infection in immunocompromised patients. *J. Pediatr.* **116**, 355.
29. Trapani, S., Ermini, M., and Falcini, F. (1999). Human parvovirus B19 infection: Its relationship with systemic lupus erythematosus. *Semin. Arthritis Rheum*. **28**, 319.
30. Kokori, S. I., Ioannidis, J. P., Voulgarelis, M., Tzioufas, A. G., and Moutsopoulos, H. M. (2000). Autoimmune hemolytic anemia in patients with systemic lupus erythematosus. *Am. J. Med.* **108**, 198.
31. Engelfreit, C. P., Overbeeke, M. A. M., and von dem Borne, A. E. G. K. R. (1992). Autoimmune hemolytic anemia. *Semin. Hematol.* **29**, 3.
32. Leddy, J. P., Falany, J. L., Kissel, G. E., *et al.* (1993). Erythrocyte membrane proteins reactive with human (warm-reacting) anti-red cell autoantibodies. *J. Clin. Invest.* **91**, 1672.
33. Oliviera, G. C. S., Hutchings, P. R., Roitt, J. M., and Lydard, P. M. (1994). Production of erythrocyte autoantibodies in NZB mice is inhibited by CD4 antibodies. *Clin. Exp. Immunol.* **96**, 297.
34. Nisitani, S., Tsubata, T., Murakami, M., *et al.* (1993). The bcl-2 gene product inhibits clonal deletion of self-reactive B lymphocytes in the periphery but not in the bone marrow. *J. Exp. Med.* **178**, 1247.
35. Murukam, M., Tsubata, T., Shinkura, R., *et al.* (1994). Oral administration of lipopolysaccharides activates B-1 cells in the peritoneal cavity and lamina propria of the gut and induces autoimmune symptoms in an autoantibody transgenic mouse. *J. Exp. Med.* **180**, 111.
36. Nisitani, S., Masao, M., and Tasuku, H. (1997). Anti-red blood cell immunoglobulin transgenic mice: An experimental model of autoimmune hemolytic anemia. *Ann. N. Y. Acad. Sci.* **815**, 246.
37. Alarcon-Segovia, D., Deleze, M., Oria, C. V., *et al.* (1989). Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. *Medicine* **68**, 353.
38. Guzman, J., Cabral, A. R., Cabiedes, J., *et al.* (1994). Antiphospholipid antibodies in patients with idiopathic autoimmune haemolytic anemia. *Autoimmunity* **18**, 51.
39. Kokori, S., Ionnidis, J. P. A., Voularelis, M., *et al.* (2000). Autoimmune hemolytic anemia in patient with systemic lupus erythematosus. *Am. J. Med.* **108**, 198.
40. Fries, L. F., Brickman, C. M., and Frank, M. M. (1983). Monocyte receptors for the Fc portion of IgG increase in number of autoimmune hemolytic anemia and other hemolytic states and are decreased by glucocorticoid therapy. *J. Immunol.* **131**, 1240.
41. Dacie, J. (2001). The immune haemolyitc anaemias: A century of exciting progress in understanding. *Br. J. Haematol.* **114**, 770.
42. Boumpas, D. T., Chrousos, G. P., Wilder, R. L., *et al.* (1993). Glucocorticoid therapy for immune-mediated diseases: Basic and Clinical Correlates. *Ann. Intern. Med.* **119**, 1198.
43. Hershko, C., Sonnenblick, M., and Ashkenazi, J. (1990). Control of steroid-resistant autoimmune hemolytic anemia by cyclosporin. *Br. J. Haematol*. **76**, 436.
44. Pignon, J. M., Poirson, E., and Rochant, H. (1993). Danazol in autoimmune hemolytic anemia. *Br. J. Haematol.* **83**, 343.
45. Von Keyserlingk, H., Meyer-Saballek, W., Arnzt, R., *et al.* (1987). Plasma exchange treatment in autoimmune hemolytic anemia of the warm antibody type with renal failure. *Vox Sang* **52**, 598.
46. Flores, G., Cunningham-Rundles, C., Newland, A. C., and Bussel, J. G. (1993). Efficacy of intravenous immunoglobulin in the treatment of autoimmune hemolytic anemia: Results in 73 patients. *Am. J. Hematol.* **44**, 237.
47. Perrotta, S., Locatelli, F., La Manna, A., *et al.* (2002). Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolytic anaemia in a patient with systemic lupus erythematosus. *Br. J. Haematol.* **116**, 465.
48. Pegels, J. G., Helmerhorst, F. M., van Leeuwen, E. F., *et al.* (1982). The Evans syndrome: Characterization of the responsible autoantibodies. *Br. J. Haematol.* **51**, 445.
49. Miescher, P. A., Tucci, A., Beris, P., and Favre, H. (1992). Autoimmune hemolytic anemia and/or thrombocytopenia associated with lupus parameters. *Semin. Hematol.* **29**, 13.
50. Le Deist, F., Emile, J.-F., Rieux-Laucat, F., *et al.* (1996). Clinical, immunological, and pathological consequences of Fas-deficient conditions. *Lancet* **348**, 719.
51. Scaradavou, A., and Bussel, J. (1995). Treatment of refractory Evans' syndrome with alternate-day cyclosporine and prednisone. *J. Pediatr. Hematol. Oncol.* **17**, 290.
52. Allard, S. A., and Scott, J. T. (1989). Systemic lupus erythematosus and acute intermittent porphyria. *Br. J. Rheumatol.* **28**, 254.
53. Camisa, C., Fox, M., Quillin., and Hibler, J. P. (1995). Coexistence of subacute cutaneous lupus erythematosus and porphyria cutanea tarda: A case report. *Cutis* **56**, 101.
54. O'Reilly, F. M., O'Loughlin, S., and Murphy, G. M. (1996). Discoid lupus erythematosus and porphyria cutanea tarda. *J. R. Soc. Med.* **89**, 523.
55. Allard, S. A., Charles, P. J., Herrick, A. L., McColl, K. E., and Scott, J. T. (1990). Antinuclear antibodies and the diagnosis of systemic lupus erythematosus in patients with acute intermittent porphyria. *Ann. Rheum. Dis.* **49**, 246.
56. Nepveu, K., and Libman, B. (1996). Hepatitis C as another possible cause of porphyria cutanea tarda and systemic lupus erythematosus. *Arthritis Rheum.* **39**, 352.

57. Filiotou, A., Vaiopoulos, G., Capsimali, V., *et al.* (2002). Acute intermittent porphyria and systemic lupus erythematosus: Report of a case and review of the literature. *Luous* **11**, 190.
58. Abu-Shakra, M., Urowitz, M. B., Gladman, D. D., and Gough, J. (1995). Mortality studies in systemic lupus erythematosus. Results from a single center. II. Predictor variables for mortality. *J. Rheumatol.* **22**, 1265.
59. Reveille, J. D., Bartolucci, A., and Alarcon, G. S. (1990). Prognosis in systemic lupus erythematosus. Negative impact of increasing age at onset, black race, and thrombocytopenia, as well as causes of death. *Arthritis Rheum.* **33**, 37.
60. Cook, R. J., Gladman, D. D., Pericak, D., and Urowitz, M. B. (2000). Predicition of short-term mortality in systemic lupus erythematosus with time dependent measures of disease activity. *J. Rheumatol.* **27**, 1892.
61. Moss, K. E., Ioannou, Y., Sultan, S. M., Haq, I., and Isenberg, D. A. (2002). Outcome of a cohort of 300 patients with systemic lupus erythematosus attending a dedicated clinic for over two decades. *Ann. Rheum. Dis*. **61**, 409.
62. Miller, M. H., Urowitz, M. B., and Gladman, D. D. (1983). The significance of thrombocytopneia in systemic lupus erythematosus. *Arthritis Rheum*. **26**, 1181.
63. George, J. N., Raskob, G. E., Shah, S. R., Rizvi, M. A., Hamilton, S. A., Osborne, S., and Vondracek, T. (1998). Drug-induced thrombocytopenia: A systematic review of published case reports. *Ann. Intern. Med*. **129**, 886.
64. Warkentin, T. E., and Greinacher, A. (Eds.) (2001). "Heparin-Induced Thrombcytopenia" Dekker, New York.
65. Kuwana, M., Kaburaki, J., and Ikeda, Y. (1998). Autoreactive T cells to platelet GPIIb:GPIX in immune thrombocytopenic purpura. Role in production of antiplatelet autoantibody. *J. Clin. Invest.* **102**, 1393.
66. Lazarus, A. H., Ellis, J., and Semple, J. W. (2000). Comparison of platelet immunity in patients with SLE and with ITP. *Transfus. Sci.* **22**, 19.
67. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders: Prevalence and clinical significance. *Ann. Intern. Med.* **112**, 682.
68. Simantov, R., LaSala, J. M., Lo, S. K., *et al.* (1995). Activation of cultured vascular endothelial cells by antiphospholipid antibodies. *J. Clin. Invest.* **96**, 2211.
69. Alarcon-Segovia, D., Deleze, M., Oria, C. V., Sanchez-Guerrero, J., Gomez-Pacheco, L., Cabiedes, J., Fernandez, L., and Ponce de Leon, S. (1989). Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. *Medicine* **68**, 353.
70. Sturfelt, G., Nived, O., Norberg, R., Thorstensson, R., and Krook, K. (1987). Anticardiolipin antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **30**, 382.
71. Stasi, R., Stipa, E., Masi, M., *et al.* (1996). Prevalence and clinical significance of elevated antiphospholipid antibodies in patients with idiopathic thrombocytopenic purpura. *Blood* **88**, 3354.
72. Karpatkin, S. (1997). Autoimmune (idiopathic) thrombocytopenic purpura. *Lancet* **349**, 1531.
73. Doan, C. A., Bouroncle, B. A., and Wiseman, B. K. (1960). Idiopathic and secondary thrombocytopenic purpura: Clinical study and evaluation of 381 cases over a period of 28 years. *Ann. Intern. Med.* **53**, 861.
74. Marcus, A. J. (1996). Platelet and their disorders. In "Disorders of Hemostasis" (O. D. Ratnoff, Ed.).
75. George, J. N., Woolf, S. H., Raskob, G. E., Wasser, J. S., Aledort, L. M., Ballem, P. J., Blanchette, V. S., Bussel, J. B., Cines, D. B., Kelton, J. G., Lichtin, A. E., McMIllan, R., Okerbloom, J. A., Regan, D. H., and Warner, I. (1996). Idiopathic thrombocytopenic purpura: A practice guideline developed by explicit methods for the American Society of Hematology, *Blood* **88**, 3.
76. Stasi, R., Stipa, E., Masi, M., Cecconi, M., *et al.* (1995). Long-term observation of 208 adults with chronic idiopathic thrombocytopenic purpura. *Am. J. Med*. **98**, 436.
77. Julia, A., Araguas, C., Rossello, J., *et al.* (1990). Lack of useful clinical predictors of response to splenectomy in patients with chronic idiopathic thrombocytopenic purpura. *Br. J. Haematol*. **76**, 250.
78. Bell, W. R., Jr. (2000). Long-term outcome of splenectomy for idiopathic thrombocytopenic purpura. *Semin. Hematol.* **37**(1 Suppl. 1), 22.
79. Park, A. E., Birgisson, G., Mastrangelo, M. J., Marcaccio, M. J., and Witzke, D. B. (2000). Laparoscopic splenectomy: Outcomes and lessons learned from over 200 cases. *Surgery* **128**, 660.
80. Scaradavou, A., Woo, B., Woloski, B. M., *et al.* (1997). Intravenous anti-D treatment of immune thrombocytopenic purpura: Experience in 272 patients. *Blood* **89**, 2689.
81. Emilia, G., Morselli, M., and Luppi, M. (2002). Long-term salvage therapy with cyclosporin A in refractory idiopathic thrombocytopenic purpura. *Blood* **99**, 1482.
82. Anderson, J. (1994). Response of resistant idiopathic thrombocytopenic purpura to pulsed high-dose dexamethasone therapy. *N. Engl. J. Med.* **330**, 1560.
83. Godeau, B., Zii, J.-M., Schaeffer, A., and Bierling, P. (1995). High-dose methylprednisolone is an alternative treatment for adults with autoimmune thrombocytopenic purpura refractory to intravenous immunoglobulins and oral corticosteroids. *Am. J. Hematol.* **48**, 282.
84. Ahn, Y. S., Harrington, W. J., Byrnes, J. J., *et al.* (1978). The treatment of idiopathic thrombocytopenic purpura with vinbastine-loaded platelets. *N. Engl. J. Med.* **298**, 1101.
85. Stasi, R., Pagano, A., Stipa, E., and Amadori, S. (2001). Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. *Blood* **98**, 952.
86. Arnal, C., Piette, J. C., Leone, J., *et al.* (2002). Treatment of severe immune thrombocytopenia associated with systemic lupus erythematosus: 59 cases. *J. Rheumatol*. **29**, 75.
87. Musio, F., Bohen, E. M., Yuan, C. M., and Welch, P. G. (1998). Review of thrombotic thrombocytopenic purpura

in the setting of systemic lupus erythematosus. *Arthritis Rheum.* **28**, 1.
88. Brunner, H. I., Freedman, M., and Silverman, E. D. (1999). Close relationship between systemic lupus erythematosus and thrombotic thrombocytopenic purpura in childhood. *Arthritis Rheum.* **42**, 2346.
89. Levy, G. G., Nichols, W. C., Lian, E. C., *et al.* (2001). Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* **413**, 488.
90. Remuzzi, G., Galbusera, M., Noris, M., *et al.* (2002). von Willebrand factor cleaving protease (ADAMTS13) is deficient in recurrent and familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. *Blood* **100**, 778.
91. Zheng, X., Chung, D., Takayama, T. K., *et al.* (2001). Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J. Biol. Chem.* **276**, 41059.
92. Furlan, M., Robles, R., Solenthaler, M., and Lammle, B. (1998). Acquired deficiency of von Willebrand factor-cleaving protease in a patient with thrombotic thrombocytopenic purpura. *Blood* **91**, 2839.
93. Gungor, T., Furlan, M., Lammle, B., Kuhn, F., and Seger, R. A. (2001). Acquired deficiency of von Willebrand factor-cleaving protease in a patient suffering from acute systemic lupus erythematosus. *Rheumatology*, 940.
94. Mitra, D., Jaffe, E. A., Weksler, B., *et al.* (1997). Thrombotic thrombocytopenic purpura and sporadic hemolytic-uremic syndrome plasma induce apoptosis in restricted lineages of human microvascular endothelial cells. *Blood* **89**, 1224.
95. Laurence, J., and Mitra, D. (1997). Apoptosis of microvascular endothelial cells in the pathophysiology of thrombotic thrombocytopenic purpura/sporadic hemolytic uremic syndrome. *Semin. Hematol.* **34**, 98.
96. Moore, J. C., Hayward, C. P., Warkentin, T. E., and Kelton, J. G. (2001). Decreased von Willebrand factor protease activity associated with thrombocytopenic disorders. *Blood* **98**, 1842.
97. Mohri, H., Motomura, S., Kanamori, H., Matsuzaki, M., Watanabe, S., Maruta, A., Kodama, F., and Okubo, T. (1998). Clinical significance of inhibitors in acquired von Willebrand syndrome. *Blood* **91**, 3623.
98. Viallard, J. F., Pellegrin, J. L., Vergnes, C., Borel-Derlon, A., Clofent-Sanchez, G., Nurdan, A. T., Leng, B., and Nurden, P. (1997). Three cases of acquired von Willlebrand disease associated with systemic lupus erythematosus. *Br. J. Haematol.* **105**, 532.
99. Rodeghiero, F., Castaman, G., and Mannucci, P. M. (1991). Clinical indications for desmopressin (DDAVP) in congenital and acquired von Willebrand disease. *Blood Rev*. **5**, 155.
100. Nived, O., Sturfelt, G., and Wollheim, F. (1985). Systemic lupus erythematosus and infection: A controlled and prospective study including an epidemiological group. *Q. J. Med*. **271**.
101. Keeling, D. M., and Isenberg, D. A. (1993). Haematological manifestations of systemic lupus erythematosus. *Blood Rev.* **7**, 199.
102. Kuster, S., Apenberg, S., Andrassy, K., and Ritz, E. (1992). Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. *Contrib. Nephrol.* **99**, 94.
103. Waldendorf, M., and Schneider, M. (1993). Antineutrophil cytoplasmic antibodies in patients with systemic lupus erythematosus. *Adv. Exp. Med. Biol.* **336**, 381.
104. Kuster, S., Apenberg, S., Andrassy, K., and Ritz, E. (1993). Antineutrophil cytoplasmic antibodies in patients with systemic lupus erythematosus. *Adv. Exp. Med. Biol.* **336**, 381.
105. Wong, S. N., Shah, V., and Dillon, M. J. (1995). Antineutrophil cytoplasmic antibodies in childhood systemic lupus erythematosus. *Eur. J. Pediatr.* **154**, 43.
106. Kurien, B. T., Newland, J., Paczkowski, C., Moore, K. L., and Scofield, R. H. (2000). Association of neutropenia in systemic lupus erythematosus (SLE) with anti-Ro and binding of an immunologically cross-reactive neutrophil membrane antigen. *Clin. Exp. Immunol.* **120**, 209.
107. Arenas, M., Abad, A., Valverde, V., *et al.* (1992). Selective inhibition of granulopoiesis with severe neutropenia in systemic lupus erythematosus. *Arthrits Rheum.* **35**, 979.
108. Liu, J. H., Ozaki, K., Matsuzaki, Y., *et al.* (1995). Suppression of haematopoiesis by IgG autoantibodies from patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **100**, 480.
109. Courtney, P. A., Crockard, A. D., Williamson, K., Irvine, A. E., Kennedy, R. J., and Bell, A. L. (1999). Increased apoptotic peripheral blood neutrophils in systemic lupus erythematosus: Relations with disease activity, antibodies to double stranded DNA, and neutropenia. *Ann. Rheum. Dis*. **58**, 309.
110. Cairns, A. P., Crockard, A. D., McConnell, J. R., Courtney, P. A., and Bell, A. L. (2001). Reduced expression of CD44 on monocytes and neutrophils in systemic lupus erythematosus: Relations with apoptotic neutrophils and disease activity. *Ann. Rheum. Dis.* **60**, 950.
111. Euler, H. H., Schwab, U. M., and Schroeder, J. O. (1994). Filgrastim for lupus neutropenia. *Lancet* **344**, 1513.
112. Starkebaum, G. (1997). Use of colony-stimulating factors in the treatment of neutropenia associated with collagen vascular disease. *Curr. Opin. Hematol.* **4**, 196.
113. Wandt, H., Seifert, M., Falge, C., and Gallmeier, W. M. (1993). Long-term correction of neutropenia in Felty's syndrome with granulocyte colony-stimulating factor. *Ann. Hematol.* **66**, 265.
114. Kondo, H., Date, Y., Sakai, Y., and Akimoto, M. (1994). Effective simultaneous rhG-CSF and methylprednisolone "pulse" therapy in agranulocytosis associated with systemic lupus erythematosus. *Am. J. Hematol.* **46**, 157.
115. Euler, H. H., Harten, P., Zeuner, R. A., and Schwab, U. M. (1997). Recombinant human granulocyte stimulating factor in patients with systemic lupus erythematosus associated with neutropenia and refractory infections. *J. Rheumatol.* **24**, 2153.

116. Starkebaum, G. (2002). Chronic neutropenia associated with autoimmune disease. *Semin. Hematol.* **39**, 121.
117. Ward, M. W., and Studenski, S. (1990). Age-associated clinical manifestations of systemic lupus erythematosus: A multivariate regression analysis. *J. Rheumatol.* **17**, 476.
118. Harley, J. B., Sestak, A. L., Willis, L. G., Fu, S. M., Hansen, J. A., and Reichlin, M. (1989). A model for disease heterogeneity in systemic lupus erythematosus. Relationships between histocompatibility antigens, autoantibodies, and lymphopenia or renal disease. *Arthritis Rheum*. **32**, 826.
119. Osman, C., and Swaak, A. J. (1994). Lymphocytotoxic antibodies in SLE: A review of the literature. *Clin. Rheumatol.* **13**, 21.
120. Greenwood, D. L., Gitlits, V. M., Alderuccio, F., *et al.* (2002). Autoantibodies in neuropsychiatric lupus. *Autoimmunity* **35**, 79.
121. Dhein, J., Walczak, H., Baumler, C., *et al.* (1995). Autocrine T-cell suicide mediated by APO-1/(Fas/CD95). *Nature* **373**, 438.
122. Brunner, T., Mogil, R. J., La Face, D., Yoo, N. J., *et al.* (1995). Cell-autonomous Fas (CD95)/Fas-ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* **373**, 441.
123. Nagafuji, K., Shibuya, T., Harada, M., *et al.* (1995). Functional expression of Fas antigen (CD95) on hematopoietic progenitor cells. *Blood* **86**, 883.
124. Yasutomo, K., Nagasawa, H., Hisaeda, H., *et al.* (1996). Fas system-mediated apoptosis suppresses lymphopoiesis. *J. Immunol.* **157**, 1981.
125. Gyimesi, E., Kavai, M., Kiss, E., *et al.* (1993). Triggering of respiratory burst by phagocytosis in monocytes of patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **94**, 140.
126. Blanco, P., Palucka, A. K., Gill, M., Pascual, V., and Banchereau, J. (2001). Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* **294**, 1540.
127. Suzuki, H., Takemura, H., and Kashiwagi, H. (1995). Interleukin-1 receptor antagonist in patients with systemic lupus erythematosus. Enhanced production by monocytes and correlation with disease activity. *Arthritis Rheum.* **38**, 1055.
128. Llorente, L., Richard-Patin, Y., Wijdenes, J., *et al.* (1993). Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur. Cytokine Netw.* **4**, 421.
129. Pelton, B. K., Hylton, W., and Denman, A. M. (1992). Activation of IL-6 production by UV irradiation of blood mononuclear cells from patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **89**, 251.
130. Yabuhara, A., Yang, F. C., Nakazawa, T., *et al.* (1996). A killing defect of natural killer cells as an underlying immunologic abnormality in childhood systemic lupus erythematosus. *J. Rheumatol.* **23**, 171.
131. Erkeller-Yusel, F., Hulstaart, F., Hannet, I., Isenberg, D., and Lydyard, P. (1993). Lymphocyte subsets in a large cohort of patients with systemic lupus erythematosus. *Lupus* **2**, 227.
132. Otsuka, T., Nagasawa, K., Harada, M., and Niho, Y. (1993). Bone marrow microenvironment of patients with systemic lupus erythematosus. *J. Rheumatol.* **20**, 967.
133. Hows, J. M. (1990). Connective tissue disorders. In Haemotologic Aspects of Systemic Disease" (I. W. Delamore and J. A. Liu Yin, Eds.), Saunders, London.
134. Morales-Polanco, M., Jimenez-Balderas, F. J., and Yanez, P. (1996). Storage histiocytes and hemophagocytosis: A common finding in the bone marrow of patients with active systemic lupus erythematosus. *Arch. Med. Res.*, 57.
135. Ramakrishna, R., Kyle, P. W., Day, P. J., and Manohara, A. (1995). Evans' syndrome, myelofibrosis and systemic lupus erythematosus: Role of procollagens in myelofibrosis. *Pathology* **27**, 255.
136. Foley-Nolan, D., Martin, M. F., Rowbotham, D., *et al.* (1992). Systemic lupus erythematosus presenting with myelofibrosis. *J. Rheumatol.* **19**, 1303.
137. Paquette, R. L., Meshkinpour, A., and Rosen, P. J. (1994). Autoimmune myelofibrosis. A steroid-responsive cause of bone marrow fibrosis associated with systemic lupus erythematosus. *Medicine* **73**, 145.
138. Aharon, A., Levy, Y., Bar-Dayan, Y., *et al.* (1997). Successful treatment of early secondary myelofibrosis in SLE with IVIG. *Lupus* **6**, 408.
139. Vasquez, S., Kavanaugh, A. F., Schneider, N. R., Wacholtz, M. C., and Lipsky, P. E. (1992). Acute nonlymphocytic leukemia after treatment of systemic lupus erythematosus with immunosuppressive agents. *J. Rheumatol.* **19**, 1625.
140. Winkler, A., Jackson, R. W., Kay, D. S., *et al.* (1988). High-dose intravenous cyclophosphamide treatment of systemic lupus erythematosus associated aplastic anemia. *Arthritis Rheum*. **31**, 693.
141. Frickhofen, N., Kaltwasser, J. P., Scherzenmeier, H., Raghavachar, A., *et al.* (1991). Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporin. *N. Engl. J. Med*. **324**, 1297.
142. Speck, B. (1991). Allogeneic bone marrow transplantation for severe aplastic anemia. *Semin. Hematol.* **28**, 319.
143. Eisner, M. D., Amory, J., Mullaney, B., *et al.* (1996). Necrotizing lymphadenitis associated with systemic lupus erythematosus. *Semin. Arthritis Rheum.* **26**, 477.
144. Tsang, W. Y., and Chan, J. K. (1994). Fine-needle aspiration cytologic diagnosis of Kikuchi's lymphadenitis. A report of 27 cases. *Am. J. Clin. Pathol.* **102**, 454.
145. el-Ramahi, K. M., Karrar, A., and Ali, M. A. (1994). Kikuchi disease and its association with systemic lupus erythematosus. *Lupus* **3**, 409.
146. Menon, S., Snaith, M. L., and Isenberg, D. (1993). The association of malignancy with SLE: An analysis of 150 patients under long-term review. *Lupus* **2**, 177.
147. Leandro, M. J., and Isenberg, D. A. (2001). Rheumatic diseases and malignancy—is there an association? *Scand. J. Rheumatol*. **30**, 185.
148. Abu-Ahakra, M., Gladman, D. D., and Urowitz, M. B. (1996). Malignancy in systemic lupus erythematosus. *Arthritis Rheum.* **39**, 1050.

149. Mellemkjaer, L., Andersen, V., Linet, M. S., *et al.* (1997). Non-Hodgkin's lymphoma and other cancers among a cohort of patients with systemic lupus erythemaotusus. *Arthritis Rheum.* **40**, 761.
150. Cibere, J., Sibley, J., and Haga, M. (2001). Systemic lupus erythematosus and the risk of malignancy. *Lupus* **10**, 394.
151. Sultan, S. M., Ioannou, Y., and Isenberg, D. A. (2000). Is there an association of malignancy with systemic lupus erythematosus? An analysis of 276 patients under long-term review. *Rheumatology* **39**, 1147.
152. Xu, Y., and Wiernik, P. H. (2001). Systemic lupus erythematosus and B-cell hematologic neoplasm. *Lupus* **10**, 841.

37

ARTICULAR MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

Paul E. Di Cesare
Craig J. Della Valle
Joseph D. Zuckerman

ABSTRACT

Articular manifestations of systemic lupus erythematosus (SLE) include both small and large joints, the spine, and the complications associated with chronic steroid use such as osteonecrosis, osteoporosis, and stress fracture. Of the large joints, the knees, and of the small joints, the proximal interphalangeal joints of the hands are most commonly involved. Less frequently involved joints are the wrists, shoulders, ankles, and elbows. Periarticular symptoms can also be seen in SLE including enthesopathy, tenosynovitis, tendinopathy, and myositis as well as periarticular calcifications and nodules.

Osteonecrosis of the hip, knee, shoulder or talus may affect up to 20% of patients with SLE. Although a given patient may have osteonecrosis at mulitple sites, the femoral head is most commonly affected and bilateral involvement is common. Because of its frequency and the significant disability that can result (particularly in the young lupus population), osteonecrosis is the articular manifestation of SLE with the most morbidity. Although the precise pathogenesis of osteonecrosis is unclear, it is clearly related to the use of corticosteroids. This chapter includes an in-depth discussion of the epidemeology, pathogenesis, diagnosis, and classification of osteonecrosis and reviews both operative and nonoperative treatment options.

INTRODUCTION

Patients with systemic lupus erythematosus (SLE) do not constitute a uniform group due to the great variability in its manifestations, thereby making difficult both the diagnosis and management of this condition. The articular manifestations that are a major symptom of SLE resemble those in other rheumatic diseases and thus are not pathognomonic. The initial presentation of SLE is often insidious, with symmetric arthralgias, and as a result many of these patients are frequently misdiagnosed to have rheumatoid arthritis (RA). A nondeforming, nonerosive arthritis is considered the most characteristic articular manifestation of SLE. Rheumatic complaints can only be attributed to SLE when they occur in a setting of other well-recognized symptoms and laboratory parameters detailed elsewhere in this book. In this chapter, we review the clinical and pathologic features of the articular manifestations of SLE.

Joint pain is one of the most common presentations of SLE, occurring in 76–100% of patients [1–5]. Clinical symptoms can range from obvious arthritis with objective evidence of inflammation (e.g., painful joint motion, tenderness, erythema, or effusion) to a painful joint without any objective evidence of inflammation. Symptomatic joints can often precede the other systemic manifestations of SLE. Morning stiffness is another

common joint complaint. The arthropathy of SLE is usually symmetric, similar to RA; however, the arthritis can be short-lived, resolving within 24 h, or behave as a persistent synovitis lasting longer than 6 months. In all cases, SLE arthropathy is characterized by an inconsistency between the severe rheumatic symptoms that can be present and modest physical and radiographic changes [6, 7].

Articular manifestations of SLE include both small and large joints, the spine, and the complications associated with chronic steroid use such as osteonecrosis, osteoporosis, and stress fracture. Of the large joints, the knees, and of the small joints, the proximal interphalangeal joints of the hands are most commonly involved. Less frequently involved joints are the wrists, shoulders, ankles, and elbows [8]. Clinical symptoms localized to the hip should alert the physician to the possibility of osteonecrosis, since this joint is rarely otherwise affected. The clinician should be alert to the possibility of septic arthritis in patients with SLE. SLE patients may have impaired host immune defenses secondary to either the disease process or immunosuppressive therapy. As a result, all new joint effusions should be promptly evaluated for infection. If an inflammatory fluid is encountered, it is suggested that antibiotics be started while awaiting synovial fluid cultures even if the initial Gram stain is not diagnostic. Septic arthritis caused by *Salmonella*, *Enterococcus*, *Neisseria gonorrhea*, and other organisms have been reported in SLE and may be particularly common in patients with a history of osteonecrosis [9–12].

"LUPUS HAND"

Jaccoud's Arthropathy

As in patients with RA, the hands of the SLE patient may be helpful to the disease diagnosis [13, 14]. In 1962, Zvaifler [15] described the characteristic flexion deformities and ulnar deviation of the metacarpophalangeal (MP) joints commonly observed in SLE patients. What was remarkable about his finding was the absence of any changes in the cartilage or bone (Fig. 1). Bywaters [16] astutely noted the similarity of the hand deformities in SLE to those of the Jaccoud's type. These nonerosive, reducible joint subluxations were thus termed Jaccoud's arthropathy, and as a result alerted clinicians that hand involvement in SLE could be deforming. The important finding in most cases was that the deformity is reducible or correctable. Swelling of the proximal interphalangeal and metacarpophalangeal

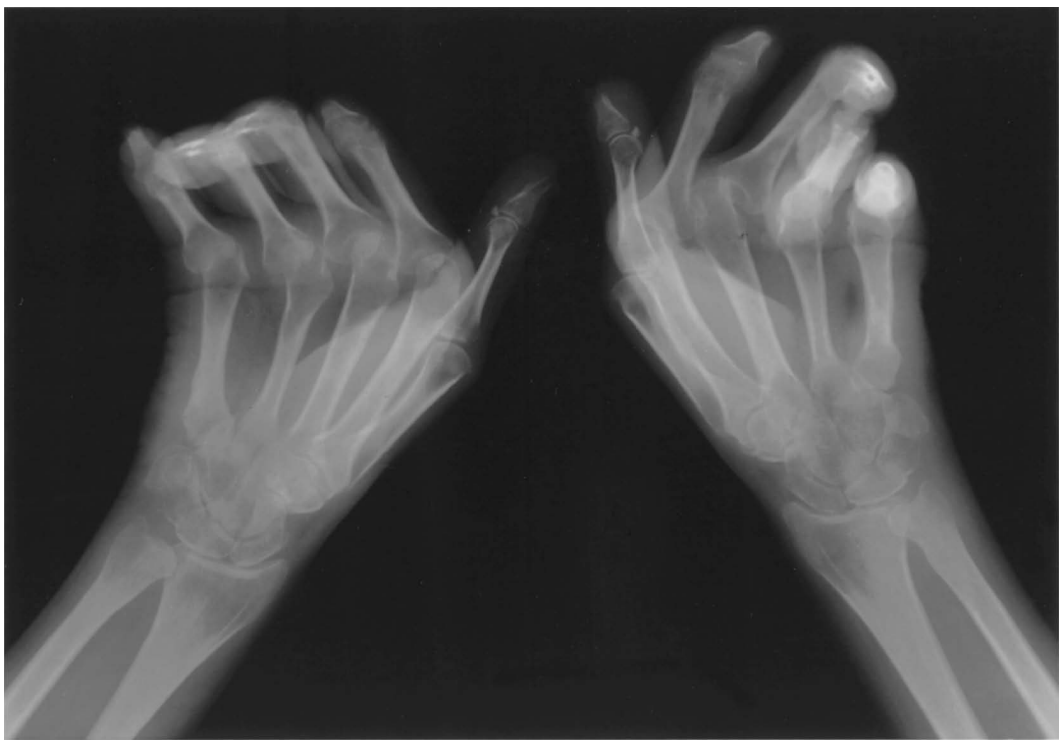


FIGURE 1 Radiographs of the hands of an SLE patient illustrating Jaccoud's arthropathy. Characteristic flexion deformities and ulnar deviation of the metacarpophalangeal joints is seen with the absence of any changes in the cartilage or bone.

joints is the predominant manifestation of SLE in the hand. Hand and wrist deformities include ulnar deviation and subluxation, swan-neck deformities, and thumb interphalangeal (IP) joint subluxations. Figures 2–6 illustrate reducible hand deformities in a patient with long-standing SLE. In SLE patients the presence of Jaccoud's arthropathy occurs more often in patients with a longer disease duration and a longer history of joint involvement [17].

Pathogenesis of Lupus Hand

The hand deformities of SLE patients appear to be secondary to involvement of periarticular tissues (i.e., joint capsule, ligaments, and tendons) resulting in hypermobile digits with reducible deformities. Hand joint deformities can occur without the erosive destruction of articular cartilage that is seen in rheumatoid arthritis. These deformities are usually the result of volar plate and ligamentous laxity as well as tendon subluxation, which lead to joint imbalance [13, 14]. Russell *et al*. [18] reported on the operative findings in several SLE patients with hand deformities. They noted in three patients at surgery (with bilateral palmar subluxation and ulnar deviation at the metacarpophalangeal joints) normal-appearing articular cartilage in all cases, a modest inflammatory synovial membrane in one case, and a marked synovial hyperplasia in another case (histology revealed only a mild mononuclear cell infiltration). These surgical findings further illustrate the differentiation of SLE hand deformities from those seen in the rheumatoid patient. In RA the appearance of joint deformities is consistent with radiographic demonstration of bony erosions and joint degeneration, in contrast to the joint deformities in SLE, which are usually nonerosive and reducible (Jaccoud's arthropathy). Joint erosions or degeneration can occur in SLE patients; however, they are usually nonprogressive and are thought to result from capsular pressure and altered joint mechanics created by joint subluxation.

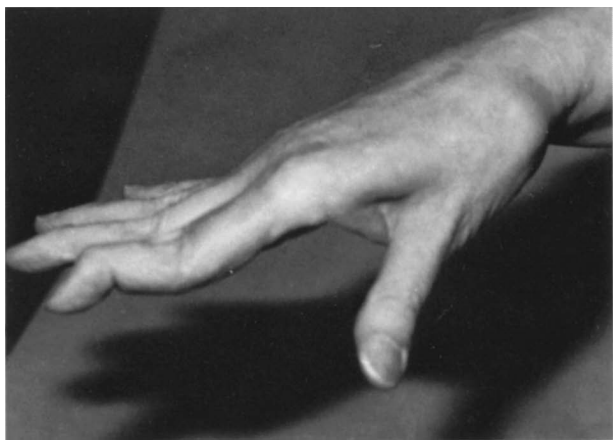


FIGURE 2 Swan-neck deformity of the second and third digits (Courtesy of Harry D. Fisher.)

Clinical Findings of Lupus Hand

In 1973, Bleifeld and Inglis [19] reported on the clinical hand findings in 50 SLE patients. They noted generalized soft tissue laxity in 50% and swan-neck deformities in 38% of patients. Most deformities were symmetric and involved two or more fingers on each hand. The swan-neck deformity was thought to result from laxity of the volar plate with dorsal displacement of the extensor tendon lateral bands. Since they noted no intrinsic muscle contractures and the proximal interphalangeal joints had maintained full flexion, the deformities were easily reducible. Hyperextension of the interphalangeal joint of the thumb was observed in 30% of patients; however, in contrast to thumb deformities seen in the rheumatoid hand, it was not associated with a flexion deformity of the first metacarpophalangeal

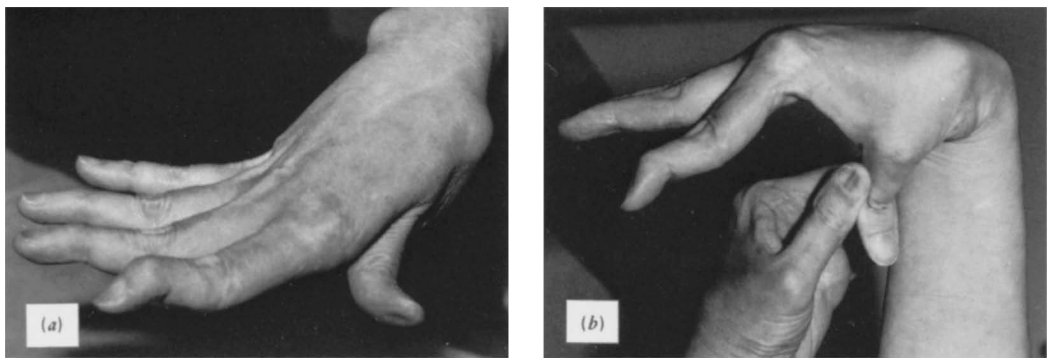


FIGURE 3 (*a*) Swan-neck deformity of the second and third digits. (*b*) Hyperextension of the first interphalangeal joint. (Courtesy of Harry D. Fisher.)

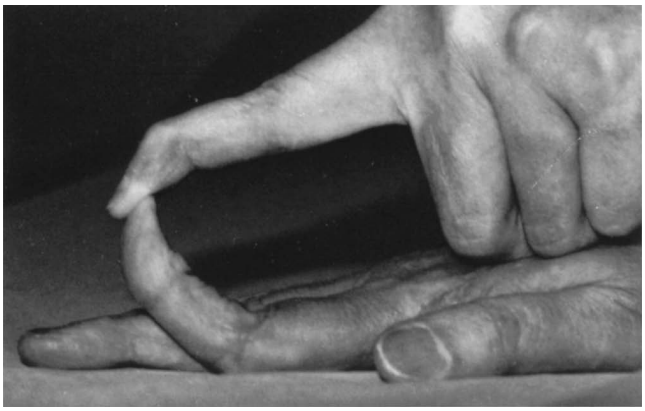


FIGURE 4 Hyperextension of the second proximal and distal interphalangeal joints. (Courtesy of Harry D. Fisher.)

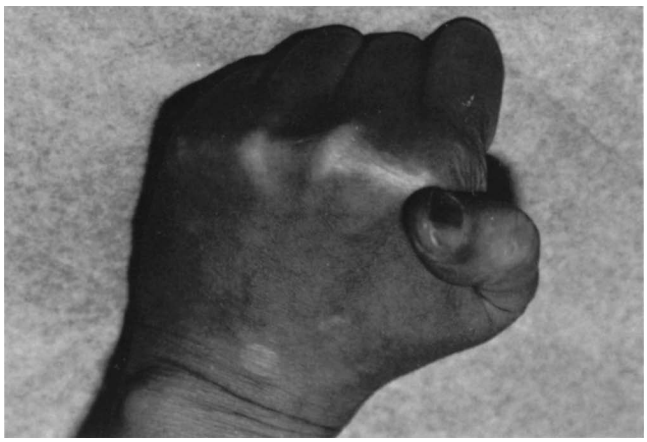


FIGURE 5 Excessive ligamentous laxity of the metacarpophalangeal joint of the thumb. (Courtesy of Harry D. Fisher.)

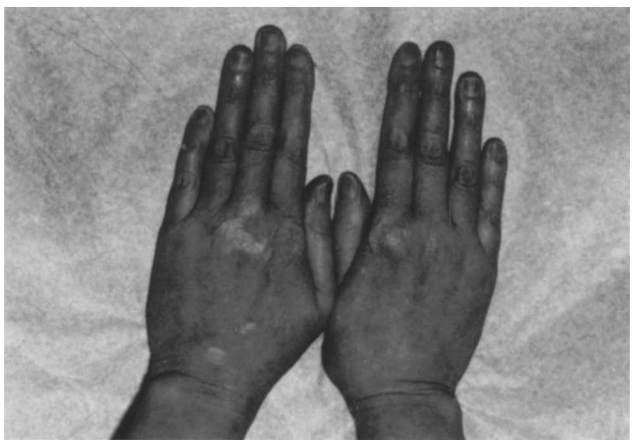


FIGURE 6 The hand of the same patient demonstrating the reducible character of the deformities. (Courtesy of Harry D. Fisher.)

joint. The carpometacarpal joint of the thumb could be subluxated in 16% of patients. Reducible ulnar deviation of the metacarpophalangeal joints occurred in 14% of patients. No cases of metacarpophalangeal joint subluxation were seen. Excessive lateral instability in the distal interphalangeal joints was common. Radiographic examination of these patients' hands revealed no articular destruction except for narrowing of the intercarpal joints in 1 of the 50 patients. The hand deformities noted previously have been since corroborated by Labowitz and Schumacher [2], Esdaile *et al*. [20], Russell *et al*. [18], and Kramer *et al*. [21].

Metacarpophalangeal Joint Abnormalities

The characteristic volar subluxation and ulnar deviation hand deformity of SLE occurs at the metacarpophalangeal (MP) joint. Patients often have full active finger flexion, but a loss of active finger extension. Initially patients can passively extend their finger, but gradually lose this ability with time. The pathologic sequence of deformity that follows includes ulnar subluxation of the extensor tendon, volar subluxation of the proximal phalanx, and intrinsic muscle contracture, while there is still preservation of the articular surface. Surgical treatment of the MP hand deformities includes intrinsic muscle lengthening, release, or transfer, metacarpal "step-cut" osteotomies, and Swanson MP arthroplasties [13].

Interphalangeal Joint Abnormalities

Involvement of the interphalangeal joints (IP) due to laxity of the supporting soft tissue structures can lead to hyperextention, flexion, and lateral deviation deformities. If the deformity remains reducible, then rebalancing surgical procedures can be performed. If the deformity becomes fixed, then fusion of the IP in a functional position is indicated. It is important to emphasize that the restoration of IP joint alignment is essential in order to maintain MP joint realignment [13].

Thumb Abnormalities

The first hand deformity to occur is often that of the thumb, as patients complain of a painless loss of pinching ability [22, 23]. Lateral subluxation of the thumb IP joint is a frequent manifestation, with preservation of the articular surfaces. In order to restore pinching ability, fusion of the thumb IP joint is the surgical treatment of choice. The thumb MP joint can also be the location of deformity as a result of extensor pollicis longus (EPL) subluxation resulting in abnormal thumb flexion. Lateral subluxation may also occur secondary

to collateral ligament laxity. Treatment for this type of thumb deformity may involve EPL rerouting if the IP joint requires fusion or MP fusion when the deformity cannot be passively corrected (13).

Carpometacarpal Joint Abnormalities

Primary subluxation or dislocation can occur at the carpometacarpal (CM) joint. If the thumb MP and IP joints are functioning well and aligned, then a CM fusion with the thumb metacarpal slightly abducted can be performed. An alternative surgical procedure is soft tissue stabilization with rerouting of the flexor carpi radialis as a sling with or without resection arthroplasty [13].

Abnormalities and Treatment of the Wrist

The ligamentous support of the wrist may also be affected, as noneroded carpal bones may sublux or dislocate. Subluxation can occur at either the midcarpal or radiocarpal joints. The most common carpal dislocation involves the lunate [23]; in these cases, surgical treatment of a limited or total wrist fusion may be indicated [13]. Another common wrist problem in SLE patients is dorsal subluxation of the ulna, as patients can present with wrist pain and limited pronation and supination. Much as in RA, untreated dorsal ulna subluxation can lead to extensor tendon rupture by attrition, and surgical treatment may be necessary; Nalebuff recommends a Darrach procedure in these cases [13].

ARTHROPATHY OF THE FEET, SACROILIAC JOINT, AND SPINE

The Foot in SLE

In SLE patients, Jaccoud's arthropathy can occur in the small joints of the foot with approximately the same frequency as in the hands, and in a similar manner [24]. Morley *et al*. [25] reported on 3 patients with bilateral subluxations of the metatarsophalangeal and interphalangeal joints of the toes. In "lupus foot," suggested by Mizutani and Quismorio [26], the most common abnormalities are metatarsophalangeal subluxation, hallux valgus, hammertoes, and forefoot widening without erosions or cystic lesions. Foot deformities were passively correctable in 12 of 14 patients. The patients most frequently affected were those with hand involvement and long-standing disease. Foot deformities can result in painful bunions and callosities, and it is recommended that proper shoe wear and meticulous foot care be instituted to prevent complications [27].

Sacroiliac Joint Abnormalities in SLE

Sacroiliitis, while typically affecting patients with seronegative arthritis, has also been reported in SLE patients [28]. Asymptomatic sacroiliitis has been observed on both radiographs and bone scans of SLE patients [29] with a male predominance [30]. Nassanova *et al*. noted that the male SLE patients with asymptomatic sacroiliitis were negative for HLA B-27 [30]. Symptomatic sacroiliitis, although rare, is seen in some SLE patients [28, 29] presenting as low back pain that is aggravated by movement. As with other acute joint inflammations in SLE patients, infection in the sacroiliac must be ruled out. Treatment with nonsteroidal anti-inflammatory medications (NSAIDs) or steroids may be needed.

Spinal Abnormalities in SLE

The spine in patients with SLE can also be involved. Pathologic lesions may involve the osseous structures, ligamentous complexes, or neural elements of the spine. The majority of spinal involvement in SLE patients occurs in the upper cervical spine, but it can also involve the subaxial spine.

Atlantoaxial Instability

Atlantoaxial instability, most commonly associated with rheumatoid arthritis, also occurs in SLE patients. In a prospective study, Babini *et al*. reported an incidence of atlantoaxial instability in 8.5% of SLE patients based on flexion–extension radiographs [31]. The mechanism by which this instability occurs is unknown, although it has been postulated to be due to generalized laxity of the cervical ligaments similar to that seen in other synovial joints (Jaccoud's arthropathy). None of the patients in this series had associated erosive bony changes. Atlantoaxial instability was associated with longer disease duration, Jaccoud's arthropathy, articular hypermobility, chronic renal failure, and elevated PTH levels. No recommendations or guidelines for the treatment of cervical instability were given, as all these patients were asymptomatic.

LESS FREQUENTLY ENCOUNTERED ARTICULAR AND PERIARTICULAR MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

The Shoulder, Hip, and Knee

The shoulders, hips, and knees can be involved in SLE, although they are less frequently involved than are the hands. Jaccoud's arthropathy has been used to

describe large joint involvement and has been reported to occur in shoulders and knees, most cases being symmetrical [32, 33]. Clinical signs of swelling, usually due to soft tissue thickening and joint effusions, are minimal, and joint subluxations or dislocations that are initially reversible can become fixed [27]. Synovitis or Jaccoud's arthropathy of the hip is rare, and therefore any hip or groin pain in an SLE patient should be considered due to osteonecrosis until proven otherwise. One rare and unusual articular manifestation of SLE is erosion of the temporomandibular joint [34, 35]. A diagnostic symptom may be complaint of recurrent earaches.

Periarticular Manifestations

Infrequently, periarticular symptoms can also be seen in SLE.

Enthesopathy and Tenosynovitis

Enthesopathy, defined as inflammation of ligaments, tendons, or joint capsules at their sites of attachment to bone, can also occur. Tenosynovitis has been reported in less than 10% of SLE patients [3]. In a study performed at the Hospital for Joint Diseases, 9 of 70 SLE patients had at least one episode of enthesopathy, including epicondylitis, rotator cuff tendonitis, achilles tendonitis, posterior tibial tendonitis, or plantar fasciitis. Some of these patients also exhibited a concurrent axial arthritis with or without sacroiliitis associated with an asymmetric lower extremity oligoarticular arthritis. Two of seven patients were positive for HLA B27, a proportion similar to the institutional control group. The dominant autoantibody in these patients was anti-nucleoprotein [36].

Tendon Ruptures

Tendon degeneration and rupture has been associated with chronic corticosteroid use, extended disease duration, and presence of other musculoskeletal complications [37]. Several investigators have described infrapatellar tendon rupture [38–40]; in some cases involvement can be bilateral. The clinical presentation is frequently acute, with both physical findings (inability to extend the knee actively, tenderness, and a palpable defect over the area of the rupture) and radiographic findings (a high-riding patella). Although only a few cases have been reported in the literature, it should be noted that there has been a tendency for male predominance in tendon rupture. Histology of ruptured tendons revealed linear hemorrhages, chronic degenerative changes, and vascular infiltration with mononuclear cells. Pritchard and Berney, seeking to identify the frequency of patellar tendon rupture in SLE patients over a 10-year period, reported this phenomenon in 4 of 180 [39]. They noted that all patients with a tendon rupture had been on prednisone for 7–15 years and had other complications associated with chronic steroid use such as osteonecrosis and vertebral compression fractures. Their review of the literature on 17 other cases of patella tendon rupture in SLE patients revealed the same risk factors for development of this complication, including chronic steroid use, extended disease duration, and presence of other musculoskeletal complications. Bilateral achilles tendon rupture has also been reported [41] and is possibly due to a combination of underlying disease and the degenerative changes that result from corticosteroid therapy.

Myositis and Myalgia

Muscle tenderness (40–48% of patients) and generalized myalgia in the proximal limb musculature are common in patients with lupus flares. Inflammatory myositis (5–11% of patients) involving the proximal musculature can be diagnosed by elevated creatinine phosphokinase levels, electromyography (EMG), and muscle biopsy and should be differentiated from proximal weakness due to a drug-induced myopathy secondary to glucocorticoids or antimalarials. There are no cases in the literature of rhabdomyolysis in SLE patients [27]. The EMG findings, which in SLE patients are similar to those observed in dermatomyositis–polymyositis patients, include spontaneous fibrillations, positive potentials, small amplitude, polyphasic potentials, and repetitive high-frequency potentials. Muscle biopsy findings can be normal or show interstitial inflammation, fibrillar necrosis, and degeneration [42].

Nodules and Soft Tissue Calcifications

Subcutaneous Nodules

Subcutaneous nodules have been observed in SLE patients [43, 44]. The histology of SLE nodules may be distinctly different from that of RA nodules. The histology from four nodule biopsies from Larson's series (44) revealed nonspecific inflammation, erythema nodosum, benign mesenchymoma, or fatty necrosis. In another report, Hahn *et al*. [45] described the histology of three biopsied periarticular subcutaneous nodules. In contrast to the observations of Larson, these nodule biopsy specimens revealed deep subcutaneous lesions with central necrosis surrounded by epithelial cells, suggesting that they resembled rheumatoid nodules. Subcutaneous nodules are usually associated with clin-

ically active SLE and may change size in conjunction with disease activity (shrink or disappear with declining disease activity, reappear or increase in size with disease exacerbation). It is speculated that theses nodules result from a common initial event of necrotizing inflammation in the small arteries of the subcutaneous tissue resulting in tissue necrosis distal to the damaged vessels followed by the formation of granulation tissue.

Soft Tissue Calcifications

Soft tissue calcifications that are more prominent in other collagen–vascular diseases (e.g., progressive systemic sclerosis, dermatomyositis, polymyositis) are uncommon in SLE. In most cases, the soft tissue calcification seen in SLE patients is noted as an incidental finding on radiographs, as patients are typically asymptomatic. In SLE, the calcific deposits, if present, are most frequently localized to the lower extremities. Minami *et al*. [46] reported two cases of subcutaneous calcification of the forearm in patients with SLE that were excised surgically. The histology revealed pure calcium phosphate crystals.

Calcinosis Universalis

Calcinosis universalis (deposits of calcium phosphate in muscle, subcutaneous nodules, or periarticular tissues) has been observed in discoid and SLE patients. Weinberger *et al*. reported on an SLE patient with calcinosis universalis who underwent spontaneous remission 1 year after systemic steroid therapy was started [47]. Only approximately 30 cases of calcinosis universalis have been reported in SLE patients [48]. The current recommended nonoperative treatment is diltiazem (a calcium channel blocker) [27].

SYNOVIAL FLUID AND TISSUE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Synovial Fluid

Joint effusions are typically infrequent and of small volume in patients with SLE. Several investigators have reported synovial fluid analysis from SLE patients. Gross description of the SLE synovial fluid is usually a clear to slightly cloudy fluid with good viscosity and mucin clot reflecting a lack of inflammation (suggesting no breakdown of hyaluronic acid by hyaluronidase). Histologic analysis has revealed the presence of lupus erythematosus cells (LE cells), and immunologic analysis has revealed the presence of anti-nuclear antibodies (ANA). Pekin and Zvaifler [49] reported on synovial fluid analysis from 26 SLE patients. In their series, the synovial fluid white blood cell count was less than 2000/mm^3 in 19 of 26 samples and greater than 10,000/mm^3 in only 2 samples. Analysis of the cells revealed that they were predominately mononuclear. Twelve of the specimens of synovial fluid from this study underwent further analysis. Four specimens had the features of a transudate, with an expected high ratio of serum/synovial fluid levels of complement, total protein, and IgG. It was postulated that the noninflammatory transudate resulted from a generalized change in the periarticular vascular permeability, thereby causing a dilution of synovial fluid by edema fluid. The other eight specimens exhibited the features of an exudate. Of these, three had serum/synovial fluid ratios (of complement, total protein, and IgG) approaching 1, indicating a proportional escape of proteins into the joint fluid, which is typical of an exudative effusion; in the other five, the ratio of serum complement to synovial fluid complement was elevated, in contrast to the ratios obtained for either total protein or IgG concentration (i.e., the synovial fluid complement concentration was lower relative to either the protein or IgG concentration), suggesting the local consumption of complement in or just before it entered the synovial fluid. The mechanism of complement consumption, however, is still unknown. The latter five exudative effusions, three of which had the highest white cell counts, with reduced complement content and higher total protein and IgG content, were reminiscent of the synovial fluid seen in RA. A distinguishing feature of SLE joint fluid may be that they typically have fewer polymorphonuclear leukocytes than rheumatoid joint fluid.

Synovial fluid analysis is not pathognomonic for SLE. Many earlier investigators relied only on the LE cell preparation for diagnosis of SLE since they did not have more specific laboratory tests (e.g., kinetoplast of the *Crithidiae luciliae* for detection of antibodies to native DNA and "immunoblot" or Western blot techniques for the detection of soluble RNA–protein conjugate antigens of Sm specificity). The presence of antibodies to both nDNA and Sm from synovial fluid samples is extremely suggestive of SLE [50].

Synovial Tissue

Most investigators have observed that the synovial histopathology in SLE is nonspecific [51–53]. Histologic analysis of synovial tissue has yielded some insights into the pathogenesis of the arthropathy in SLE patients. In 1959, Cruickshank [54], examining histologic specimens obtained from autopsies of 10 SLE patients, observed diminished synovial lining cells, minimal inflammation,

and hematoxylin bodies in the synovial cell layer (thought to be tissue analogs of the LE cell inclusion bodies containing nuclear debris). A thick layer of superficial fibrin-like material deposited along the synovial surface and within the synovium was also observed, with only minimal perivascular inflammation.

Subsequent histologic analyses of needle biopsy synovial fluid specimens have noted greater lining cell proliferation, inflammation, and vascular lesions. Labowitz and Schumacher [2] using light microscopy, observed superficial fibrin-like material on the synovial membrane, focal or diffuse synovial lining cell proliferation, variable inflammation (predominantly of perivascular mononuclear cells), and occasional small vessel wall infiltration suggestive of vasculitis. Many of the vessel lumens were obliterated due to enlarged endothelial cells, inflammatory cells, and organized thrombi. Histologic analysis of specimens from two patients without clinical signs of joint inflammation showed synovial inflammation and vascular lesions. Electron microscopy revealed superficial fibrin-like material in most specimens in the absence of typical fibrin periodicity; lining-cell proliferation consisting of type A (phagocytic), type B (synthesize hyaluronidate), and type C (intermediate and secretory) cells with no clear cellular predominance; necrotic cells and nuclear debris scattered throughout the superficial and deep synovial lining layer; disorganized and multilaminated vascular basement membranes; and enlarged vascular endothelial cells occluding some lumens. Erythrocytes and polymorphonuclear leukocytes were also observed in some specimens in gaps between venular epithelial cells. Aggregates of microtubules reminiscent of paramyxoviruses were seen as vascular endothelial inclusions in some samples. In only one specimen was there a small amount of electron-dense material lying beneath the vascular endothelium similar to that described in the glomerulus of the lupus patient observed. These authors suggested that the small vessel changes contribute to the pathogenesis of the articular disease.

TREATMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS ARTHROPATHY

The arthropathy of SLE tends to be nonaggressive, and thus treatment is generally medical, with either salicylates or nonsteroidal anti-inflammatory drugs (NSAIDs). The goals of medical management include suppression of inflammation, pain relief, and maintenance of normal joint function and muscular strength.

Salicylates

Patients with short-duration arthralgias may not require medication. For patients whose symptoms are more pronounced, initial medical management with salicylates (daily dose of approximately 3 g/day) may be indicated as long as there is no evidence of hepatic inflammation from systemic SLE disease activity. Long-acting salicylate preparations (e.g., zero-order release aspirin) can also be used, allowing for fewer daily doses and longer dosage intervals for medication intake, usually resulting in greater patient compliance. Salicylate levels can be monitored to maintain a therapeutic level of 20–25 mg/100 ml. In the authors' experience, salicylates have been well tolerated in SLE patients; however, the clinician should monitor patients for gastrointestinal intolerance and hepatocellular abnormalities.

Nonsteroidal Anti-inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) have also been used successfully to treat SLE arthropathy. Cases of aseptic meningitis have been reported with the use of ibuprofen. Results on the use of specific prostaglandin and/or leukotriene inhibitors show promise but still await controlled clinical trials. NSAID selection depends on cost, patient tolerance, drug half-life, and individual patient side effects.

Antimalarial Drugs

Antimalarial drugs—hydroxychloroquine (starting dose of 200–400 mg/day), taken in the evening to reduce gastrointestinal side effects such as nausea—may be a useful adjunct in the management of SLE arthralgias and polyarthritis and can be used in addition to NSAIDs [55, 56]. As antimalarial drugs are slower-acting than salicylates or NSAIDs, the beneficial effects may not be seen during the first month of treatment. Ophthalmologic follow-up is mandatory on all patients treated with antimalarial drugs.

Corticosteroids

Corticosteroids should be used only to treat patients who have failed a trial of other medications (e.g., salicylates, NSAIDs, or antimalarial drugs) and whose activities of daily living are severely impaired. When corticosteroids are prescribed for articular involvement without other accompanying signs or symptoms of SLE disease activity, one should use low doses (equivalent of 10 mg prednisone/day or lower) for as brief duration as is possible.

OSTEONECROSIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

Epidemiology of Osteonecrosis in SLE

Osteonecrosis represents a collection of pathologic conditions of various etiologies that result in impairment of local blood supply to periarticular bone, resulting in bone cellular death. The host response to osteonecrosis can lead to architectural collapse of subchondral bone, joint incongruency, and eventual secondary degenerative arthritis. The reported incidence of osteonecrosis in SLE patients is 4–16%, although it may be higher; the femoral head is involved in 80% of these patients and involvement is often bilateral [57–59]. Because of its frequency and the significant disability that can result (particularly in the young lupus population), osteonecrosis is the articular manifestation of SLE with the most morbidity.

It is interesting to note that there were no reported cases of osteonecrosis in SLE patients prior to 1960; corticosteroid therapy for SLE patients began in the 1950s. Most cases of osteonecrosis in SLE patients are believed to be due in part to steroid treatment, for there is a positive correlation in patients so treated between incidence of osteonecrosis and both dosage and duration of treatment (including pulse steroid therapy) [58–63]. Other possible causes of osteonecrosis include fat emboli, Raynaud's phenomenon, small vessel vasculitis, and the anti-phospholipid antibody syndrome [64–67].

Dubois and Cozen [68] were the first to described osteonecrosis in patients with SLE in 1960. They reported on 11 cases of osteonecrosis in a population of 400 SLE patients who were known to have SLE for a mean of 5.1 years. The mean age of these patients was 36 years old. Eight patients had bilateral and one had unilateral femoral head involvement; the remaining two patients had bilateral knee involvement. These authors noted a different characteristic pattern of clinical symptoms (persistent painful motion localized to a single joint) suggesting osteonecrosis rather than an arthralgia or arthritis due to lupus disease activity. Patients with knee osteonecrosis noted initial clinical symptoms of unilateral swelling and joint effusion (with no increase in local warmth), which were completely relieved by rest. Patients with hip osteonecrosis noted a limp, groin pain, or pain radiating down one side of the thigh, and occasionally thigh muscle spasm. Histology from biopsy of the femoral head of one of these patients revealed osteonecrosis without evidence of vasculitis. There also appeared to be an association between onset of symptoms of osteonecrosis and corticosteroid therapy. In an attempt to determine the incidence of asymptomatic osteonecrosis, these authors examined the hip radiographs of 70 asymptomatic SLE patients, noting only a single case of early osteonecrosis.

Subsequent reports more firmly established the relationship between steroid therapy and osteonecrosis in SLE patients. In 1962, Siemsen *et al*. [69] reported on 3 patients with SLE and osteonecrosis. One male patient with discoid lupus, who never received systemic steroids, had mild membranous glomerulonephritis (positive ANA's were never detected) and osteonecrosis of both femoral heads. Histology of biopsy specimens from this patient revealed evidence of vasculitis in the synovial tissue and muscle but not in bone. Each of the 2 other SLE patients had unilateral femoral head osteonecrosis and had been treated with at least 20 mg prednisone/day for more than 12 months. Leventhal and Dorfman [70] described the cases of 11 women with femoral head osteonecrosis; 10 in patients with SLE and 1 in a patient with discoid lupus. All patients were being treated with or had been treated with steroids. Surgical specimens analyzed by histology from 10 patients (13 femoral heads) failed to demonstrate any evidence of a recent, healed, or chronic vasculitis.

Klipper *et al*. [71] reported on 23 SLE patients with stage 2 osteonecrosis (radiographic changes without evidence of subchondral collapse), and attempted to determine the risk factors for development of osteonecrosis in SLE patients. All but 1 patient was female, and there was no racial predominance. The average interval between diagnosis of SLE and the occurrence of osteonecrosis was 3.4 years (range 0.6–16 years). All patients had received steroids (duration of treatment 40 months, range 7–132 months) with maximum daily dosages of 20–100 mg except for a single patient who was taking 12.5 mg/day. Multiple joints were affected in 83% of patients; femoral head osteonecrosis occurred in 21 patients (15 patients with bilateral involvement), humeral head osteonecrosis in 7 patients, tibial plateau osteonecrosis in 3 patients, and scaphoid osteonecrosis in 1 patient. Eighty-seven percent of SLE patients with osteonecrosis had either Raynaud's phenomenon and/or central nervous system involvement. These authors suggested that osteonecrosis was the result of a generalized vascular pathology. Surgical specimens from 6 patients were analyzed; however, there were no cases of endarteritis within the femoral heads. One histologic specimen did demonstrate the deposition of fibrinoid material in and on the synovial membrane and vessels with fibrous perivascular cuffing (perhaps reflecting a healed vasculitis). These authors concluded that either vasospasm, vasculitis, or both in addition to glucocorticoid therapy might cause occlusion of the microcirculation with resultant bone ischemia.

Dimant *et al*. [72] analyzed 22 patients with osteonecrosis from a cohort of 234 SLE patients. Using life table analysis, they calculated that the projected frequency of osteonecrosis approached 30% in patients who had had SLE for 10–15 years. All the affected patients were women (mean age of onset 30.7 years) who had received steroid treatment; the majority had been diagnosed with osteonecrosis at 4–7 years following the diagnosis of SLE. Osteonecrosis involved the femoral heads in 18 patients and the knees in 4 patients. These authors, however, noted no correlation between the development of osteonecrosis and any particular clinical feature of SLE. Raynaud's phenomenon was present in 36% with evidence of osteonecrosis and in 43% of patients without such evidence. No correlation was found between osteonecrosis and duration, peak dose, cumulative dose, or average monthly dose of corticosteroid therapy; however, a trend toward higher peak and cumulative corticosteroid dose in patients who developed osteonecrosis was observed, possibly reflecting the longer duration of follow-up in these patients. No pathologic specimens were available from patients in this study.

Investigating the possibility of other predisposing factors, Klippel *et al*. [73], who reviewed 31 patients diagnosed with osteonecrosis (confirmed by plain radiographs) from a cohort of 375 SLE patients, noted no differences with regard to sex, racial distribution, age of onset, or select manifestations of SLE between the group that had osteonecrosis and the group that did not. The most commonly affected sites were the large weight-bearing joints of the lower extremities, with a predominance for bilateral involvement. All patients with osteonecrosis had received corticosteroids, and there was a direct correlation between patients with osteonecrosis and treatment with high doses of steroids. An important contribution of this study was the radiographic evaluation of 31 corticosteroid-treated SLE patients without articular complaints. The X rays of bilateral hips, knees, shoulders, ankles, elbows, and wrists revealed radiographic evidence of stage 2 osteonecrosis in 26% of these asymptomatic patients. The authors suggested that the true frequency of osteonecrosis in adult SLE patients might exceed 30% and that as many as 75% of SLE patients with osteonecrosis are asymptomatic.

In a prospective study, Zizic *et al*. [74] studied 54 SLE patients and found that 28 had osteonecrosis. Osteonecrosis was present in multiple sites in 26 of them (93 locations overall); the hips, knees, and shoulders were most commonly affected (in decreasing order of frequency). Demographic characteristics as well as clinical and laboratory findings were similar in patients with and without osteonecrosis, except for cushingoid changes (24 patients [86%] with osteonecrosis vs 4 [15%] patients without). In patients with osteonecrosis, there was a strong correlation between mean steroid dose and number of bony sites involved; mean daily steroid dose for the highest single month (93% of osteonecrosis patients had received greater than 20 mg/day) as well as the greatest number of consecutive 3-, 6-, and 12-month periods of corticosteroid therapy were both significantly higher in patients with osteonecrosis. In patients with Raynaud's phenomenon, even lower mean steroid doses resulted in osteonecrosis. A summary of the literature on osteonecrosis in patients with SLE appears in Table 1.

Pathogenesis of Osteonecrosis

Much of the following discussion is based on studies of osteonecrosis of the femoral head, as this is the site most commonly involved.

Blood Supply of the Femoral and Humeral Head

The frequency of femoral head involvement may be explained, in part, by its vascular supply. The femoral head derives its blood supply from three sources: the intraosseous cervical vessels, retinacular vessels, and the artery of the ligamentum teres. Since each vascular source serves a specific area of the femoral head and there are few vascular anastomoses, disruption of blood supply from one arterial source cannot be compensated for by collateral vasculature. The humeral head has a similarly tenuous blood supply, with the ascending branch of the anterior humeral circumflex artery supplying the largest portion of the articular surface with little collateral overlap.

Vasculitis and Osteonecrosis in SLE

Several pathogenic mechanisms have been proposed to result in osteonecrosis in SLE, including both intrinsic factors due to the underlying disease activity, such as vasculopathy [72], and extrinsic factors such as glucocorticoid treatment. It is possible that a vascular insult, such as vasospasm or inflammation, could interfere with bone microcirculation. This hypothesis is supported by the finding that SLE patients with Raynaud's phenomenon have a higher incidence of osteonecrosis [71, 74]. Vasospasm alone is not a sufficient agent, as there are no reports of osteonecrosis in patients with progressive systemic sclerosis and Raynaud's phenomenon. Klipper *et al*. [71], in a study of 77 patients with progressive systemic sclerosis, and Frayha *et al*. [75], in a retrospective review of 180 such cases, both failed to show osteonecrosis of either the femoral or humeral heads.

TABLE 1 Major Studies on Patients with SLE and Osteonecrosis

Author	Patients with ON[a]/total patients	Presence of Raynaud's and/or CNS involvement	Sites involved[b]	Pathology data	Relationship to corticosteroid therapy
Dubois and Cozen [68]	11/400	NA[c]	Femoral heads, femoral condyles	NA	10/11 had received steroids, dose NA
Leventhal and Dorfman [70]	11/No control group	1 had Raynaud's, 3 had CNS involvement, 1 had vasculitic ulcers	Femoral heads, humeral heads	No evidence of vasculitis or fat emboli	All patients had received steroids: 1 only received steroids for a month, 20 years prior to ON[a]
Klipper *et al*. [71]	23/No control group	87% had Raynaud's and/or CNS involvement	Femoral heads, humeral heads, navicular (scaphoid); tibial plateau	No evidence of vasculitis	All patients had received steroids, maximum dose 20–100 mg/day, except 1, who received 12.5 mg/day. Therapy lasted 9–132 months. One had not received steroids for 13 years prior to ON
Smith *et al*. [142]	7/99—Used control group of 7 patients without ON	3 had Raynaud's and ON vs none in control group; 1 had CNS involvement vs none in control group	Hips, knees, shoulders	NA	All patients with ON had received steroids; no difference from 7 control patients
Delanois *et al*. [155]	14/32 (pediatric group)	No differences between groups with or without ON	Distal femoral condyles, femoral heads, talus, metatarsal, patella, capitulum	NA	All patients with ON had received 54 g (average) compared with 31 g in patients without ON. No difference was found in average annual dose
Dimant *et al*. [72]	22/234	No differences between group with or without ON	Hips, knees	NA	All patients with ON had received steroids. Those with ON received a mean cumulative dose of 43.7 g compared with 30.6 g in patients without ON
Abeles *et al*. [61]	17/365	18% with ON had Raynaud's, 18% without ON had Raynaud's, 2 with ON had CNS involvement	Hips, knees, navicular (scaphoid), capitate	NA	Patients with ON received an average total dose of 32 g steroids compared with 31.8 g for patients without ON. Patients with ON had higher prednisone intake during the initial 6 months of therapy
Klippel *et al*. [73]	31/375	26% with ON had Raynaud's, 35% without ON had Raynaud's, 18% with ON had CNS involvement, 4% without ON had CNS involvement	Femoral head, femoral condyles, talus, humoral head, radial head, metacarpal head, metatarsal head, navicular (scaphoid) lunate	NA	All patients with ON had received steroids. Of those with ON, 32% received higher doses of steroids than those without ON
Zizic *et al*. [74]	28/54	Patients with ON and Raynaud's received less prednisone than those with ON without Raynaud's	Hips, knees, shoulders	NA	Patients with ON received 43.3 g steroids compared with 41.8 g for patients without ON. Patients with ON had higher mean dose of steroids for the higher single month

[a] ON, osteonecrosis.
[b] Anatomic sites are written as they are described by the respective authors.
[c] NA, not available.

Vasculitis is not always seen in pathologic specimens of osteonecrosis in SLE patients; however, it may be undetectable due to its transient presence and possible absence in later stage of the disease [70]. Vasculitis, characterized by inflammation of vessel walls with intravascular activation of polymorphonuclear leukocytes, diapedesis, and release of toxic oxygen species, may stimulate polymorphonuclear leukocytes to self-aggregate (leukoemboli), resulting in intravascular thrombi. Given *et al.* [76] observed leukoaggregating factors such as complement split products in the sera of lupus patients. Increased surface statement of the complement receptor CR3 (involved in cell–cell adhesion) on neutrophils isolated from SLE patients supports the possibility of white cell vascular plugging [77]. Possibly a combination of vasospasm, vasculitis, and white cell vascular thrombi help bring about osteonecrosis in bony sites that lack sufficient collateral circulation (e.g., the femoral head).

Glucocorticoids and Osteonecrosis in SLE

The mechanism of steroid-induced osteonecrosis is still not fully understood. McFarland and Frost [78] suggested that steroids suppress osteoblastic function and therefore interfere with the normal host repair response to microfractures, while other investigators have postulated that the analgesic effect of steroids results in a neuropathic (Charcot-like) arthropathy [79]. Other possible factors include the effects of steroids on fat metabolism, storage (causing fatty liver [80]), and asymptomatic systemic fat emboli. Fisher and Bickel [81, 82], using the oil red O staining technique, were able to identify intravascular fat emboli in patients on corticosteroids who had femoral head osteonecrosis. They concluded that systemic fat emboli (arising from coalescent hepatic fat cysts due to corticosteroid therapy) caused a mechanical vascular obstruction and osteonecrosis. Jones and Sakovich [83] induced osteonecrosis in the femoral heads of rabbits by the infusion of iodized poppy seed oil into their distal aortas. Histologic analysis revealed intraosseous fat emboli and obstructed subchondral arterioles. The presence of steroid-induced fat emboli, however, may be transient, as Leventhal and Dorfman [70] could not detect intravascular fat emboli from multiple human femoral sections stained with oil red O.

Lipid Metabolism and Osteonecrosis

Other investigators have suggested altered lipid metabolism (e.g., increase in fat cell size and/or number) resulting in intramedullary lipocyte proliferation as a cause of osteonecrosis. An increase in the number and/or size of lipocytes could increase intraosseous pressure, reduce sinusoidal circulation, and subsequently infarct the marrow and bone [84, 85]. This theory is supported by the observations of Zizic and co-workers [74, 86], who noted a higher incidence of osteonecrosis in patients with cushingoid features (increased truncal adipose tissue, possibly as a result of increased intramedullary lipocytes). Bluemke *et al.*, using magnetic resonance imaging (MRI) and spectroscopic evaluation in SLE and normal patients, noted a higher marrow fat content in SLE patients in the femoral head and neck, with an inverse relationship to the perfusion of the femoral head [87].

Thrombophilia and Osteonecrosis

A hypercoagulable state may also predispose to osteonecrosis in SLE patients. Asherson *et al.* noted that patients with a hypercoagulable state due to antiphospholipid antibodies had an increased tendency to develop osteonecrosis [88]. Finally, corticosteroid treatment may induce a hypercoagulable state promoting venous stasis and ultimately decreasing oxygenation of affected bone [89, 90].

Conclusions: Pathogenesis of Osteonecrosis

In conclusion, no one pathogenic mechanism can explain the cause of osteonecrosis in SLE. The cause is probably multifactorial and may be either intrinsic or extrinsic causes or both. Certainly, SLE patients are at higher risk for developing osteonecrosis if their mean daily steroid dose for 1 month or more is high [74], if they have evidence of vasospasm (e.g., Raynoud's phenomenon), or have evidence of altered lipid metabolism (e.g., cushingoid features). The reversibility of these risk factors in patients without osteonecrosis, or how long they remain at risk for osteonecrosis, is not known.

Diagnosis Studies

Although many diagnostic radiologic modalities are available (e.g., scintigraphy, computed tomography), magnetic resonance imaging (MRI), because it permits early diagnosis of clinically and radiographically silent cases of osteonecrosis, is the diagnostic tool of choice [91–97]. Magnetic resonance imaging has a diagnostic accuracy rate of 90–99% and a sensitivity and specificity of 80–85%. Computerized tomography and bone scintigraphy are less accurate and may miss early osteonecrotic lesions. Bone scan has an accuracy rate of approximately 85% and sensitivity and specificity of 68–83% [92, 98–100]. Bone biopsy and culture may be necessary to differentiate atypical sites of osteonecrosis

from osteomyelitis [101]. Early recognition of osteonecrosis is important for proper patient counseling, prognosis, and surgery if core decompression with or without structural grafting will be performed. SLE patients who present with osteonecrosis at one anatomic site should undergo a thorough investigation for lesions at other sites including the knee, ankle, and shoulder as many patients eventually develop involvement of multiple joints.

Staging and Classification for Osetonecrosis of the Femoral Head

There have been several classification systems for osteonecrosis. This has led to much confusion in the literature regarding results of treatment and prognosis of early lesions. Although the etiology of osteonecrosis is diverse, the disease process typically progresses through definable stages. Staging of osteonecrosis is based on a combination of pathologic, radiographic, and/or clinical criteria and is important in order to determine prognosis and appropriate treatment [98, 100, 102]. The osteonecrosis staging system proposed by Ficat and Arlet [102], previously in greatest use, has been replaced by a uniform staging system introduced by the Association Research Circulation Osseous (ARCO). In this five-stage system, stage 0 is histology only, stage 1 a positive diagnostic test, stage 2 a positive X ray with no radiographic collapse, stage 3 a positive X ray with collapse, and stage 4 a positive X ray with osteoarthritis. Each stage can be subclassified according to the percentage of head involvement or surface collapse: Subclass A—minimal, with <15% femoral head involvement or <2 mm of surface collapse; Subclass B—moderate, with 15–30% head involvement or with 2–4 mm of surface collapse; Subclass C—extensive, with >30% femoral head involvement or >4 mm of surface collapse. Not all cases of osteonecrosis develop joint degeneration. The critical stage of this new classification system, as of previous ones, is the development of the crescent sign or subchondral collapse. Once this sign is present, the joint will invariably degenerate and orthopedic reconstructive surgery will be necessary (Fig. 7).

Stage 0

This initial stage precedes clinical and radiographic manifestations of the disease. By definition, radiographs, bone scan, and MRI are normal. This stage is usually only diagnosed in the contralateral femoral head in a patient with known osteonecrosis of the opposite hip. Contralateral hip osteonecrosis occurs in 90% of SLE patients [71, 73, 91, 94, 103, 104]. As a result of the disruption of vascular supply to a segment of periarticular bone, cell death occurs [62, 63, 105–108]. Necrotic changes in the marrow elements and empty lacunae representing osteocyte death are the earliest events observed by histology. At this stage, bone maintains its normal trabecular architecture and density.

Stage 1

Stage 1 osteonecrosis usually represents the onset of clinical signs without concomitant radiographic abnormalities. This is referred to as the *silent hip* of osteonecrosis, where histologic changes can be seen and in some cases hemodynamic changes are present [108]. Following cell death, the host repair process begins with a reactive hyperemia to the bone adjacent to the infarcted area, resulting in bone resorption (demineralization and trabecular thinning of the bone). The host repair process continues as new capillaries and granulation tissue invade the area of necrotic bone. The necrotic bone and cellular debris are resorbed by creeping substitution by osteoclasts and phagocytes, respectively. Diagnosis is based on a positive diagnostic test such as a positive bone scan or MRI. Localization of the area of bone involvement may help guide those surgeons who are advocates of core decompression to the precise area of involvement [92, 93].

Symptomatic patients usually complain of intermittent and mild to moderate groin pain. Physical examination at this stage may show a mildly restricted range of motion, particularly in internal rotation [99]. Diagnostic evaluation should start with X rays of the hip, an anteroposterior view (taken with the hip internally rotated 15° for best presentation of the femoral head and neck), and a lateral view. X rays at this stage are by definition normal; however, in some cases subtle changes in the trabecular architecture may be evident when compared with the opposite (normal) side. For patients at risk for osteonecrosis, such as SLE patients, MRI is indicated. Bone scan and biopsy, both of which have been advocated in the past, should be reserved only for those situations in which MRI is not available.

Stage 2

Patients with stage 2 osteonecrosis have a positive X ray finding with no radiographic collapse. These patients most often complain of dull, aching groin pain on weight bearing and occasionally at rest [99]. Physical examination usually reveals a global decrease in range of motion and a slight limp. Radiographic changes are variable; the infarcted area may appear sclerotic in comparison with the surrounding area of the femoral head and neck due to the reactive hyperemia and osteoporosis that occur

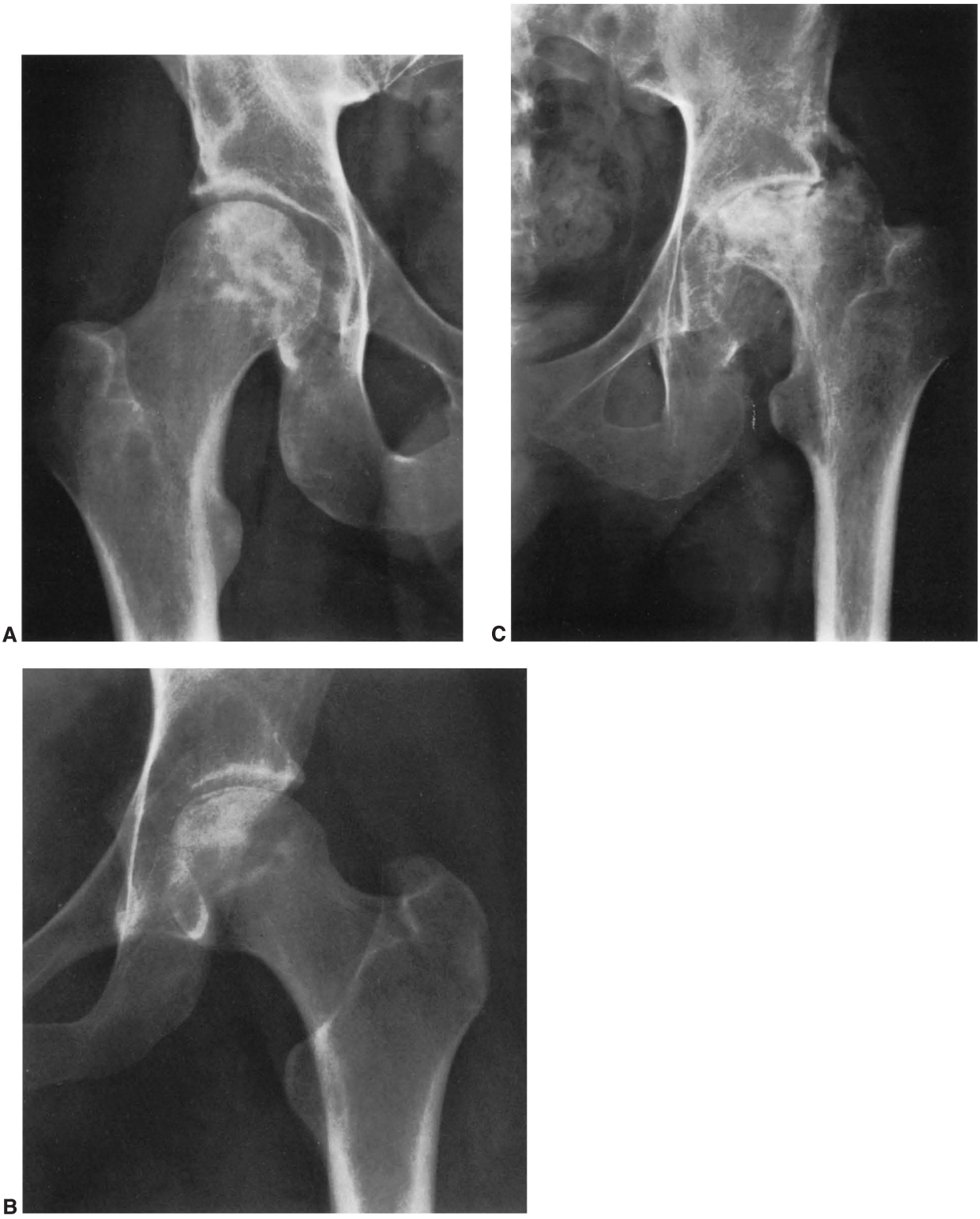


FIGURE 7 Anteroposterior radiographs of hips with osteonecrosis demonstrating the various stages: (A) stage 2 osteonecrosis showing areas of sclerosis and cyst formation without evidence of subchondral collapse. (B) stage 3 osteonecrosis reveals the crescent sign. The crescent sign marks the progression from stage 2 to stage 3 osteonecrosis. (C) stage 4 osteonecrosis showing extensive femoral head collapse, joint space narrowing, and secondary degenerative changes.

at the vascularized perimeter of the infarct; the infarcted area may be well delineated by a peripheral rim of increased density due to new bone formation at the periphery of the infarct; or the infarcted area may appear osteoporotic or cystic as the repair process progresses. An important radiographic finding is maintenance of both the joint space and the sphericity of the femoral head. The crescent sign represents the end of stage 2 and the start of stage 3.

Stage 3

Stage 3 osteonecrosis is characterized by a positive X ray with subchondral collapse. As the necrotic subchondral bone is resorbed, the overlying articular cartilage may be left without sufficient structural support for a significant length of time. During this period, either weight-bearing or joint reactive forces may cause the weakened subchondral bone to fracture or collapse, leading to joint incongruity. Radiographically, a crescent-shaped subchondral lucency, termed the *crescent sign*, can be observed. The host response at this point continues to be reparative; however, it is now no longer possible to restore the sphericity of the femoral head and joint congruity.

Patients at this stage usually have an exacerbation of symptoms about the hip, and pain at rest is more common [99]. Many patients will also note a distinct clicking sensation during movement of the hip due to the joint incongruity. On physical examination, the hip range of motion is reduced and a limp is evident. Standard radiographs are pathognomonic; flattening of the femoral head is evident, particularly on the lateral view due to mechanical failure of the osteonecrotic area of the femoral head. The acetabulum is radiographically unaffected.

Stage 4

Stage 4 osteonecrosis, characterized by a positive X ray with secondary degenerative joint disease, represents end-stage disease [99]. With time, due to a combination of joint incongruity and lack of diffusion nutrition to the overlying articular cartilage from the subchondral bone, secondary degenerative joint changes occur.

Patients will complain of increasing joint pain, stiffness, and a deterioration of function. Radiographs reveal flattening of the femoral head, progressive loss of joint space osteophytes, and subchondral cysts on either the femoral or acetabular side or both. These end-stage radiographic changes at times may be indistinguishable from the changes of long-standing osteoarthritis.

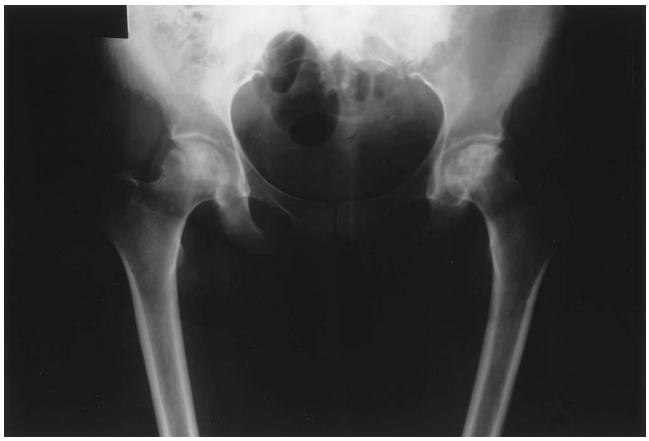
Treatment of Osteonecrosis of the Femoral Head

Treatment of femoral head osteonecrosis is based on the stage of presentation, location and percentage of bone involvement, and status of the articular cartilage [100]. Once degenerative joint changes occur, cartilage sacrificing procedures such as joint replacement may be required (Fig. 8) [109, 110]. Since there have been few studies of the management of osteonecrosis of SLE patients, the algorithm of treatment of osteonecrosis from all causes is generally applied. Nevertheless, in the following discussion, results from studies of SLE patients and osteonecrosis will be emphasized where available and appropriate.

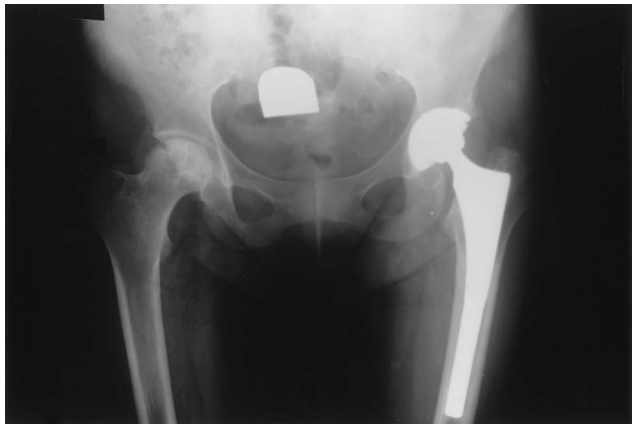
The treatment of the early stages of osteonecrosis (stages 0, 1, and 2) is controversial. Prior to the development of the crescent sign (stage 3), treatment options include observation, restriction of weight bearing, electrical stimulation, surgical core decompression with or without structural bone grafting (with either avascular or vascularized fibular graft), and proximal femoral osteotomies. There are two different philosophies of how to treat early femoral head osteonecrosis. One group of physicians believes that the die is cast at the time of the vascular insult and thus that symptomatic management is all that is needed. Prognosis for later subchondral collapse and early degenerative joint disease is based primarily on the percentage and location of bone involvement. The other group of physicians believes in the possibility that surgical treatment can succeed in promoting revascularization, healing the osteonecrotic segment, and preventing collapse. Due to problems in older staging classification systems, it has been difficult to compare outcomes among the various treatment options or to determine the natural history of osteonecrotic lesions. As a result, there have been several nonoperative and operative treatments for osteonecrosis that have had varying results.

Nonoperative Treatment

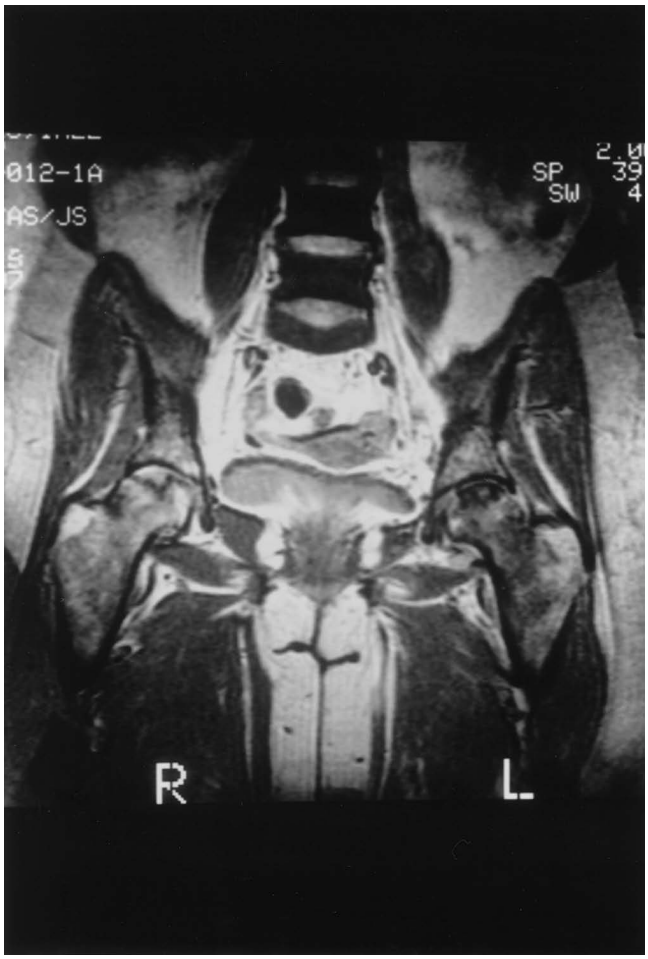
Nonoperative treatment with the use of either nonsteroidal anti-inflammatory or analgesic medications can help to decrease symptoms and improve function, but have no known effect on the possible progression to subchondral collapse. Restriction of weight bearing using either a cane or crutches for periods when the joint is more symptomatic can help ameliorate pain; however, such measures have not been shown to affect the ultimate outcome from the osteonecrosis probably due to the high joint reactive forces that cross the hip joint with movement. The results of nonoperative treatment have been variable—again as a result of



A



C



B

FIGURE 8 (A) Anteroposterior radiograph of the hips of a patient with SLE with hip pain localized to the left groin. Marked sclerosis of the femoral head with subchondral collapse is seen consistent with stage 3 osteonecrosis. (B) MRI demonstrates osteonecrosis in both femoral heads compromising more than 30% of the femoral head on the left thereby staging this to 3C osteonecrosis. (C) This patient later went on to stage 4C osteonecrosis and underwent successful treatment with an uncemented total hip arthroplasty.

nonuniform osteonecrosis staging. It is generally believed that patients in whom >50% of the femoral head is osteonecrotic, especially in the weight-bearing area, are likely ultimately to experience subchondral collapse and degenerative arthritis, whereas patients with <15% femoral head involvement will probably heal without collapse. Early reports of poor prognosis for patients with early-stage osteonecrosis did not evaluate the percentage of bone involvement and in some cases reported poor outcomes with this approach, thereby suggesting operative management [98].

Core Decompression

Core decompression is one surgical approach to treating the early stages of osteonecrosis [99,102,

111–113]. In stage 0–2 osteonecrosis, core decompression can be performed; however, its use remains controversial in the orthopedic literature. Ficat and Arlet [102] and Hungerford and Zizic [111] have postulated that elevation of bone marrow pressure within a closed, rigid space such as the femoral neck leads to occlusion of intraosseous blood vessels, resulting in bone ischemia and the clinical symptoms of pain prior to collapse. To diagnose osteonecrosis, these same authors described the "functional exploration of bone" in order to identify pre-radiographic osteonecrosis.

Surgery (core decompression) is performed in order to measure bone marrow pressure, release or decompress elevated bone marrow pressure, and perform a core biopsy for both diagnostic and therapeutic purposes. The surgical procedure for core decompression consists of drilling through the femoral neck into the femoral head with a hollow coring device (6–10 mm in diameter) in order to reduce the elevated intraosseous pressure and improve vascularization. Ficat and Arlet [102] have used this modality extensively with excellent results; however, other authors have not been able to replicate these results [114–116]. Using their own staging system, Ficat and Arlet reported 94% good or excellent clinical results in 82 hips with stage 1 osteonecrosis and 86% good or excellent clinical results in 51 hips with stage 2 osteonecrosis (14% developed radiographic signs of collapse) at an average follow-up of 9.6 years [99,102]. Aaron *et al*. [114], utilizing only clinical criteria, reported only a 44% success rate in patients treated with core decompression. Tooke *et al*. [115] reported a 100% success rate when treating stage 1 hips but only a 58% success rate when treating stage 2 hips with core decompression. Mont *et al*. [117] conducted a cross-sectional study of osteonecrosis in 31 hips in SLE patients and in 48 hips in those who were on steroid medication for other reasons, all treated with core decompression. At an average follow-up of 12 years, only 53% of all core decompression patients had a satisfactory result (68% of SLE patients compared to 33% of other steroid-treated patients had undergone hip replacement surgery). A matched subset of these patients for age, gender, steroid dose, osteonecrosis stage, and length of follow-up showed no statistically significant differences with regard to clinical outcome. The authors noted a worse prognosis for patients who were treated in stage 3 osteonecrosis and who had a greater extent of femoral head involvement. In light of these variable clinical outcomes with core decompression alone, clinicians have sought out other surgical treatments in order to try to preserve articular cartilage and improve outcomes of patients with femoral head osteonecrosis [116].

The issue of cartilage sparing procedures in SLE patients with osteonecrosis who require continued steroid treatment remains unanswered. Some authors note no effect on surgical outcomes in patients who are on steroids [114], whereas others have noted poorer outcomes in patients with continued steroid treatment who have undergone core decompression (with or without concomitant bone grafting) than in similar patients whose steroid treatment had been terminated [115].

Proponents of core decompression cite intraosseous hypertension as a source of joint pain and further bone necrosis, while opponents cite venous hypertension as either an artifact or a result of the necrosis and propose that removal of bone from the femoral head further weakens the subchondral bone, leading to further collapse. Papers citing the success of core decompression can be contrasted to those that show either no difference or worse results as well as both intraoperative and postoperative femoral neck fractures occurring in a small percentage of patients (also reported in SLE patients following the procedure) [118]. Based on these findings, the majority of surgeons believe that core decompression should not be performed for stage 3 or 4 osteonecrosis.

It is probable that the controversy in the literature is at least in part due to the nonuniform staging systems used in the past. It is currently believed that there exists a strong correlation between the size of osteonecrosis of the femoral head and the prognosis for collapse, with significantly better results with smaller-size lesions and sparing of the lateral column. The probability of progression to end-stage (requiring arthroplasty) osteonecrosis has been found to vary between 20 and 60% of cases.

Core Decompression Combined with Structural Bone Grafting

The less-than-desirable outcomes of core decompression alone are believed to be due to a further compromise of the subchondral bone by removal of a core of bone, making it more susceptible to collapse [119]. As a result, some surgeons recommend core decompression with structural bone grafting. The types of grafts used have been either transplanted autografts, allografts, and, more recently, vascularized autograft bone [120–124]. The purpose of a structural bone graft is to act as a mechanical support for the subchondral bone, preventing collapse while enhancing revascularization and healing of the necrotic bone segment. As with the results of core decompression alone, the outcomes of this approach have been quite variable, again probably secondary to the staging criteria used [108,120–125].

More recent results with vascularized fibular strut graft have been encouraging in short-term follow-up and suggest a distinct advantage over core decompression alone for Ficat stage II and III disease [126]; however, as with core decompression alone, if bone grafting is to be performed, a better prognosis is expected for those cases without radiographic evidence of collapse.

Electrical Stimulation and Pulsed Electromagnetic Fields

Adjunctive treatments such as electrical stimulation and capacitative coupling have also been used, with varying results. Electrical stimulation (pulsed electromagnetic fields) is a treatment alternative that is still under investigation and whose approval by the FDA is pending. Tranick and Lanceford [123] reported on the use of electrical stimulation alone in the treatment of osteonecrosis and found that 100% of patients with stage 2 disease had further deterioration of function. Steinberg *et al*. [125], however, noted that electrical stimulation combined with core decompression and bone grafting may improve outcomes of treatment. Aaron *et al*. [114] and Eftekar *et al*. [127], reporting on the use of pulsed electromagnetic fields, were encouraged by a superior success rate to that of core decompression alone. Studies of the use of external capacitative coupling in addition to core decompression and bone grafting report no improvement in treatment outcomes [128].

Proximal Femoral Osteotomy

Proximal femoral osteotomies (cutting and realignment of the proximal femur) have been used in order to replace an osteonecrotic bone segment in the weight-bearing portion of the femoral head with a normal segment of bone [129–131]. In contrast to the previously described procedures, osteotomy may be performed after collapse of the femoral head (stage 3 osteonecrosis)—but before degeneration of the acetabulum occurs (stage 4 osteonecrosis). Two types of osteotomy (angulation and rotational) have been proposed for the treatment of osteonecrosis. A rotational osteotomy, in which the femoral head is rotated 90°, was reported to yield good to excellent results in 80% of 200 hips [129]. However, this procedure is technically demanding, and few centers have been able to repeat these results. An angular osteotomy should is performed to direct a nonnecrotic segment of the femoral head into the weight-bearing portion of the joint; varus osteotomies are used for central or medial head involvement and valgus osteotomies are used for superolateral head defects. Ganz, and Buchler [130] reported that only 8 of 17 patients that had undergone an intertrochanteric osteotomy for osteonecrosis had satisfactory results at 3 years. Gottschalk [131] hypothesized that the poor results observed may be due to continued corticosteroid therapy or metabolic bone abnormalities and concluded that this procedure should be reserved for posttraumatic, idiopathic, or alcohol-induced osteonecrosis. In both applications (rotational or angulation osteotomies) the failure rate increases with the size of the femoral head defect. Beyond 50% involvement, the procedure is contraindicated by virtue of poor outcome (over 70% conversion to total hip arthroplasty at 10 years).

Treatment of Stage 4 Osteonecrosis: Hip Arthroplasty

Treatment of stage 4 osteonecrosis (significant subchondral collapse with secondary degenerative joint changes) is initially aimed at decreasing symptoms (anti-inflammatory drugs, mild analgesics, rest, and assistive devices for ambulation) until pain and disability warrant joint replacement surgery. Because of the high incidence of bilateral osteonecrosis in SLE patients, hip arthrodesis (surgical fusion of the joint) is not a surgical option. Since the femoral head is primarily involved and the acetabulum is relatively spared, some authors have suggested the use of bipolar hemiarthroplasty (replacement of the femoral head and neck with a prosthesis and preservation of the acetabulum). However, results of this approach have been disappointing, and most of these patient need be converted to a total hip arthroplasty as the "normal" acetabulum is typically not normal due to changes that have occurred during the joint deterioration and osteoporosis that occurs secondary to long-term steroid therapy. Surface replacement hemiarthroplasty of the hip (a more limited resurfacing prosthesis) has been proposed as another alternative to total hip arthroplasty; however, conversion rates to total hip arthroplasty approach 40% at 10 years of follow-up [132].

Hanssen *et al*. [133] reported the results of 43 hip reconstructions over a 12-year period in SLE patients. Patients who had undergone total hip arthroplasty had results superior to those who underwent bipolar replacement at a mean follow-up of 57 months. As a result, total hip replacement is the treatment of choice for SLE patients with stage 4 osteonecrosis who have failed nonoperative treatment. It should be noted that the clinical outcomes and survivorship of total hip arthroplasty performed for osteonecrosis (when all etiologies are considered) are worse than those done for other conditions such as osteoarthritis [86]. Although

total hip replacement using cemented femoral and acetabular components has yielded excellent pain relief and a success rate of over 90%, due to the younger age of SLE patients with osteonecrosis, prosthesis inserted without cement (that offer the prospect of biologic fixation) have been used in order to limit the long-term problems of aseptic loosening that have been observed with components inserted with cement.

Hanssen *et al*. [133] reported good to excellent results in 28 of 31 SLE patients who underwent total hip replacement at a mean follow-up of 5.5 years. Although SLE disease activity was quiescent at the time of surgery, complication rates were higher than those for patients undergoing hip replacement without SLE. Twenty-five percent of patients had local wound complications; however, no correlation between corticosteroid use perioperatively and wound complications was noted. Twenty-five percent of the patients in this study had died at final follow-up due to SLE complications unrelated to the total hip arthroplasty. Huo *et al*. [134] reported a survivorship rate of 94.6% at 5 years and 81.8% at 9 years for total hip arthroplasty in SLE patients. The clinical results were good or excellent in all patients at a mean follow-up of 47 months. Other studies have confirmed the effectiveness of total hip replacement for the treatment of stage 3 and 4 osteonecrosis in lupus patients [93,94]. The early results (4- to 8-year follow-up) of total hip arthroplasty performed with porous-coated components inserted without cement in SLE patients have yielded 62.5% good or excellent clinical results—which, however, is still inferior to those in patients so treated for other conditions [135]. Newer total joint component designs and articulating surfaces should improve the long-term outcomes of SLE patients with joint replacements.

Thromboembolic Prophylaxis for SLE Patients Undergoing Total Hip Arthroplasty

All patients who undergo hip replacement surgery are at risk for venous thrombosis, and as a result it is recommended that all such patients receive some form of prophylaxis. In the authors' institution, low-molecular-weight heparin is used for thrombosis prophylaxis. Patients with SLE who have significant serum levels of anti-cardiolipin antibodies (prevalence of 30–40%) and/or lupus anticoagulant are at increased risk for venous thrombosis [88,136–138]. It is still not known whether these patients are at a further increased risk for thrombosis following joint replacement surgery, nor whether our current prophylaxis regimens are adequate. Thrombosis prophylaxis with warfarin may be more appropriate in this subgroup of SLE patients. Patients with a history of embolic phenomena and antiphospholipid antibody have had uncomplicated joint replacement surgery by short-term withdrawal of coumadin and continued aspirin and steroid treatment [139]. SLE patients with lupus anticoagulants have also undergone surgical procedures without complications [140].

Osteonecrosis of Other Sites

Osteonecrosis has been observed in the femoral condyles, talus, humeral head, metatarsal heads, radial head, carpal bones, proximal and distal tibia, and metacarpal heads; it can occur in multiple sites in the same patient, and bilateral involvement is frequent [71,91,101,141–150]. SLE patients who present with osteonecrosis at one anatomic site should undergo a thorough investigation for lesions at other sites including the hip, ankle, and shoulder as many patients eventually develop involvement of multiple joints.

Osteonecrosis About the Knee

Osteonecrosis about the knee may affect one or both femoral condyles, the proximal tibia, or the patella and poses many of the same problems as femoral head involvement. While combined lesions of the distal femur and proximal tibia are common, isolated involvement of the proximal tibia is unusual [144,151–153]. As the knee is a major weight-bearing joint, subchondral collapse, joint incongruity, and progressive joint deterioration can occur. The natural history of osteonecrosis of the knee appears to be poor with the majority of symptomatic patients requiring operative treatment [153]. MRI of the knee in patients without X-ray changes is diagnostic in clinically symptomatic patients and has been found to be more useful than nuclear medicine scans [153]. As in the disease progression for hip osteonecrosis, early radiographic changes include a sclerotic appearance of the involved area, followed by a crescent sign, and later progression to flattening of the condyles, with eventual development of secondary degenerative changes.

Treatment of Osteonecrosis of the Knee

Early treatment of femoral condyle osteonecrosis consists of anti-inflammatory medications, quadriceps-strengthening exercises, and use of a cane or crutches for limited weight bearing in order to decrease symptoms and improve function. As with the hip, the size of the osteonecrotic lesion appears to determine prognosis with larger lesions having a worse prognosis. The combined necrotic angle, as described by Kerboul *et al*. [154], has been shown to be the most useful method for

categorizing the size of the lesion. With this method, a goniometer is used to measure the arc of the angle of the surface involved on both anteroposterior and lateral projections. Lesions with a combined necrotic angle of greater than 250° are categorized as large and have the worst prognosis [153].

Core decompression of the osteonecrotic area to stimulate revascularization has been reported to result in resolution of symptoms in some patients [153]; however, it is not known if it alters the ultimate natural history of the disease. In some cases, the cartilage overlying the osteonecrotic bone may become detached, acting as a loose body within the joint and obstructing knee motion, and should be removed by arthroscopic surgery; an intra-articular injection of lidocaine can be useful in determining whether a given patient's symptoms are related to intra-articular pathology or are related to pain from bone necrosis. If osteonecrosis is localized to only one femoral condyle, a realignment osteotomy designed to shift the weight-bearing forces to the uninvolved condyle may be helpful. If both femoral condyles have osteonecrosis and if secondary degenerative changes or collapse have occurred, then total knee replacement is indicated (Fig. 9). The results of total knee arthroplasty in patients with osteonecrosis of the knee and SLE have not been as good as for patients with osteoarthritis or other inflammatory arthropathies. SLE patients have a reported higher rate of early prosthetic loosening, poorer clinical outcomes, and increased rate of infection [153]. Knee arthrodesis is usually not indicated for SLE patients due to the frequency of polyarticular and bilateral osteonecrosis.

Osteonecrosis of the Talus

Treatment of osteonecrosis of the talus is similar to that of femoral condyle osteonecrosis. If the plain radiographs are normal in a symptomatic patient MRI is diagnostic. Nonoperative treatment includes the use of anti-inflammatory medications and limited weight bearing. Involvement is frequently bilateral and patients may have osteonecrotic lesions of the other bones of the foot and ankle [155]. Arthrotomy or arthroscopic surgery may be required if loose-bodies develop secondary to detachment of a cartilaginous fragment and symptoms develop. Core decompression has been reported by Delanois *et al.* to be useful in patients who do not have subchondral collapse [155]. If subchondral collapse and degenerative joint disease occurs that is resistant to nonsurgical treatment, then tibiotalar arthrodesis is indicated. Total ankle replacement is not an option for these patients, as it has fallen out of general use due to a high incidence of unsatisfactory results compared to the excellent results of ankle arthrodesis [156,157].

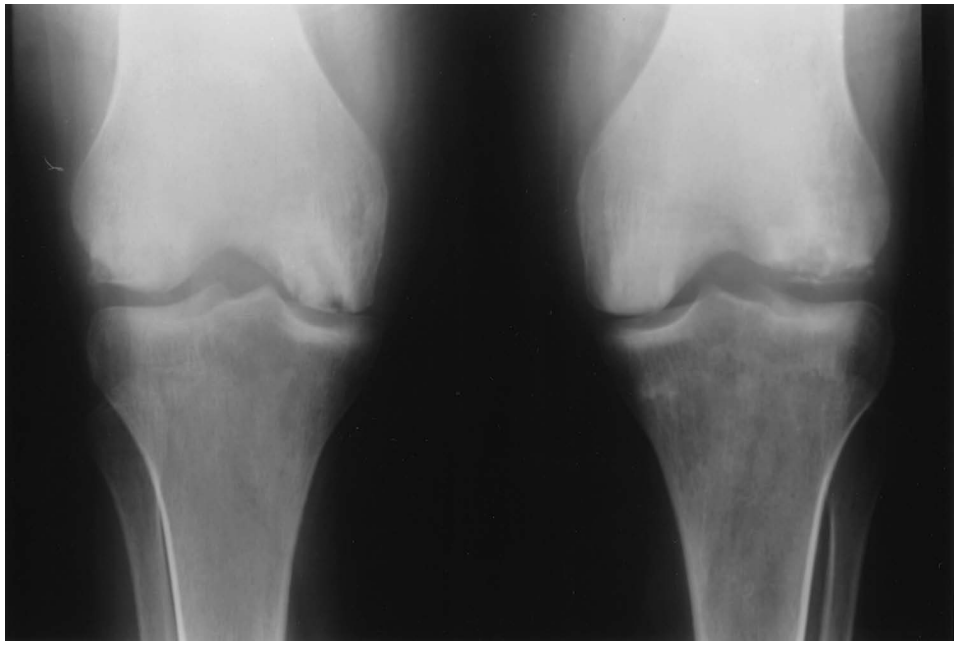
Osteonecrosis of the Humeral Head

Osteonecrosis of the humeral head is initially treated with anti-inflammatory medication and exercises designed to maintain shoulder range of motion (Fig. 10) [145]. As the glenohumeral joint is not subjected to the same kind of weight-bearing forces as the large joints of the lower extremity, osteonecrosis of the humeral head seems to have a better prognosis than osteonecrosis of the hip, knee, or ankle. The glenohumeral joint is also less constrained than the large joints of the lower extremity and scapulothoracic motion can in part compensate for lost glenohumeral motion; thus greater amounts of deformity are tolerated before function is severely compromised. Patients with less than 50% involvement of the humeral head have a better prognosis than those who have involvement of more than 75% of the humeral head [158].

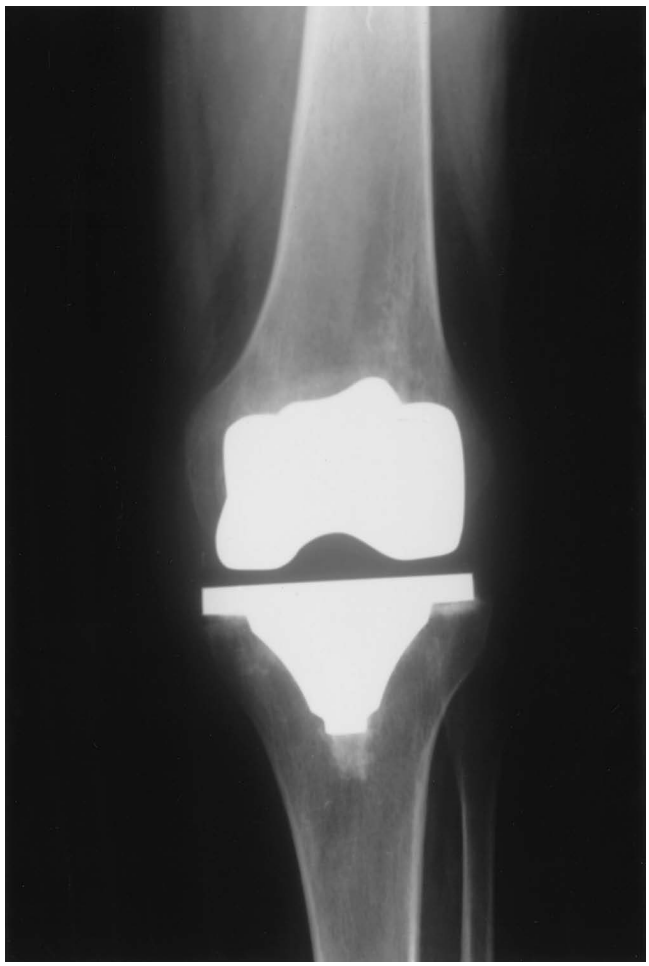
Osteonecrosis of the humeral head is relatively uncommon; however, in one series, 25 of 72 patients with humeral head osteonecrosis also had SLE [159]. If significant symptoms persist despite conservative measures and humeral head deformity or degenerative changes occur, humeral head hemiarthroplasty or total shoulder arthroplasty is indicated [158–161]. Core decompression of the humeral head has also been utilized in patients without signs of collapse of the articular surface; however, only moderate success has been demonstrated with this procedure [159, 162]. Patients with symptomatic osteonecrosis of the shoulder should be referred to an orthopedic surgeon early in the course of their disease as severe bone loss can occur which makes total shoulder arthroplasty technically more difficult.

Osteonecrosis of the Metatarsal Head, Radial Head, and Carpal Bones

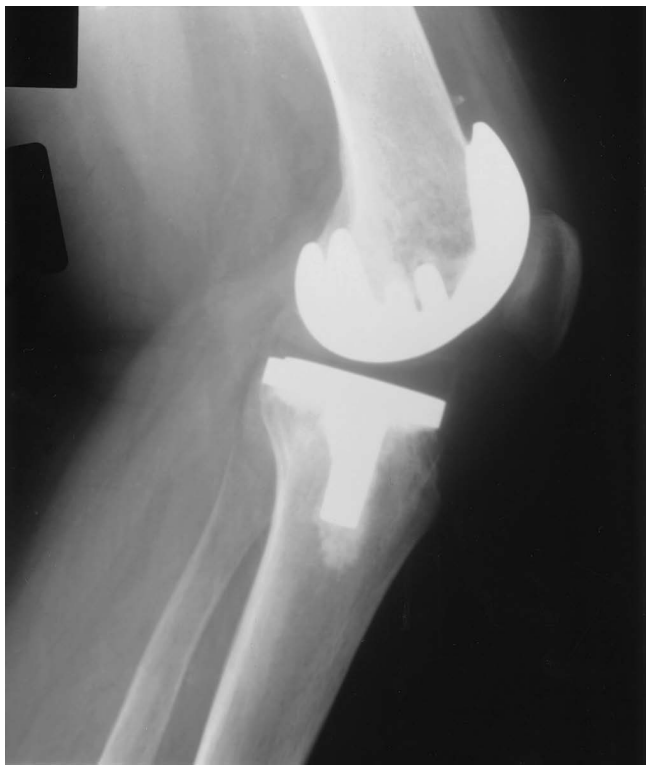
Osteonecrosis of the metatarsal head is usually treated with medication, limited weight bearing, and shoe modification; however, if these fail, then surgical excision of the metatarsal head may be indicated. Radial head osteonecrosis is also treated with medication and exercises to maintain range of motion, and if these fail then surgical excision of the radial head is the procedure of choice. The carpal bones usually involved with osteonecrosis are the scaphoid, lunate, and capitate; this can be treated with medication and splinting. If symptoms persist and early collapse is evident, surgical treatment by a limited wrist fusion is indicated.



A



B



C

FIGURE 9 (A) Anteroposterior standing knee radiographs of an SLE patient with bilateral knee pain, worse on the left. Osteonecrosis with subchondral collapse is noted in the lateral femoral condyle on the left knee, and in the medial femoral condyle of the right knee. Anteroposterior (B) and lateral (C) radiographic views of the same patient successfully managed with cemented total knee arthroplasty.

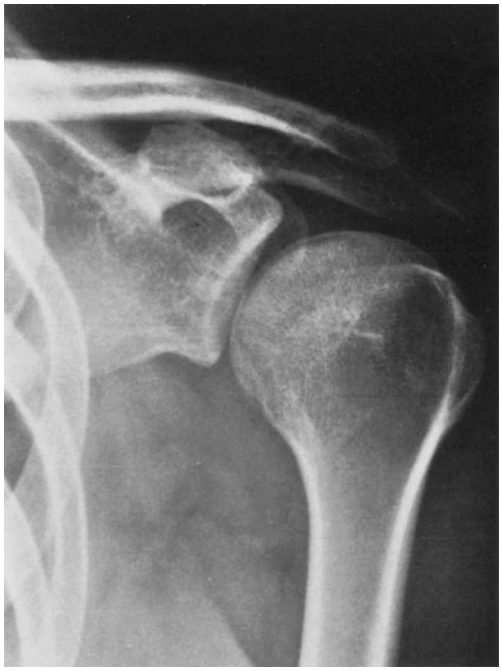


FIGURE 10 Anteroposterior shoulder radiograph demonstrated osteonecrosis of the humeral head. A crescent sign seen as a subchondral lucency is present.

References

1. Ropes, M. W. (1976). "*Systemic Lupus Erythematosus*." Harvard Univ. Press, Cambridge, Massachusetts.
2. Labowitz, R., and Schumacher, H. R. J. (1971). Articular manifestations of systemic lupus erythematosus. *Ann. Intern. Med*. **74**, 911–921.
3. Rothfield, N. (1985). Clinical features of systemic lupus erythematosus. In "Textbook of Rheumatola" W. N., Kelly, E. D., Harris, S., Ruddy, and C. B., Sledge, (Eds. p. 1070.), Saunders, Philadelphia.
4. Parke, A., and Rothfield, N. F. (1988). "Systemic lupus erythematosus, in Diagnosis and Management of Rheumatic Diseases." Lippincott, Philadelphia.
5. Cronin, M. E. (1988). Musculoskeletal manifestations of lupus erythematosus. *Rheum. Dis. Clin. North Am*. **14**, 99–116.
6. Silver, M., and Steinbrocker, O. (1961). The musculoskeletal manifestations of systemic lupus erythematosus. *JAMA* **176**, 1001.
7. Stevens, M. B. (1983). Musculoskeletal manifestations. In P, I. S. (ed.), "Clinical Management of Systemic Lupus Erythematosus." (I. S. P, Ed.), p. 63. Grune & Stratton, New York.
8. Fessel, W. J. (1974). Systemic lupus erythematosus in the community. *Arch. Intern. Med.* **134**, 1027–1035.
9. Edelen, J. S., Lockshin, M. D., and LeRoy, E. C. (1971). Gonococcal arthritis in two patients with active systemic lupus erythematosus. *Arthritis Rheum*. **14**, 557–559.
10. Gomez-Rodriguez, N., Ferreiro-Seoane, J. L., Formigo-Rodriguez, E., and Ibanez-Ruan, J. (1996). Septic arthritis caused by *Salmonella enteritidis* in systemic lupus erythematosus. *Acta Med. Interna*. **13**, 87–92.
11. Markov, G., Dobro, J., Shankman, S., and Belmont, H. M. (1996). Enterococcal arthritis with avascular necrosis in a lupus patient. *Br. J. Rheumatol*. **35**, 595–597.
12. Phillips, F. M., and Pottenger, L. A. (1988). Acute septic arthritis in chronic osteonecrosis of the hip. *J. Rheumatol*. **15**, 1713–1716.
13. Nalebuff, E. A. (1996). Surgery of systemic lupus erythematosus arthritis of the hand. *Hand Clin*. **12**, 591–602.
14. Paredes, J. G., Lazaro, M. A., Citera, G., Da Representaco, S., and Maldonado Cocco, J. A. (1997). Jaccoud's arthroplathy of the hands in overlap syndrome. *Clin. Rheumatol*. **16**, 65–69.
15. Zvaifler, N. J. (1962). Chronic postrheumatic fever (Jaccoud's arthritis). *N. Engl. J. Med*. **267**, 10.
16. Bywaters, E. G. L. (1975). Jaccoud's syndrome: A sequel to the joint involvement of systemic lupus erythematosus. *Clin. Rheum. Dis*. **1**, 125.
17. Spronk, P. E., terBorg, E. J., and Kallenberg, C. G. (1992). Patients with systemic lupus erythematosus and Jaccoud's arthropathy: A clinical subset with an increased C reactive protein response? *Ann. Rheum. Dis*. **51**, 358–361.
18. Russell, A. S., Percy, J. S., Rigal, W. M., and Wilson, G. L. (1974). Deforming arthropathy in systemic lupus erythematosus. *Ann. Rheum. Dis*. **33**, 204–209.
19. Bleifeld, C. J., and Inglis, A. E. (1974). The hand in systemic lupus erythematosus. *J. Bone Joint Surg*. **56A**, 1207.
20. Esdaile, J. M., Danoff, D., Rosenthall, L., and Gutkowski, A. (1981). Deforming arthritis in systemic lupus erythematosus. *Ann. Rheum. Dis*. **40**, 124.
21. Kramer, L. S., Ruderman, J. E., Dubois, E. L., and Frious, G. J. (1970). Deforming non-erosive arthritis of the hands in chronic systemic lupus erythematosus. *Arthritis Rheum*. **13**, 329.
22. Dray, G. J. (1989). The hand in systemic lupus erythematosus. *Hand Clin*. **5**, 145–155.
23. Dray, G. J., Millender, L. H., Nalebuff, E. A., and Philips, C. (1981). The surgical treatment of hand deformities in systemic lupus erythematosus. *J. Hand Surg*. **6**, 339–345.
24. Reilly, P. A., Evison, G., Mc Hugh, N. J., and Maddison, P. J. (1990). Arthropathy of the hands and feet in systemic lupus erythematosus. *J. Rheumatol*. **17**, 777–784.
25. Morley, K. D., Leung, A., and Rynes, R. I. (1982). Lupus foot. *Br. Med. J*. **284**, 557–558.
26. Mitzutani, W., and Quismorio, F. P., Jr. (1984). Lupus foot: Deforming arthropathy of the feet in systemic lupus erythematosus. *J. Rheumatol*. **11**, 80.
27. Wallace, D. J. (1997). "The musculoskeletal system. in Dubois' Lupus Erythematosus." Wilkins & Wilkins, Baltimore.
28. Lee, S. S. (1995). Symptomatic unilateral sacroiliitis in systemic lupus erythematosus. *Lupus* **4**, 328–329.
29. Kohli, M., and Bennett, R. M. (1994). Sacroiliitis in systemic lupus erythematosus. *J. Rheumatol*. **21**, 170–171.
30. Nassonova, V. A., Alekberova, Z. S., Folomeyev, M. Y., and Mylov, N. M. (1984). Sacroiliitis in male systemic lupus erythematosus. *Scand. J. Rheumatol*. **52**, 23–29.

31. Babini, S. M., Cocco, J. A., Babini, J. C., de la Sota, M., Arturi, A., and Marcos, J. C. (1990). Atlantoaxial subluxation in systemic lupus erythematosus: Further evidence of tendinous alterations. *J. Rheumatol*. **17**, 173–177.
32. Siam, A. R. M., and Hammoudeh, M. (1992). Jaccoud's arthropathy of the shoulders in systemic lupus erythematosus. *J. Rheumatol*. **19**, 980–981.
33. de la Sota, M., and J.A.M.-C. (1989). Jaccoud's arthropathy of the knees in systemic lupus erythematosus. *Clin. Rheumatol*. **8**, 416–417.
34. Liebling, M. R., and Gold, R. H. (1981). Erosions of the temporomandibular joint in systemic lupus erythematosus. *Arthritis Rheum*. **24**, 948–950.
35. Gerbracht, D., and Shapiro, L. (1982). Temporomandibular joint erosions in systemic lupus erythematosus. *Arthritis Rheum*. **25**, 597.
36. Shookster, L., and Solomon, G. Unpublished data.
37. Furie, R. A., and Chartash, E. K. (1988). Tendon rupture in systemic lupus erythematosus. *Semin. Arthritis Rheum*. **18**, 127–133.
38. Morgan, J., and McCarty, D. J. (1974). Tendon ruptures in patients with systemic lupus erythematosus treated with corticosteroids. *Arthritis Rheum*. **17**, 1033–1036.
39. Pritchard, C. H., and Berney, S. (1989). Patella tendon rupture in systemic lupus erythematosus. *J. Rheumatol*. **16**, 786–788.
40. Clement, B., Vasey, F. B., Germain, B. F., and Espinoza, L. R. (1983). Subacute infrapatellar tendon rupture in systemic lupus erythematosus. *J. Rheum*. **10**, 164–165.
41. Khan, M. A., and Ballou, S. P. (1981). Tendon rupture in systemic lupus erythematosus. *J. Rheumatol*. **8**, 308–310.
42. Vippula, A. (1972). Muscular disorders in some collagen vascular diseases. A clinical electromyographic and biopsy study. *Acta Med. Scand*. **540**, 1–47.
43. Dubois, E. L. (1966). "Lupus Erythematosus." McGraw-Hill, New York.
44. Larson, D. L. (1961). "Systemic Lupus Erythematosus." p. 47. Little Brown, Co., Boston.
45. Hahn, B. H., Yardley, J. H., and Stevens, M. B. (1970). "Rheumatoid" nodules in systemic lupus erythematosus. *Ann. Intern. Med*. **72**, 49–58.
46. Minami, A., Suda, K., Kaneda, K., and Kumakiri, M. (1994). Extensive subcutaneous calcification of the forearm in systemic lupus erythematosus. *J. Hand Surg*. **19B**, 638–641.
47. Weinberger, A., Kaplan, J. G., and Myers, A. R. (1979). Extensive soft tissue calcification in systemic lupus erythematosus. *Ann. Rheum. Dis*. **38**, 384–386.
48. Cousins, M. A., Jones, D. B., Whyte, M. P., and Monafo, W. W. (1997). Surgical management of calcinosis cutis universalis in systemic lupus erythematosus. *Arthritis Rheum*. **40**, 570–572.
49. Pekin, T. J., and Zvaifler, N. J. (1970). Synovial fluid findings in systemic lupus erythematosus. *Arthritis Rheum*. **13**, 777–785.
50. Notman, D. D., Kurata, N., and Tan, E. M. (1975). Profiles of antinuclear antibodies in systemic rheumatic diseases. *Ann. Intern. Med*. **83**, 464–469.
51. Rodnan, G. P., Yonis, E. J., and Totten, R. S. (1960). Experience with punch biopsy of synovium in the study of joint disease. *Ann. Intern. Med*. **53**, 319.
52. Schwartz, S., and Cooper, N. (1961). Synovial membrane punch biopsy. *Arch. Intern. Med*. **108**, 122.
53. Goldberg, D. I., and Cohen, A. S. (1978). Synovial membrane histopathology in the differential diagnosis of rheumatoid arthritis, gout, pseudogout, systemic lupus erythematosus, infectious arthritis and degenerative joint disease. *Medicine* **57**, 239.
54. Cruickshank, B. (1959). Lesions of joints and tendon sheaths in systemic lupus erythematosus. *Ann. Rheum. Dis*. **18**, 111.
55. Meinao, I. M., Sato, E. I., Andrade, L. E., Ferraz, M. B., and Atra, E. (1996). Controlled trial with chloroquine diphosphate in systemic lupus erythematosus. *Lupus* **5**, 237–241.
56. Williams, H. J., Egger, M. J., Singer, J. Z., Willkens, R. F., Kalunian, K. C., Clegg, D. O., Skosey, J. L., Brooks, R. H., Alarcon, G. S., Steen, V. D., *et al*. (1994). Comparison of hydroxychloroquine and placebo in the treatment of the arthropathy of mild systemic lupus erythematosus. *J. Rheumatol*. **21**, 1457–1462.
57. Dubois, E. L., and Tuffanelli, D. L. (1964). Clinical manifestations of systemic lupus erythematosus. *JAMA* **190**, 104.
58. Velayos, E. E., Leidholt, J. D., Smyth, C. J., and Priest, R. (1966). Arthropathy associated with steroid therapy. *Ann. Intern. Med*. **64**, 759.
59. Williams, P. L., and Corbett, M. (1983). Avascular necrosis of bone complicating corticosteroid replacement therapy. *Ann. Rheum. Dis*. **42**, 276–279.
60. Dubois, E. L., and Cozen, L. (1960). Avascular necrosis associated with systemic lupus erythematosus. *JAMA* **174**, 966.
61. Abeles, M., Urman, J. D., and Rothfield, N. F. (1978). Aseptic necrosis of bone in systemic lupus erythematosus. *Arch. Intern. Med*. **138**, 750–754.
62. Glimcher, M. J., and Kenzora, J. E. (1978). The biology of osteonecrosis of the human femoral head and its clinical implications: An abridged communication. *Clin. Orthop*. **130**, 47–50.
63. D'Aubiane, R. M., Postel, C. M., Mazabrand, A., *et al*. (1965). Idiopathic necrosis of the femoral head in adults. *J. Bone Joint Surg*. **47B**, 612.
64. Nagawasa, K., Tsukamoto, H., Tada, Y., Mayumi, T., Satoh, Onitsuka, H., Kuwabara, Y., and Niho, Y. (1994). Imaging study on the mode of development and changes in avascular necrosis of the femoral head in systemic lupus erythematosus: Long-term observations. *Br. J. Rheumatol*. **33**, 343–347.
65. Migliaresi, S., Picillo, U., Abrosone, L., Di Palma, G., Mallozzi, M., Tesone, E. R, and Tirri, G. (1994). Avascular osteonecrosis in patients with SLE: Relation to corticosteroid therapy and anticardiolipin antibodies. *Lupus* **3**, 37–41.
66. Weiner, E. S., and Abeles, M. (1989). Aseptic necrosis and glucocorticosteroid in systemic lupus erythematosus: A reevaluation. *J. Rheumat*. **16**, 604–608.

67. Massardo, L., Jacobelli, S., Leissner, M., Gonzalez, M., Villarroel, L., and Rovero, S. (1992). High-dose intravenous methylprednisolone therapy associated with osteonecrosis in patients with systemic lupus erythematosus. *Lupus* **1**, 401–405.
68. Dubois, E. L., and Cozen, L. (1960). Avascular (aseptic) bone necrosis associated with systemic lupus erythematosus. *JAMA* **174**, 108.
69. Siemsen, J. K., Brook, J., and Mister, L. (1962). Lupus erythematosus and avascular bone necrosis: A clinical study of three cases and review of the literature. *Arthritis Rheum*. **5**, 493.
70. Leventhal, G. H., and Dorfman, H. D. (1974). Aseptic necrosis of bone in systemic lupus erythematosus. *Semin. Arthritis Rheum*. **4**, 73–93.
71. Klipper, A. R., Stevens, M. B., Zizic, T. M., and Hungerford, D. S. (1976). Ischemic necrosis of bone in systemic lupus erythematosus. *Medicine* **55**, 251–257.
72. Dimant, J., Ginzler, E. M., Diamond, H. S., Schlesinger, M., Marino, C. T., Weiner, M., and Kaplan, D. (1978). Computer analysis of factors influencing the appearance of aseptic necrosis in patients with systemic lupus erythematosus. *J. Rheumatol*. **5**, 136–141.
73. Klippel, J. H., Gerber, L. H., Pollak, L., and Decker, J. L. (1979). Avascular necrosis in systemic lupus erythematosus, silent symmetric osteonecrosis. *Am. J. Med*. **67**, 83–87.
74. Zizic, T. M., Marcoux, C., Hungerford, D. S., Dansereau, J. V., and Stevens, M. B. (1985). Corticosteroid therapy associated with ischemic necrosis of bone in systemic lupus erythematosus. *Am. J. Med*. **79**, 596–604.
75. Frayha, R. A., Scarola, J. A., and Shulman, L. E. (1973). Calcinosis in scleroderma, a re-evaluation of the CREST syndrome, abstracted. *Arthritis Rheum*. **16**, 542.
76. Given, W. P., Edelson, H. S., Kaplan, H. B., Aisen, P., Weissmann, G., and Abramson, S. B. (1984). Generation of C5-derived peptides and other immune reactants in the sera of patients with systemic lupus erythematosus. *Arthritis Rheum*. **27**, 631–637.
77. Buyon, J., Shaddick, N., Berkman, R., Hopkins, P. L., Dalton, J., Weissman, G., Winchester, R., and Abramson, S. B. (1988). Surface expression of Gp 165/95, the complement receptor of CR3, as a marker of disease activity in systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **46**, 141–149.
78. McFarland, P. H., and Frost, H. M. (1961). A possible new cause for aseptic necrosis of the femoral head. *Henry Ford Hosp. Med. Bull*. **9**, 115.
79. Sweetman, D. R., Mason, R. M., and Murray, R. G. (1960). Steroid arthropathy of the hip. *Br. Med. J*. **1**.
80. Jones, J. P., Engleman, E. P., and Steinbach, H. L. (1965). Fat embolization as a possible mechanism producing avascular necrosis. *Arthritis Rheum*. **8**, 449.
81. Fisher, D. E., Bickel, W. H., and Holley, K. E. (1969). Histologic demonstration of fat emboli in aseptic necrosis associated with hypercortisonism. *Mayo Clin. Proc*. **44**, 252–259.
82. Fisher, D. E., and Bickel, W. H. (1971). Corticosteroid induced avascular necrosis. *J. Bone Joint Surg*. **53A**, 859–873.
83. Jones, J. P., and Sakovich, L. (1966). Fat embolism of bone, a roentgenographic and histological investigation, with use of intra-arterial lipidol in rabbits. *J. Bone Joint Surg*. **(A)48**, 149–164.
84. Gruess, R. L. (1981). Steroid-induced osteonecrosis: A review. *Can. J. Surg*. **24**, 567.
85. Solomon, L. (1979). Avascular necrosis of the femoral head preclinical changes and their bearing on pathogenesis and treatment, abstracted. *J. Bone Joint Surg*. **61B**, 126.
86. Zizic, T. M. (1991). Osteonecrosis. *Curr. Opin. Rheumatol*. 481–489.
87. Bluemke, D. A., Petri, M., and Zerhouni, E. A. (1995). Femoral head perfusion and composition: MR imaging and spectroscopic evaluation of patients with systemic lupus erythematosus and at risk for avascular necrosis. *Radiology* **197**, 433–438.
88. Asherson, R. A., Liote, F., Page, B., Meyer, O., Buchanan, N., Khamshta, M. A., Jungers, P., and Hughes, G. R. (1993). Avascular necrosis of bone and antiphospholipid antibodies in systemic lupus erythematosus. *J. Rheumatol*. **20**, 284–288.
89. Cosgriff, S. W., Diefenbach, A. F., and Vogt, W., Jr. (1950). Hypercoagulability of the blood associated with ACTH and cortisone therapy. *Am. J. Med*. **9**, 752.
90. Goldie, I., Tibblin, G., and Scheller, S. (1967). Systemic lupus erythematosus and aseptic bone necrosis. *Acta Med. Scand*. **182**, 55–63.
91. Meyers, M. H. (1988). Osteonecrosis of the femoral head. *Clin. Orthop*. **231**, 51–61.
92. Hauzeur, J. P., Pasteels, J. L., Schoutens, A., Hinsenkamp, M., Appelboom, T., Chochrad, I., and Perlmutter, N. (1989). The diagnostic value of magnetic resonance imaging in nontraumatic osteonecrosis of the femoral head. *J. Bone Joint Surg*. **71A**, 641–649.
93. Robinson, H. J., Hartleben, P. D., Lund, G., and Schreimen, J. (1989). Evaluation of magnetic resonance imaging in the diagnosis of osteonecrosis of the femoral head. *J. Bone Joint Surg*. **71A**, 650–663.
94. Beltran, J., Knight, C. T., Zuelzer, W. A., Morgan, J. P., Shwendeman, L. J., Chandnani, V. P., Mosure, J. C., and Shaffer, P. B. (1990). Core decompression of the femoral head: Correlation between long-term results and preoperative MR staging. *Radiology* **175**, 533–536.
95. Halland, A. M., Klemp, P., Botes, D., Van Heerden, B. B., Loxton, A., and Scher, A. T. (1993). Avascular necrosis of the hip in systemic lupus erythematosus: The role of magnetic resonance imaging. *Br. J. Rheumatol*. **32**, 972–976.
96. Sugano, N., Ohzono, K., Masuhara, K., Takaoka, K., and Ono, K. (1994). Prognostication of osteonecrosis of the femoral head in patients with systemic lupus erythematosus by magnetic resonance imaging. *Clin. Orthop*. 190–199.
97. Pollack, M. S., Dalinka, M. K., Kressel, H. Y., Lotke, P. A., and Spritzer, C. E. (1987). Magnetic resonance imaging in the evaluation of suspaected osteonecrosis of the knee. *Skeletal Radiol*. **16**, 121–127.

98. Steinberg, M. E. (1988). Early diagnosis of avascular necrosis of the femoral head. *Instruct. Course Lect*. **37**, 51.
99. Ficat, R. P., and Arlet, J. (1980). "Ischemia and Necroses of Bone." Williams & Wilkins, Baltimore.
100. Stulberg, B. N., Bauer, T. W., Belhobek, G. H., Levine, M., and Davis, A. (1989). A diagnostic algorithm for osteonecrosis of the femoral head. *Clin. Orthop*. **249**, 176–182.
101. Stolow, J., Parikh, S., Shybut, G., Robinson, P., and Pope, R. M. (1991). An atypical site of osteonecrosis in a patient with systemic lupus erythematosus. *J. Rheumatol*. **18**, 1623–1626.
102. Ficat, R. P. (1983). Treatment of avascular necrosis of the femoral head. In "The Hip: Proceedings of the Eleventh Open Scientific Meeting of the Hip Society" (D.S. Hungerford, Ed.), p. 279. Mosby, St. Louis, Missouri.
103. Kulkarni, M. V., Tarr, R. R., Kim, E. E., McArdle, C. B., and Partain, C. L. (1987). Potential pitfalls of magnetic resonance imaging in the diagnosis of avascular necrosis. *J. Nucl. Med*. **28**, 1052.
104. Markisz, J. A., Knowles, R. J., Altchek, D. W., Schneider, R, Whalen, J. P., and Cahill, P. T. (1987). Segmental patterns of avascular necrosis of the femoral heads: Early detection with MRI imaging. *Radiology* **162**, 717–720.
105. Dalnka, M. K., Alavi, A., and Forste, D. H. (1977). Aseptic (ischemic) necrosis of the femoral head. *JAMA* **238**, 1059.
106. Kenzora, J. E., Steele, R. E., Yosepavitch, Z. H., and Glimcher, M. J. (1978). Experimental osteonecrosis of the femoral head in adult rabbits. *Clin. Orthop*. **130**, 8–46.
107. Meyers, M. H. (1983). Avascular necrosis of the femoral head diagnostic techniques, reliability and relevance. In "The Hip: (D.S. Hungerford, Ed.), pp. 263–278. Proceedings of the Eleventh Open Scientific Meeting of the Hip Society." Mosby, St. Louis, Missouri.
108. Marcus, N. D., Enneking, W. F., and Massam, R. A. (1975). The silent hip in idiopathic aseptic necrosis. Treatment by bone grafting. *J. Bone Joint Surg*. **55A**, 1351–1366.
109. Springfield, D. S., and Enneking, W. J. (1978). Surgery for aseptic necrosis of the femoral head. *Clin. Orthop*. **130**, 175–185.
110. Enneking, W. F. (1979). The choice of surgical procedures in idiopathic aseptic necrosis. In "The Hip: Proceedings of the Seventh Open Scientific Meeting of the Hip Society" (D.S. Hungerford, Ed.), p. 238. Mosby, St. Louis, Missouri.
111. Hungerford, D. S., and Zizic, T. M. (1983). Pathogenesis of ischemic necrosis of the femoral head. In "The Hip: Proceedings of the Eleventh Open Scientific Meeting of the Hip Society" (D.S. Hungerford, Ed.), pp. 249–262. Mosby, St. Louis, Missouri.
112. Hungerford, D. S., and Zizic, T. M. (1978). Alcoholism associated ischemic necrosis of the femoral head: Early diagnosis and treatment. *Clin. Orthop*. **130**, 144–153.
113. Steinberg, M. E., Brighton, C. T., Steinberg, D. R., Tooze, S. E., and Hayken, G. D. (1984). Treatment of avascular necrosis of the femoral head by a combination of bone grafting, decompression and electrical stimulation. *Clin. Orthop*. **186**, 137–153.
114. Aaron, R. K., Lennox, D., Bunce, G. E., and Ebert, T. (1989). The conservative treatment of osteonecrosis of the femoral head. *Clin. Orthop*. **249**, 209–218.
115. Tooke, S. M., Nugent, P. J., Bassett, C. W., Nottingham, P., Mirra, J., and Jinnah, R. (1988). Results of core decompression for femoral head osteonecrosis. *Clin. Orthop*. **228**, 99.
116. Camp, J. F., and Colwell, C. W. (1986). Core decompression of the femoral head in osteonecrosis. *J. Bone Joint Surg*. **68A**, 1313–1319.
117. Mont, M. A., Fairbank, A. C., Ptri, M., and Hungerford, D. S. (1997). Core decompression for osteonecrosis of the femoral head in systemic lupus erythematosus. *Clin. Orthop.* **334**, 91–97.
118. Usui, M., Inoue, H., Yukihiro, S., and Abe, N. (1996). Femoral neck fracture following avascular necrosis of the femoral head. *Acta Med. Okayama*. **50**, 111–117.
119. Ganczarczyk, M. L., Lee, P., and Fornasier, V. L. (1968). Early diagnosis of osteonecrosis in systemic lupus erythematosus with magnetic resonance imaging. Failure of core decompression. **13**, 814–817.
120. Boettcher, W. G., Bonfiglio, M,. and Smith, K. (1970). Nontraumatic necrosis of the femoral head. Part II: Experiences in treatment. *J. Bone Joint Surg*. **52A**, 322.
121. Baksi, P. P. (1991). Treatment of osteonecrosis of the femoral head by drilling and muscle-pedicle bone grafting. *J. Bone Joint Surg*. **73B**, 241.
122. Hari, Y. (1981). Revitalization of the osteonecrotic femoral head by vascular bundle transplantation. In "Segmental Idiopathic Necrosis of the Femoral Head," p. 47. Springer-Verlag, New York.
123. Tranick, T., and Lanceford, E. (1990). The effect of electrical stimulation on osteonecrosis of the femoral head. *Clin. Orthop*. **256**, 120.
124. Malizos, K. N., Soucacos, P. N., Beris, A. E., Korobilias, A. B., and Xenakis, T. A. (1994). Osteonecrosis of the femoral head in immunosuppressed patients: Hip salvaging with implantation of a vascularized fibular graft. *Microsurgery* **15**, 485–491.
125. Steinberg, M. E., Brighton, C. T., Corces, A., Hayken, G. D., Steinberg, D. R., Strafford, B., Tooze, S. E., and Fallon, M. (1989). Osteonecrosis of the femoral head: Results of core decompression and grafting with and without electrical stimulation. *Clin. Orthop*. **249**, 199–208.
126. Scully, S. P., Aaron, R. K., and Urbaniak, J. R. (1998). Survival analysis of hips treated with core decompression or vascularized fibular grafting because of avascular necrosis. *J. Bone Joint Surg*. **80A**, 1270–1275.
127. Eftekar, N. S., Schink, M. M., Ascani, M. M., and Mitchell, S. N. (1983). Osteonecrosis of the femoral head treated by pulsed electromagnetic fields (PEMFs): A preliminary report. In "The Hip: Proceedings of the Eleventh Open Scientific Meeting of the Hip Society" (D.H. Hungerford, Ed.), p. 306. Mosby, St. Louis, Missouri.
128. Steinberg, M. E., Brighton, C. T., Bandy, R. E., and Hartman, K. M. (1990). Capacitive coupling as an adjunctive treatment for avascular necrosis. *Clin. Orthop*. **261**, 11.

129. Sugioka, Y. (1978). Transtrochanteric anterior rotational osteotomy of the femoral head in the treatment of osteonecrosis affecting the hip, a new osteotomy operation. *Clin. Orthop*. **130**, 191–201.
130. Ganz, R., and Buchler, V. (1983). Overview of attempts to revitalize the dead head in aseptic necrosis of the femoral head: Osteotomy and revascularization. In "The Hip: Proceedings of the Eleventh Open Scientific Meeting of the Hip Society" (D.S. Hungerford, Ed.), pp. 296–305. Mosby, St. Louis, Missouri.
131. Gottschalk, F. (1989). Indications and results of intertrochanteric osteotomy in osteonecrosis of the femoral head. *Clin. Orthop*. **249**, 219–222.
132. Hungerford, M. W., Mont, M. A., Scott, R., Fiore, C., Hungerford, D. S., and Krackow, K. A. (1998). Surface replacement hemiarthroplasty for the treatment of osteonecrosis of the femoral head. *J. Bone Joint Surg*. **80A**, 1656–1664.
133. Hanssen, A. D., Cabanela, M. E., and Michet, C. J. (1987). Hip arthroplasty in patients with systemic lupus erythematosus. *J. Bone Joint Surg*. **69**, 807–814.
134. Huo, M. H., Salvati, E. A., Browne, M. G., Pellicci, P. M., Sculco, T. P., and Johansen, N. A. (1992). Primary total hip arthroplasty in systemic lupus erythematosus. *J. Arthroplasty* 7, 51–56.
135. Brinker, M. R., Rosenberg, A. G., Kull, L., and Galante, J. O. (1994). Primary total hip arthroplasty using noncemented porous-coated femoral components in patients with osteonecrosis of the femoral head. *J. Arthroplasty* **9**, 457–468.
136. Nojima, J., Suehisa, E., Akita, N., Toku, M., Fushimi, R., Tada, H., Kuratsune, H., Machii, T., Kitani, T,. and Amino, N. (1997). Risk of arterial thrombosis in patients with anticardiolipin antibodies and lupus anticoagulant. *Br. J. Haemotol*. **96**, 447–450.
137. Fijnheer, R., Horbach, D. A., Donders, R. C., Vile, H., vonOort, E., Nieuwenhuis, H. K., Gmelig-Meijling, F. H., deGroot, P. G., and Derksen, R. H. (1996). Factor V Leiden, antiphospholipid antibodies and thrombosis in systemic lupus erythematosus. *Thromb. Haemos*. **76**, 514–517.
138. Alarcon-Segovia, D., Deleze, M., Oria, C. V., Sanchez-Guerro, J., Gomez-Pacheco, L., Cabiedes, J., Fernandez, L., and Ponce de Leon, S. (1989). Antiphospholipid antibodies and the antiphospholiopid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. *Medicine* **68**, 353–365.
139. Schnitz, W. M., Lister, K. A., and McCarty, G. A. (1992). Management of antiphospholipid antibody positivity and elective orthopedic procedures. *Lupus* **1**, 187–189.
140. Minezaki, T., and Ichikawa, Y. (1993). Surgical experience on patients with serum lupus anticoagulants. A report of two cases. *Tokai Journal of Experimental & Clinical Medicine* **18**, 95–97.
141. Griffiths, I. D., Maini, R. N., and Scott, J. T. (1979). Clinical and radiological features of osteonecrosis in systemic lupus erythematosus. *Ann. Rheum. Dis*. **38**, 413–422.
142. Smith, F. E., Sweet, D. E., Brunner, C. M., and Davis, J. S. (1976). Avascular necrosis in systemic lupus erythematosus. An apparent predilection for young patients. *Ann. Rheum. Dis*. **35**, 227–232.
143. Urman, J. D., Abeles, M., Houghton, A. N., and Rothfield, N. F. (1979). Aseptic necrosis presenting as wrist pain in systemic lupus erythematosus. *Arthritis Rheum*. **20**, 825–828.
144. Bauer, G. C. (1978). Osteonecrosis of the knee. *Clin. Orthop*. **130**, 210–217.
145. Cruess, R. L. (1978). Experience with steroid induced avascular necrosis of the shoulder and etiologic considerations regarding osteonecrosis of the hip. *Clin. Orthop*. **130**, 86–93.
146. Lightfoot, R. W. J., and Latke, P. A. (1972). Osteonecrosis of the metacarpal heads in systemic lupus erythematosus: Value radiostrontium scintimetry in differential diagnosis. *Arthritis Rheum*. **15**, 486–492.
147. Hirohata, S., and Ito, K. (1992). Aseptic necrosis of unilateral scaphoid bone in systemic lupus erythematosus. *Intern. Med*. **31**, 794–797.
148. Wright, T. C., and Dell, P. C. (1991). Avascular necrosis and vascular anatomy of the matacarpals. *J. Hand Surg*. **16**, 540–544.
149. Fishel, B., Caspi, D., Eventov, I., Avrahami, E., and Yaron, M. (1987). Multiple sclerosis lesions in systemic lupus erythematosus. *J. Rheumatol*. **14**, 601–604.
150. Outwater, E., Oates, E., and, R. C. S. (1989). Bilateral distal tibial osteonecrosis in systemic lupus erythematosus. *Am. J. Roentgenol*. **152**, 895–896.
151. Chancelier, M. D., Helenon, O., Page, B., Rousselin, B., Legendre, C., and Moreau, J. F. (1992). Aseptic osteonecrosis of the knee induced by corticoids. MRI aspects. *J. Radiol*. **73**, 191–201.
152. Rozing, P. M., Insall, J., and Bohme, W. H. (1980). Spontaneous osteonecrosis of the knee. *J. Bone Joint Surg*. **62A**, 2–7.
153. Mont, M. A., Baumgarten, K. M., Rifai, A., Bluemke, D. A., Jones, L. C., and Hungerford, D. S. (2000). Atraumatic osteonecrosis of the knee. *J. Bone Joint Surg*. **82A**, 1279–1290.
154. Kerboul, M., Thomine, J., Postel, M., and Merle d'Aubigne, R. (1974). The conservative surgical treatment of idiopathic aseptic necrosis of the femoral head. *J. Bone Joint Surg*. **56B**, 291–296.
155. Delanois, R. E., Mont, M. A., Yoon, T. R., Mizell, M., and Hungerford, D. S. (1998). Atraumatic osteonecrosis of the talus. *J. Bone Joint Surg*. **80A**, 529–536.
156. Demottaz, J. D., Mazur, J. M., Thomas, W. H., Sledge, C. B., and Simon, S. R. (1979). Clinic study of total ankle replacement with gait analysis. *J. Bone Joint Surg*. **61A**, 976.
157. Newton, S. E. (1982). Total ankle arthroplasty: Clinical study of 50 cases. *J. Bone Joint Surg*. **64A**, 104–111.
158. Hattrup, S. J., and Cofield, R. H. (1999). Osteonecrosis of the humeral head: Relationship of disease stage, extent, and cause to natural history. *J. Shoulder Elbow Surg*. **8**, 559–564.

159. Mont, M. A., Payman, R. K., Laporte, D. M., Petri, M., Jones, L. C., and Hungerford, D. S. (2000). Atraumatic osteonecrosis of the humeral head. *J. Rheumatol*. **27**, 1766–1773.
160. Neer, C. S., Watson, K. C., and Stanton, F. J. (1982). Recent experience in total shoulder replacement. *J. Bone Joint Surg*. **64A**, 319–337.
161. Cofield, R. C. (1984). Total shoulder arthroplasty with Neer prosthesis. *J. Bone Joint Surg*. **66A**, 899.
162. LaPorte, D. M., Mont, M. A., Mohan, V., Pierre-Jacques, H., Jones, L. C., and Hungergord, D. S. (1998). Osteonecrosis of the humeral head treated by core decompression. *Clin. Orthop*. **355**, 254–260.

38

OSTEOPOROSIS AND METABOLIC BONE DISEASE IN LUPUS

Oscar Gluck
Michael J. Maricic

OSTEOPOROSIS

Introduction

Osteoporosis is conceptually defined as "a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. Bone strength reflects the integration of two main features: bone density and bone quality" [1]. Clinically, osteoporosis can be diagnosed without the use of bone density measurements in patients who have had vertebral compression fractures or nonvertebral fragility fractures. The World Health Organization (WHO) has developed criteria for the diagnosis of osteoporosis in postmenopausal Caucasian women based on measurements of bone mineral density (BMD) [2]. The number of standard deviations (SD) below the mean peak adult value at the skeletal site measured is expressed as a "T-score." A T-score of equal or greater than 2.50 SD below peak adult bone mass allows for the diagnosis of osteoporosis without the presence of fragility fractures [2] (Table 1).

The WHO criteria make it possible to assess the prevalence of osteoporosis in patients with lupus and to examine associated risk factors. Several cross-sectional and prospective studies in patients with systemic lupus have suggested a reduction of bone mass and an increased risk for fragility fractures [3, 4]. Therefore, it is advisable to measure bone mineral density in patients with lupus at risk for bone loss.

Factors that play a role in the association between lupus and bone loss include the inflammatory disease itself, disease-related comorbidities and therapy, specifically exposure to glucocorticoids [5]. Recognition of the role of cytokines and other inflammatory mediators in the regulation of bone remodeling has led to a better understanding of the etiology of bone loss in inflammatory diseases, including systemic lupus [6].

EPIDEMIOLOGY OF BONE LOSS AND FRACTURES IN SYSTEMIC LUPUS

The prevalence of patients with lupus having low bone mass varies from 15–46% (osteopenia), to 6–15% (osteoporosis) in different studies [5, 7]. Decreases in both cortical and trabecular bone compared to age matched controls have been reported [8], and low bone mineral density is evidenced in both early and late disease [9, 10]. Thus, the duration of systemic lupus does not seem to be a major factor. Low bone mineral density is seen across multiple skeletal sites, and both the prevalence and incidence of low bone mass reported varies according to the site of measurement (lumbosacral spine, total hip, femoral neck, trochanteric region), gender, and age of the patient studied.

1. Postmenopausal women: Along with other factors, improved survival has increased the population at risk for the development of reduced BMD, osteopenia, and osteoporosis in postmenopausal women with lupus [7].
2. Premenopausal women: In many studies, a higher incidence of osteopenia and osteoporosis by WHO

TABLE 1 World Health Organization Criteria

WHO diagnostic categories	
Diagnosis	**T-score**
Normal	Greater than 1.0 SD below the average value of peak average bone mineral density (BMD)
Osteopenia	More than 1.0 SD below the average value of peak average BMD but not more than 2.5 below
Osteoporosis	More than 2.5 SD below the average value of peak average BMD
Severe osteoporosis	More than 2.5 SD below the average value of peak average BMD and the presence of a fragility fracture

criteria has been noted in premenopausal women with lupus compared to controls [11]. Significant loss of lumbosacral spine bone mass reflecting predominately trabecular bone has been detected in these younger women [5, 7].

3. Males: In men with lupus, the prevalence of low bone density is controversial. A few small studies showed no relationship between low BMD and men with systemic lupus [12]. As in women, glucocorticoid in men seemed to be a major risk factor for low bone mass.

4. Juveniles and children: Patients with juvenile systemic lupus showed a significant loss of bone mass of the lumbosacral spine, specifically while treated with glucocorticoids [13].

In most but not all studies, a higher frequency of osteoporosis at all sites is noted compared to age-matched controls [5]. In one study, equally low bone mineral density values were noted in patients with rheumatoid arthritis or systemic lupus [14].

An association between low bone mineral density and cardiovascular disease (atherosclerosis) in patients with lupus has more recently been described [15], emphasizing the role of inflammatory mediators such as cytokines in both entities.

FRACTURES

There are very few studies that provide data on the incidence of fragility fractures in patients with systemic lupus. In the Baltimore Cohort Study [16], 32 out of 364 SLE patients had fractures, and of those, 24 were defined as atraumatic or fragility fractures. The risk factors for fragility fractures in this study included advanced age, cumulative and highest daily glucocorticoid dose, the presence of previous osteonecrosis of bone, postmenopausal status, and prior identification of osteopenia on X-ray.

Although many anecdotal reports have described multiple vertebral compression fractures in patients with lupus, prospective studies are not yet available. In an extensive retrospective study of 702 women with lupus [17] with a total of 5951 person years, the risk of fracture was noted to be increased (odds ratio = 4.7) in the lupus cohort compared to control women of similar age. Older age at lupus diagnosis and longer duration of glucocorticoid use seemed to be independent determinates of fractures in patients with lupus. In all published recent pharmaceutical trials of patients testing bisphosphonates for glucocorticoid-induced osteoporosis (in which some of these patients were those with systemic lupus), it was noted that most fractures occurred in the postmenopausal female population [18, 19].

PATHOPHYSIOLOGY OF LOW BONE MASS AND OSTEOPOROSIS IN PATIENTS WITH SYSTEMIC LUPUS

Several cytokines, including interleukin 1 (IL1), interleukin 6 (IL6), and tumor necrosis factor-α (TNF-α), play a role in the recruitment, differentiation, activation, and function of osteoclasts *in vivo* [6]. In some experimental models of systemic lupus, levels of IL1, IL6, and TNF-α are increased compared to normal controls [20]. In patients with systemic lupus erythematosus, the spontaneous production of bone resorptive lymphokines (IL1, IL6, and TNF) by B cells has been noted [21].

These cytokines stimulate the release of RANK (receptor for activator of nuclear factor-κB) ligand (RANKL) by vascular endothelium, activated T cells and mesenchymal cells, principally osteoblasts. Synovial fibroblasts may also produce RANKL [22, 23] and thus promote osteoclast activation and differentiation without the participation of other bone cells [24].

RANKL binds to its receptor RANK on preosteoclasts and osteoclasts, thereby inducing osteoclast differentiation and increased activity of mature osteoclasts. A natural soluble inhibitor of RANKL called osteoprotegrin (OPG) (Fig. 1) is a product of osteoblastic/stromal cells [25] and is under cytokine control. The balance between RANKL and OPG production determines the level of osteoclast differentiation and activity. More recently, increased levels of osteoprotegrin have been noted in patients with lupus

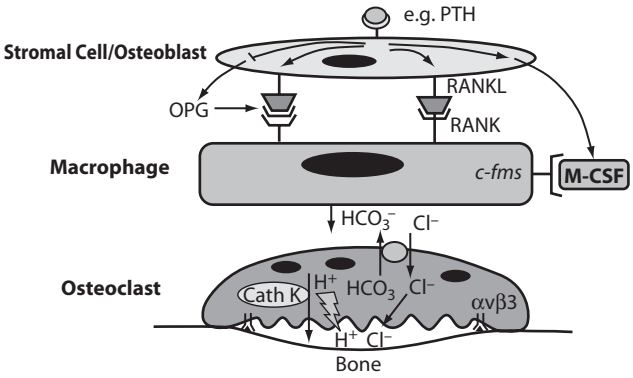


FIGURE 1 Osteoclastogenesis and bone resorption [24].

[26], which could have a protective effect on bone or merely reflect a high turnover state.

Abnormal response of T-helper cells is involved in the pathogenesis of systemic lupus. The release of several TH1 and TH2 derived cytokines may have direct effects on bone resorption. Activation and clonal expansion of CD4 positive autoreactive T cells also release cytokines such as IL6, which play a prominent role in bone reabsorption [6].

The relevance of the observations regarding cytokines in animal models of lupus to human disease is difficult to infer, but the process of bone loss is certainly orchestrated by the vast cocktail of cytokines, secreted both by T and B cells, fibroblasts, osteoblastic cells, and mononuclear cells. The systemic effects of these mediators could explain the relationship between disease activity and osteopenic bone disease in systemic lupus [27].

RISK FACTORS OF OSTEOPOROSIS IN PATIENTS WITH SYSTEMIC LUPUS

Risk factors found to be significantly related to low bone mineral density in patients with lupus include Caucasian race, older age at diagnosis, higher cumulative glucocorticoid dose, higher systemic lupus erythematosus disease activity index (SLEDAI), and postmenopausal status [28]. In most studies, low bone mineral density strongly correlates with cumulative organ damage and high lupus activity index, but not necessarily with duration of disease. Systemic lupus women with osteoporosis, compared to controls, have a history of lower daily calcium intake, are more likely to smoke, and use less oral contraceptives [17]. Glucocorticoid therapy, used in moderate and severe systemic lupus, is usually the pivotal risk factor for bone loss in this patient population [7, 29].

ABNORMAL VITAMIN D METABOLISM

Abnormal vitamin D metabolism has been described in patients with systemic lupus and renal disease [30]. Due to the lack of sun exposure, often required by treatment, patients with lupus are susceptible to vitamin D deficiency. Of course, if there is renal insufficiency, further impairment of vitamin D dihydroxylation and secondary hyperparathyroidism may occur [31].

OVARIAN DYSFUNCTION

Amenorrhea and early menopause have been recognized as risk factors for low bone mass in patients with lupus [5]. Ovarian dysfunction due to the disease activity, or therapy with cytotoxic drugs and/or glucocorticoids has a detrimental effect on bone mineral density. Low estrogen levels lead to increased release of cytokines, such as IL1, TNF, and IL6, and other TH1 linked factors, increased production of RANKL over OPG, and a high turnover bone resorptive state [6].

Patients with lupus may have reduced estrogen exposure due to their concern over estrogen replacement therapy inducing flares of their disease, a controversial issue that is being studied (the Selena trial) but not yet clarified [32]. To date, there are no prospective data demonstrating a deleterious effect of exogenous estrogens on disease activity in human lupus [33]. A more recent case control study [34] found no association between lupus activity and current use or duration of use of hormone replacement therapy or oral contraceptives. In both males and females with lupus, elevated estradiol levels have been described [35], but there have not yet been studies relating this alteration in estrogen metabolism with bone mass in lupus patients.

OTHER HORMONAL FACTORS

Patients with lupus have low DHEA levels [36] and therapy with DHEA may improve bone mass in this population [37]. In addition, increased testosterone oxidation and low plasma androgens have been reported in patients with lupus [38]. The relationship between circulating levels of androgen and bone mass has been addressed in patients with lupus, with women showing a significantly positive relationship between serum dehydroepiandosterone levels (DHEAS) and higher BMD in the lumbar and femoral neck [36]. Low testos-

terone levels have been reported in premenopausal women with lupus, with a high frequency of osteopenia and osteoporosis.

PHYSICAL ACTIVITY AND BONE MASS

In patients with systemic lupus, low bone mass is associated with a lack of physical activity. The presence of arthritis, muscle pain, and weakness reduces mobility and impairs weight-bearing exercises [39]. Studies on physical disability in lupus show a correlation between changes in the disability index, glucocorticoid usage, and the severity of bone mass loss. The disease activity, as measured by the SLICC ACR damage index, can give us information on physical function and disability in patients with lupus. The index score correlates with the physical function scores of the SF36.

LUPUS AND GLUCOCORTICOID-INDUCED OSTEOPOROSIS

One of the most important common causes of low bone mass in systemic lupus is iatrogenic osteoporosis due to chronic glucocorticoid therapy (GIOP). Patients with moderate or severe systemic lupus are commonly exposed to glucocorticoids. There is a rapid initial phase of glucocorticoid induced bone loss (up to 12% of bone mass loss over 6–12 months), followed by a subsequent slower phase of bone loss [40]. Glucocorticoid-induced osteoporosis affects trabecular or cancellous bone (metabolically active bone) more than cortical bone. Alternate day oral therapy with glucocorticoids is also associated with bone loss [41].

Osteoporotic fractures are the most common serious adverse events associated with this therapy [42]. The fragility fracture incidence in the first year of glucocorticoid therapy may be as high as 15% [43]. Cumulative glucocorticoid dose correlates with the risk of fracture in most cross-sectional studies with lupus [5] and prolonged exposure to lower doses also results in bone loss [44]. In the only prospective study to examine this issue, daily rather than cumulative glucocorticoid dose was a strong predictor for vertebral compression fractures [45]. In this study, glucocorticoid users had considerably higher fracture risk than no-users at similar bone density levels. Postmenopausal women on chronic therapy have the highest incidence of fractures because in addition to their systemic lupus and glucocorticoid exposure, estrogen deficiency and aging further contribute to bone loss and microarchitectural deterioration.

PATHOPHYSIOLOGY OF GLUCOCORTICOID-INDUCED OSTEOPOROSIS IN PATIENTS WITH LUPUS

Most forms of osteoporosis result from an increase in the rate of bone reabsorption, coupled by a concomitant decrease in the rate of bone formation. In the case of glucocorticoid therapy, the decrease in rate of bone formation seems to be the predominant mechanism.

The major cytokines controlling bone formation and resorption and their coupling are RANKL (receptor activated nuclear kinase ligand), RANK, and OPG (osteoprotegrin). RANKL is a cytokine produced predominantly by cells of the osteoblast/stromal cell lineage that binds to RANK, its specific receptor on the surface of osteoclasts, leading to osteoclastogenesis and bone reabsorption. OPG, also produced by osteoblasts, acts as a natural decoy inhibitor of RANK. Glucocorticoids inhibit OPG and stimulate the expression of RANKL in human osteoblasts *in vitro* [46]. Thus, during the initial phase of glucocorticoid-induced osteoporosis, osteoclasts may be stimulated. However, osteoclastic function eventually diminishes as demonstrated by inhibition of bone resorption markers [47].

One of the major mechanisms leading to decreased bone mass in GIOP is increased apoptosis or cell death of osteoblasts [48]. Profound suppression of osteoblast numbers and activity subsequently occurs, reflected by significant suppression of markers of bone formation such as bone specific alkaline phosphatase and serum osteocalcin. Histologically, one can demonstrate decreased bone formation rate, decreased trabecular wall thickness, and apoptosis of bone cells.

In addition to their local effects on apoptosis and cytokine production, glucocorticoids also have a number of systemic effects that adversely affect calcium balance [49]. They decrease gastrointestinal calcium absorption and increase urinary calcium excretion, leading to a state of secondary hyperparathyroidism. In addition, glucocorticoids decrease gonadal production of estrogen, testosterone, and adrenal androgens.

DRUG-INDUCED OSTEOPOROSIS IN LUPUS

Cyclophosphamide, methotrexate, and other chemotherapeutic drugs used in lupus have been reported as decreasing bone mineral density or bone mass [50]. Most of these medications are used in lupus concomitantly with glucocorticoid therapy and it is quite difficult to ascertain whether the loss of bone mass

in these patients is due to the chemotherapeutic agent, additional immunosuppressive agents or glucocorticoid therapy. Warfarin impairs the gamma-carboxylation of osteocalcin [51] and thus may have an adverse effect on bone formation. However, clinical decreases in bone density in patients on warfarin have not been demonstrated.

OSTEOMALACIA IN PATIENTS WITH LUPUS

Osteomalacia can be present in patients with lupus. Osteomalacia is a disorder of mineralization of newly formed matrix in adults. Osteomalacia is a syndrome rather than a disease and as such demands a search for the primary etiology. Causes of osteomalacia can be broadly broken down into (1) defective calcium and/or vitamin D absorption and metabolism, (2) calcium or phosphate renal wasting, and (3) inhibitors of mineralization. Insufficient dietary intake of calcium and/or vitamin D, and disorders of malabsorption such as postgastrectomy syndrome or sprue may lead to defective bone mineralization. Patients with lupus often have low sun exposure (as they are often told to avoid the sun) and the dietary intake of vitamin D may also be low (particularly in the elderly). In addition, patients with systemic lupus and/or Sjögren's syndrome with predominant tubulointerstitial disease can develop renal tubular acidosis. Systemic acidosis can contribute to low bone mass by a compensatory enhanced reabsorption of calcium from the skeleton [52].

Anticonvulsants such as phenobarbital, phenytoin, and carbamazepine may cause osteomalacia due to increased hepatic degradation of 25-hydroxyvitamin D [53] through inducement of a metabolizing enzyme that enhances cytochrome P450 liver metabolism. Other anticonvulsants, such as sodium valproate, which do not induce this type of drug metabolizing enzyme, have little or no impact on 25-hydroxyvitamin D levels. Phenytoin and phenobarbital are often utilized in lupus to manage seizures. Patients taking anticonvulsants who are unable to be exposed to sunlight (such as institutionalized patients) are more likely to develop osteomalacia, and usually require an enhanced intake of vitamin D and calcium to achieve positive calcium balance. There is evidence of increased hip fractures in patients on phenytoin [54].

Osteomalacia may also occur in the presence of chronic renal failure due to defective 1α-hydroxylation of 25-hydroxyvitamin D. In the setting of chronic renal failure, osteomalacia is often accompanied by osteitis fibrosa cystica (due to secondary hyperparathyroidism) and osteoporosis. The exact contribution of each disorder to decreased bone density is complex and individual in each patient.

TABLE 2 Laboratory Differential Diagnosis in Osteomalacia

	Calcium	Phosphorus	Alkaline phosphatase
Osteoporosis	Normal	Normal	Normal
Vitamin D deficiency	Low/nl	Low	Elevated
Urinary phosphate wasting disorders	Normal	Low	Normal
Hypophosphatasia	Normal	Normal	Low

Hypophosphatemia most commonly occurs in conditions characterized by defective renal resorption of phosphorus (Fanconi's syndrome, myeloma, lupus, primary Sjögren's syndrome). Hypophosphatemia may occur with or without renal tubular acidosis, which causes osteomalacia by exchanging hydrogen anions with calcium from bone.

Osteomalacia from inhibition of mineralization by etidronate, fluoride, or aluminum is rare, but should be considered in the absence of more obvious etiologies. Adult hypophosphatasia is a rare syndrome characterized by low serum alkaline phosphatase levels which results in abnormal bone mineralization.

Patients with osteomalacia often present with nonspecific bone pain due to insufficiency fractures especially in the areas of the ribs, pubis, or lesser trochanter. An elevated alkaline phosphatase level (due in most cases to secondary hyperparathyroidism) is an important clue that one may be dealing with osteomalacia rather than osteoporosis (Table 2).

BONE MINERAL DENSITY MEASUREMENT

Bone mineral density (BMD) measurements have become an invaluable tool for the assessment of fracture risk in patients and for monitoring response to therapy [55]. There are currently a large number of techniques that permit accurate and precise measurement of bone mass at a variety of skeletal sites. These include central and peripheral densitometry techniques. (Table 3).

Central Techniques

DXA

Dual energy X-ray absorptiometry (DXA) is currently considered to be the gold standard for the assess-

TABLE 3 Methods of Bone Density Measurment

Methods	Major measurement sites	*Short-term precision error (%)	*Accuracy error (%)	Radiation source	Radiation exposure (skin does)
QUS (quantitative ultrasound)	Heel	2–4	3–6	None	None
DXA (pencil-beam and fan-array)	Spine, proximal femur, total body, and forearm	1–2	0–15	X ray	2–5 mrem
PDXA	Peripheral bones	1–2	—	X ray	2–20 mrem
SXA	Forearm and os calcis	1–2	3–5	X ray	1.3 mrem
QCT and PQCT	Spine, forearm	1–3	5–15	X ray or isotopes	100–300 mrem

ment of bone mineral density. The advantages of DXA over other techniques include the vast pool of scientific literature correlating DXA measurements to future fracture risk, low precision errors which make DXA the only device with proven ability to monitor individual response to therapy, and ability to measure multiple sites (usually the spine and the hip) [56]. The ability to measure more than one site helps to negate one of the major limitations of most peripheral devices (the problem of discordance) [57].

Discordance relates to the fact that bone density and T-scores are not the same at all sites. Discordance may be due to differences in peak bone mass, different rates of bone loss after menopause in primarily cortical vs trabecular bones, artifactual elevation of bone density in the spine after age 65, and differences in normative databases between devices and manufacturers. DXA overcomes many of these problems by measuring both cortical (hip) and trabecular (spine) sites and by using a well-established and accepted normative database, the NHANES III total hip database [58].

The majority of cross-sectional data used to establish the diagnosis of osteoporosis in lupus without fragility fractures has been obtained with DXA. The lowest bone mineral density obtained from the lumbosacral spine or any of three sites of the hip (total hip, femoral neck, or trochanteric area), may be used to establish the diagnosis of osteoporosis [59].

Quantitative CT (QCT)

As opposed to the areal bone density obtained with DXA, QCT can take three-dimensional measurements and determine true volumetric bone density [60]. Although techniques are now available for measuring either peripheral (wrist, hip) or central sites, most studies have been performed measuring predominantly cancellous (trabecular) bone of the spine. Since trabecular bone has a greater surface area and thus greater metabolic rate than cortical bone, QCT's major advantage over DXA (which measures a composite of cortical and trabecular bone) has been in assessing the rate of change of trabecular bone mass in patients with high turnover states (e.g., glucocorticoid use, parathyroid therapy) [61].

Peripheral Techniques

Portable peripheral techniques for bone mineral density measurements have been developed. Those include the use of peripheral dual energy X-ray absorptiometry (usually performed at the wrist, heel, or phalanx, single X-ray absorptiometry of the heel, peripheral quantitative computerized tomography of the wrist or hip and quantitative ultrasound (QUS), predominantly performed at the heel [62]. However, in most studies in patients with lupus, these techniques have been used much less than dual energy X-ray absorptiometry in studying patients with lupus [63]. QCT has a special interest in lupus, specifically in patients on corticosteroids, since they are most likely to have involvement of cancellous or trabecular bone.

In summary, in lupus, bone density measurements are used to make a diagnosis of osteoporosis, assess fracture risk, and monitor the effects of therapy. Although both central and peripheral devices are very valuable for fracture risk assessment, at the present time, only DXA has the ability to monitor the effectiveness of therapy.

THERAPY OF OSTEOPOROSIS IN PATIENTS WITH SYSTEMIC LUPUS

As in any at-risk patient, reduction of risk factors and adequate nutrition are important components of intervention in patients with lupus who have low bone mass,

osteoporosis, or other metabolic bone diseases. Physical activity, such as weight-bearing exercises, can enhance muscle mass and strength, coordination, and balance. Education regarding fall prevention and an exercise regimen individualized to the patient's ability are also very useful. Modification of adverse behaviors, such as smoking and inactivity, and prevention of recurrent falls is crucial to avoid fragility fractures.

Calcium and vitamin D in combination have been found to prevent glucocorticoid induced osteoporosis. Multiple studies have shown that dietary calcium with appropriate supplementation is important. The goal is an optimal daily intake of 1000–1500 mg of elemental calcium. Patients with inflammatory diseases, such as lupus, may have impaired calcium intake and decline in gastrointestinal absorption. In patients with lupus, especially if they avoid sun exposure, vitamin D is crucial in the management of low bone mass. Vitamin D increases calcium and phosphorus absorption from the gastrointestinal tract and maintains muscle strength, which is important and plays a role in protection against falls and weakness. At least 400–800 IU of vitamin D a day is appropriate, and in patients with glucocorticoid-induced osteoporosis, this therapy, in addition to calcium, has shown to be protective in a meta-analysis [64].

In patients who are hypogonadal, either postmenopausal women or men with androgen deficiency, hormonal replacement has been suggested [49]. Use of estrogen in patients with lupus, including estrogen replacement therapy, remains controversial. The evidence that estrogens, such as oral contraceptives, can induce anti-nuclear antibodies and the linkage between estrogen exposure with the onset of lupus has been described both in prospective cohort studies and in case reports [65]. Prospective trials of the effects of these hormones on disease activity are not yet available. Thus, estrogen replacement therapy exposes patients to a higher risk of systemic lupus, but there may not be an association with activity of lupus once it is already present.

In postmenopausal women, the long-term benefits vs risks of estrogen replacement therapy are being debated. In July 2002, the hormone replacement therapy (HRT) arm of the Women's Health Initiative (WHI) was stopped prematurely (planned follow-up 8.5 years, actual follow-up 5.2 years) because of an increased risk of breast cancer and an unacceptable rate of the global outcome index [66]. The study did demonstrate fracture risk reduction in hip (34%) and clinical vertebral (34%) fractures for the first time in a prospective study. However, this was offset by the increased relative risk of coronary heart disease (29%) and invasive breast cancer (26%), leading a number of groups including the U.S. Prevention Task Force [67] to recommend caution in prescribing long-term hormone replacement preparations to postmenopausal women for chronic disease prevention (e.g., osteoporosis).

Alendronate

Currently the most widely used therapies in patients with lupus for glucocorticoid-induced osteoporosis and postmenopausal osteoporosis are the bisphosphonates, medications which primarily decrease osteoclastic bone reabsorption. In 1998, Saag and colleagues [18] presented alendronate data from two double-blind trials of 560 patients receiving at least 7.5 mg of prednisone or equivalent daily. The patients had been randomized to three groups: those receiving glucocorticoids for up to 4 months, those receiving them for 4 to 12 months, and those receiving them for more than 12 months. All the data were pooled. As in most trials of osteoporosis, all patients received calcium and appropriate vitamin D. Patients receiving at least 5–10 mg of alendronate per day had significantly increased bone mineral density (BMD) of the lumbosacral spine, trochanter, and femoral neck. Total body BMD was also significantly increased, but only in patients receiving 10 mg of alendronate. In this trial, patients on estrogen were allowed to continue on that therapy.

The second year extension of this trial [68] included 208 patients from the initial cohort receiving 5 or 10 mg of alendronate or placebo. An increase in BMD in the lumbosacral spine and trochanter was demonstrated in patients receiving alendronate, and maintenance of improvement in the femoral neck was also seen. Bone reabsorption markers decreased in the first year, but remained within normal ranges during year 2 for men and premenopausal women. When the alendronate vertebral fracture data was compared to that of controls in this study at 24 months, a significant lowering of vertebral fracture was seen: 0.7% in the alendronate group vs 6.8% in the control group ($P < 0.05$), with 90% reduction of vertebral fractures, year 2, in the pooled data on alendronate. No significant vertebral fracture difference was demonstrated in the first year, but it was seen in the second year [68].

Risedronate

In the prevention trial [69], Risedronate was shown to be efficacious in maintaining BMD in patients initiating glucocorticoid therapy. The study population, including 228 patients, was randomized to 2.5 mg of Risedronate, 5 mg of Risedronate, or placebo. Risedronate was found to be well tolerated, with a distribution of adverse events similar across all groups. In this trial, patients had been receiving glucocorticoid therapy

for less than 3 months. At 12 months of follow-up, BMD in the lumbosacral spine, femoral neck, and trochanter was maintained in patients treated with Risedronate, while patients receiving placebo demonstrated significant progressive bone loss at all sites ($P < 0.05$).

In the treatment trial of Risedronate [70], this agent was proven effective in treating glucocorticoid induced osteoporosis in patients on long-term glucocorticoid therapy, defined as at least 6 months on 7.5 mg of prednisone or prednisone equivalent. The 3-month double-blind multicenter trial studied 290 patients being treated primarily for rheumatic disease. Patients were randomized to either risedronate or vitamin D and calcium. Risedronate significantly increased BMD in both the hip and the spine compared to baseline and compared to placebo (1.76% and 2.9%, respectively. Additionally, assessment of bone turnover markers demonstrated no evidence of oversuppression of bone turnover.

When the data from both the prevention and treatment trials with Risedronate were pooled, there was a 70% reduction in the incidence of vertebral fractures in patients on 5 mg of Risedronate compared to controls within the first year of treatment ($P = 0.016$). The mean incidence of vertebral fractures was 16.3% in the control group and 5.45% in the Risedronate group [70].

Calcitonin

Calcitonin, a 32 amino acid polypeptide hormone that directly suppresses osteoclast activity, is not indicated in glucocorticoid-induced osteoporosis. The Food and Drug Administration (FDA) approved its use in women at least 5 years after the menopause with postmenopausal osteoporosis; however, studies in patients receiving glucocorticoids have been inconclusive.

Meta-analysis of Therapy

A meta-analysis to examine how various drug treatments for glucocorticoid-induced osteoporosis differed by efficacy has been performed [64]. Sixty-one randomized controlled trials lasting at least 6 months in patients using oral glucocorticoids were evaluated. The use of calcium, vitamin D, calcitonin, and bisphosphonates was assessed. BMD was measured in the lumbosacral spine by either QCT, DXA, or DPA. Studies were included in the analysis if changes of BMD from baseline could be determined. Thirty-three studies were eligible for analysis. The data showed that of all the treatments that significantly increased lumbosacral spine BMD, bisphosphonates proved substantially more effective than calcitonin or vitamin D plus calcium ($P < 0.001$). This data, coupled with the data from clinical trials, demonstrated an increase in bone mass and significant reduction of vertebral fractures, providing a strong rationale for the use of bisphosphonate therapy for the treatment and prevention of glucocorticoid-induced osteoporosis.

Parathyroid Hormone Therapy

Parathyroid hormone was utilized in a randomized clinical trial of postmenopausal women (mean age was 63 years) with osteoporosis who were taking glucocorticoids and hormone replacement therapy. Of the 51 patients, 8 had systemic lupus erythematosus. In this study [71], parathyroid administration significantly increased bone mineral density by QCT and by DXA ($P < 0.001$) in comparison to those patients on estrogen plus calcium and vitamin D alone.

During the first 3 months of parathyroid hormone (PTH) therapy, markers of bone formation increased significantly. Parathyroid hormone dramatically increased bone mineral density in the central skeleton of postmenopausal women, some with lupus, with glucocorticoid-induced osteoporosis who were taking hormone replacement therapy.

In glucocorticoid-induced osteoporosis, the addition of parathyroid hormone increased dramatically bone mineral density in the lumbosacral spine and hip over estrogen alone. In the initial study, no patient on parathyroid hormone plus estrogen had any fragility fracture, and those patients, followed for another year, continued to have an increase in bone mineral density. One year after discontinuation of PTH, while the patients continued to receive hormone replacement therapy, lumbar spine bone mineral density remained stable while total hip bone mass increased 5% over baseline levels [61]. Although PTH treatment in postmenopausal osteoporotic women has been shown to reduced incidence in radiographic vertebral fractures, there are no data yet regarding PTH in bisphosphonate treated patients who later receive PTH.

ACR Guidelines

The recommendations for the prevention and treatment of glucocorticoid induced osteoporosis from the American College of Rheumatology [72] include the modification of lifestyle and risk factors for osteoporosis (e.g., smoking cessation or avoidance, reduction of alcohol consumption if excessive, and instruction in weight-bearing physical exercises), initiation of supplementation with calcium and vitamin D, plain or activated form, and the prescription of bisphosphonates,

which should be used with caution in premenopausal women. Patients receiving long-term glucocorticoid therapy (prednisone or equivalent equal to or over 5 mg per day) should be included in these recommendations. The replacement of gonadal hormones, if deficient, needs to be performed with caution, especially in females. The measurement of bone mineral density at the lumbosacral spine and/or hip is required in those patients on chronic glucocorticoid therapy, and if the BMD is not normal, adding a bisphosphonate or a second like agent, including calcitonin, could be considered. In patients on therapy, bone mineral density can be measured as frequently as every 6 months to a year.

Unapproved Therapies for Glucocorticoid-Induced Osteoporosis

Disodium Etidronate

Disodium etidronate, given cyclically, has been shown to be effective in glucocorticoid-induced osteoporosis in a large randomized-controlled double-blind placebo-controlled trial. A 12-month, randomized, placebo-controlled study of intermittent etidronate (400 mg per day for 14 days) followed by calcium (500 mg per day for 76 days), given for four cycles, was performed in 141 men and women (age, 19 to 87 years) who had recently begun high-dose corticosteroid therapy [73]. The mean (+/–SE) bone density of the lumbar spine and trochanter in the etidronate group increased 0.61 +/– 0.54 and 1.46 +/– 0.67%, respectively, as compared with decreases of 3.23 +/– 0.60 and 2.74 +/– 0.66%, respectively, in the placebo group. There was an 85% reduction in the proportion of postmenopausal woman with new vertebral fractures in the etidronate group as compared with the placebo, a secondary endpoint of the trial. Etidronate is not approved in the United States for osteoporosis.

Pamidronate

Intravenous pamidronate has been studied in the prevention of glucocorticoid-induced osteoporosis [74]. A total of 27 patients who required first-time, long-term corticosteroid therapy at a daily dose of at least 10-mg prednisolone were studied. Patients allocated to pamidronate treatment received a baseline intravenous infusion of 90-mg pamidronate at the initiation of their steroid treatment, then received 30-mg pamidronate, intravenously, every 3 months for as long as steroid therapy was continued. The control patients (calcium group) were put on a daily 800-mg elemental calcium supplement given as calcium carbonate. Over 1 year, the pamidronate group showed a significant BMD increase in the lumbar spine (3.6%), and at all sites of the hip (2.2% at the femoral neck). In the calcium group, a significant BMD reduction was registered at the lumbar spine (–5.3%) and at the femoral neck (–5.3%). Differences between the groups were significant at all sites measured. Pamidronate is not approved in the United States for osteoporosis.

DHEA

DHEA, which has not been approved by the FDA as therapy for musculoskeletal symptoms of lupus, has been shown to decrease the glucocorticoid requirement in some patients [37]. It is widely used over-the-counter by patients with lupus.

Hydroxychloroquine

Hydroxychloroquine was found by Lakshminarayanan [75] to be the only factor associated with higher BMD of the hip and spine in patients with systemic lupus, and it remains predictive of higher BMD in a post-hoc analysis. Hydroxychloroquine may be protective against low BMD in glucocorticoid patients with systemic lupus; however, duplicate studies are needed.

THERAPY OF OSTEOMALACIA AND DRUG-INDUCED OSTEOMALACIA

After measuring 25-hydroxy and 1,25-dihydroxyvitamin D, 20–30 μg of 25-hydroxyvitamin D or 0.15–0.5 μg of 1,25-dihydroxyvitamin D were utilized where appropriate. Alternatively, 50,000 units of vitamin D in a pill or 1000 units of vitamin D given intramuscularly could be used. The dose and frequency of administration needs to be adjusted to the serum levels of calcium, phosphorus, alkaline phosphatase, and 25-hydroxyvitamin D level.

RENAL TUBULAR ACIDOSIS

Renal tubular acidosis can be present in patients with lupus and Sjögren's syndrome. In this disorder, the kidney is incapable of conserving bicarbonate and the patients have low serum or plasma bicarbonate concentration and systemic metabolic acidosis.

Patients with renal tubular acidosis can develop osteomalacia, particularly if there is proximal tubular

involvement. Patients with lupus and Sjögren's can have these types of complications. Therapy for renal tubular acidosis includes correction of the systemic disease, the use of oral sodium bicarbonate, and occasionally the use of thiazides to reduce plasma volume and enhance tubular bicarbonate reabsorption.

RENAL OSTEODYSTROPHY

Renal osteodystrophy may occur in patients with lupus and end stage kidney disease. Patients can have high turnover bone disease with secondary hyperparathyroidism or low turnover disease with osteomalacia. Osteitis fibrosis is the most common high turnover lesion of renal osteodystrophy. This represents the response of bone to persistent high levels of PTH. In these patients, there is histological evidence of active bone reabsorption with increases in the number and size of osteoclasts and in the number of reabsorption bays or lacuna within cancellous bone. Fibrous tissue is found immediately adjacent to bone trabecula and may accumulate extensively within the bone marrow space. In contrast, low turnover bone disease with osteomalacia is also seen in patients with renal osteodystrophy.

GLUCOCORTICOID-INDUCED OSTEONECROSIS

See Chapter 37. Osteonecrosis has been reported in 3–30% of patients with systemic lupus erythematosus. This wide range reflects the use of different techniques in defining this condition. Although osteonecrosis has been described in patients exposed to glucocorticoid therapy, in lupus, it occurs even in patients not exposed to glucocorticoids. In osteonecrosis, bone and marrow cells are destroyed, contributing significantly to bone loss, particularly of the femoral head, although the knees, shoulders, and other bones may also be involved. Osteonecrosis in the femoral head has been noted to occur more in subjects taking glucocorticoid therapy for their lupus than in nonusers of this medication. Although it is highly probable that both local and systemic mechanisms are at work, as shown later, in the development of glucocorticoid-induced osteonecrosis, many of these patients may have, in addition to the glucocorticoid exposure and their systemic lupus, smoking, hypercholesterolemia, and hypertriglyceridemia. Symptomatic osteonecrosis occurred in 14.5% of 744 patients with lupus, described in the Baltimore cohort [4], and on many occasions involved multiple joints. Osteonecrosis was not associated with increased mortality, but it was associated with physical disability.

CLINICAL MANIFESTATIONS OF OSTEONECROSIS

The most common presenting symptoms of osteonecrosis include groin, thigh, and buttock pain, and weight-bearing and motion-induced pain. In addition, two-thirds of patients complain of rest pain and night pain. A small group of patients may be asymptomatic.

Other bones involved, in addition to the humeral head, include the femoral condyle, proximal tibia, vertebrae, and small bones of the hands and feet. Many patients have bilateral involvement [76], and some have pain in multiple anatomical areas. Therefore, if a patient presents with osteonecrosis in one hip, the contralateral hip should be assessed for this condition. On physical examination, patients have pain and limitation of motion, particularly with internal rotation and abduction.

Radiographic Evaluation

The plain radiograph may be normal initially in osteonecrosis. Early findings are mild changes in density, followed by sclerosis and cysts. The crescent sign, a subchondral radiolucency, is felt to be pathognomonic [77]. Eventually joint space narrowing and periarticular sclerosis can occur.

Technetium-99 scanning can show decreased uptake in the affected bone. Magnetic resonance imaging is significantly more specific and sensitive than plain radiography or bone scanning. The sensitivity is felt to be up to 91% [78]. Changes can be seen early in the disease, even when other studies are negative. Focal lesions are well demarcated and homogeneous on T1-weighted images, and high intensity line can appear on T2-weighted images, representing hypervascular granulation tissue. This is felt to be a pathognomonic double line sign.

In a study of systemic lupus patients, 23 glucocorticoid-treated patients had no hip pain and negative hip X rays, but a positive MRI. Eight (35%) had evidence of osteonecrosis of the femoral head on MRI, and six, 26%, by bone scanning. Over a 3-year period, only two of the eight patients with initial abnormal MRI had a lesion that could be seen by X-ray. So, overtreatment can occur also in this condition [79].

Staging

There are multiple staging systems for osteonecrosis. The Association of Research Circulation Osseous (ARCO) developed a staging system that attempts to bring conformity to clinical trials and strategies. In stage zero, all diagnostic studies are normal. In stage one, X

rays are normal but MRI imaging is positive or the biopsy is positive for necrosis. The extent of involvement can be less than 15%, 15–30%, or greater than 30%, respectively. In stage two, X rays are positive but there is no collapse, and the extent of involvement remains between 15 and 30% or greater than 30%. In stage three, there is flattening and a crescent sign, indicating subchondral collapse. In stage four, in addition to the flattening of the femoral head with joint space narrowing, there is evidence of early osteoarthritis.

Treatment

Conservative or nonoperative therapy, including bed rest and avoiding weight bearing with crutches has been ineffective. In osteonecrosis of the shoulder, results of conservative therapy may be more efficacious than in the hip.

The results of total hip arthroplasty in osteonecrosis are not as significantly good as with total hip replacement for osteoarthritis [80], which leads to a higher revision rate in patients with osteonecrosis. Nevertheless, arthroplasty is the next best treatment available for this condition. Total arthroplasty is also utilized in osteonecrosis of the knee or shoulder.

The failure of conservative management and relative poor long-term survival of prosthetic devices, total hips, and knees, created the need for other interventions. Core decompression was initially used as a diagnostic tool to measure bone marrow pressure and other biopsy specimens. Small case series in retrospective studies have shown that cord decompression for osteonecrosis of the hip is successful, especially in stage one or stage two hip disease. Core decompression for osteonecrosis of the shoulder or knee has not been properly ascertained. Osteotomy also has been used for this purpose.

In summary, the treatment for osteonecrosis includes conservative therapy, joint replacement, including bed rest and avoiding weight bearing with crutches, core decompression, and osteotomy.

PATHOGENESIS OF GLUCOCORTICOID-INDUCED OSTEONECROSIS

In osteonecrosis, bone and marrow cells are destroyed. Patients with systemic lupus have accompanying small vessel vasculitis and venous endothelial cell changes that lead to stasis, which can increase vascular pressure and eventually cause osteonecrosis. In the earlier medical literature, severe necrosis of the femoral neck was reported to be primarily due to thrombosis and compression of blood vessels, with increased vascular pressure, or to microfractures of bone, or simply to fat and fluid retention [81].

This theory suggested that demineralization and thinning of trabecular bone occurred as a result of interrupted blood flow to the bone, and until more recently, provided the only possible explanation for the profound and rapid necrosis that is sometimes found in patients with osteoporosis of the femoral head.

A more recent theory of pathogenesis suggests that osteonecrosis is a result of a disruption of the complex interplay of osteoblasts and osteoclasts in the bone remodeling cycle. Significant glucocorticoid use leads to the suppression of osteoblastogenesis (the development of mature osteoblasts). Studies in mice given glucocorticoids [82] have shown a threefold increase in apoptosis of osteoblasts compared to normal controls. Osteoblasts that form new bone, once apoptotic, may in turn assimilate into bone matrix or become lining cells of the bone in the process of reabsorption. Histological studies have indicated that only 20–50% of osteoblasts are recovered in glucocorticoid-exposed patients [83], suggesting that programmed cell death of a majority of osteoblasts occurs during each formation reabsorption cycle in osteonecrosis. Apoptosis of osteoclasts are also associated with glucocorticoid use. It appears that part of the function of the osteoclasts may be to signal detection of microdamage and to initiate the bone remodeling process. In glucocorticoid-induced osteonecrosis, the initial signal for remodeling is prevented by apoptosis of the osteoclasts.

Research efforts are now focusing on hormones and cytokines that intervene early in the bone remodeling cycle to prevent apoptosis of osteoblasts and enhance osteoblastogenesis and therefore prevent bone loss from glucocorticoid use. The role of, for example, recombinant PTH on this process is still under study.

CONCLUSION

In patients with lupus, metabolic bone disease can be a significant cause of morbidity. Physicians caring for patients are now able to recognize and intervene to manage these complications. Most notably, dramatic advances in the ability to diagnose low bone mass before the first fracture, together with proven therapies to prevent bone loss are likely to alter fracture occurrence in patients with lupus treated with glucocorticoids.

References

1. NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy. (2001). *JAMA* **285**, 785–795.

2. World Health Organization. (1994). "Assessment of Fracture Risk and its Application to Screening for Post-Menopausal Osteoporosis." Technical Report Series 843, WHO, Geneva.
3. Sinigaglia, Varenna, M., Binelli, L., Zucchi, F., Ghiringbell, D., and Fantini, F. (2000). Bone mass in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **18**, S527–S534.
4. Redlich, K., Ziegler, S., Kiener, M. P., Spitzaur, S., *et al.* (2000). Bone mineral density and biochemical parameters of bone metabolism in female patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **59**, 308–310.
5. Fen, D., and Keen, R. W. (2001). Osteoporosis in systemic lupus erythematosus: Prevalence and therapy. *Lupus* **10**, 227–232.
6. Rodan, A. G., and Martin, J. T. (2001). Therapeutic approaches to bone diseases. *Science* **289**, 1508–1514.
7. Kipen, Y., Buchbinder, R., Forbes, A., Strauss, B., Littlejohn, G., and Morand, E. (1997). Prevalence of reduced bone mineral density in systemic lupus erythematosus and the role of steroids. *J. Rheumatol.* **24**, 1922–1928.
8. Kalla, A. A., Van Wyk, K. T., and Meyers, O. L. (1992). Metacarpal bone mass in systemic lupus erythematosus. *Clin. Rheumatol.* **11**, 475–482.
9. Kalla, A. A., Fataar, A. D., Jessop, S. J., and Bewerunpe, L. (1993). Loss of trabecular bone mineral density in systemic lupus. *Arthritis Rheum.* **36**, 1726–1734.
10. Houssiau, F. A., Legebure, C., Depresseox, G., Lambert, M., Devogelaer, J. P., Nagant, D. E., and Deuxchaisnes, C. (1996). Trabecular and cortical bone loss in systemic lupus erythematosus. *Br. J. Rheumatol.* **35**, 244–247.
11. Formiga, F., Moga, I., Nolla, J. M., Pac, M., Mitjavila, E., Rolg, E. S., and Cofet, D. (1995). Loss of bone mineral density in pre-menopausal women with systemic lupus erythematosus. *Ann. Rheum. Dis.* **54**, 274–276.
12. Formiga, F., Nolla, J. M., Mitjavila, F., Bonnin, R., Dnavanvo, M. A., and Moga, I. (1996). Bone mineral density and hormonal status in men with SLE. *Lupus* **5**, 623–626.
13. Trapane, S., Civimine, R., Crimini, H., Paci, E., and Falleimi, F. (1998). Osteoporosis in juvenile systemic lupus erythematosus: A longitudinal study on the effects of steroids on bone mineral density. *Rheumatol. Int.* **18**, 45–49.
14. Gilboe, I. M., Kvien, T. K., Haugeberg, J., and Husby, G. (2000). Bone mineral density in SLE. Comparisons to rheumatoid arthritis and healthy controls. *Ann. Rheum. Dis.* **59**, 110–115.
15. Ramsey-Goldman, R., and Mamzi, S. (2001). Association of osteoporosis and cardiovascular disease in women with systemic lupus erythematosus. *Arthritis Rheum.* **44**, 2338–2341.
16. Petrie, M. (1995). Musculoskeletal complications of systemic lupus erythematosus in the Hopkins Lupus Cohort: An update. *Arthritis Care Res.* **8**, 137–145.
17. Ramsey-Goldman, R., Dunn, J. E., Hone, C., *et al.* (1999). Frequency of fractures in women with systemic lupus erythematosus. *Arthritis Rheum*. **42**, 882–890.
18. Saag, K. G., Emkey, R., Schnitzer, T. J., Brown, J. P., Hawkins, F., Groemaere, S., Thamsborg, G., Liberman, U. A., Delmas, P. D., Malice, M. P., Czachur, M., and Daifotis, A. G. (1998). Alendronate for the prevention and treatment of glucocorticoid induced osteoporosis. *N. Engl. J. Med.* **339**, 292–299.
19. Cohen, S., Levy, R. M., Keller, M., Boling, E., Emkey, R. D., Greenwald, M., Zizic, T. M., Wallach, S., Sewell, K. L., Lukert, B. P., Axelrod, D. W., and Chines, A. A. (1999). Risedronate therapy prevents corticosteroid induced bone loss: A twelve month, multicenter, randomized, double-blind, placebo controlled, parallel group study. *Arthritis Rheum.* **42**, 2309–2318.
20. Theofilopoulos, A. N., and Lawjon, B. R. (1999). Tumor necrosis factor and other cytokines in immune lupus. *Ann. Rheum. Dis.* **58**(Suppl. 1), 149–155.
21. Tanaka, Y., Watanobe, K., Suzuki, *et al.* Spontaneous production of bone resorbing lymphokines by B-cells in patients with systemic lupus erythematosus. *J. Clin. Immunol.* **9**, 415–420.
22. Gravallese, E. M., Manning, C., Tsay, A., Naito, A., Pan, C., Amento, E., and Goldring, S. R. (2000). Synovial tissue in rheumatoid arthiritis is a source of osteoclast differentiation factor. *Arthritis Rheum.* **43**, 250–258.
23. Romas, E., Bakharevski, O., Hards, D. K., Kartsogiannis, V., Quinn, J. M., Ryan, P. F., Martin, T. J., and Gillespie, M. T. (2000). Expression of osteoclast differentiation factor at sites of bone erosion in collagen-induced arthritis. *Arthritis Rheum.* **43**, 821–826.
24. Teitelbaum, S. L. (2000). Bone resorption by osteoclasts. *Science* **289**, 1504–1508.
25. Hofbauer, L. C., Khosla, S., Dunstan, C. R., Lacey, D. L., Boyle, W. J., and Riggs, B. L. (2000). The roles of osteoprotegerin and osteoprotegrin ligand in the paracrine regulation of bone resorption. *J. Bone Miner. Res.* **15**, 2–12.
26. Ramsey-Goldman, R., Bongu, A., Langman, C. N., Price, H., Spies, S., *et al.* (2001). A pilot study of serum osteoprotegerin (sOPG), bone biochemical markers and bone mineral density (sBMD) in lupus and control women. *Arthritis Rheum.* **44**, S260.
27. Pineau, C. A., Urowitz, M. B., Fortin, P. R., Ibanez, D., and Gladman-Torres, D. D. (2001). Osteoporosis in SLE II: Prevalence and associated clinical factors in women. *Arthritis Rheum.* **44**, S334.
28. Swaak, A. J., van der Brink, H. G., Smeenk, R. J., *et al.* (1999). Systemic lupus erythematosus: Clinical features in patients with a disease duration of over 10 years, first evaluation. *Rheumatology* **38**, 953–958.
29. Sels, F., Deoueker, J., Verwilghen, J., and Mboxmuamba, S. M. (1996). SLE and osteoporosis: Dependence and/or independence on glucocorticoids. *Lupus* **5**, 89–92.
30. Di Munno, O., Delle Sedie, A., Mazzantini, M., Frigelli, S., and Metelli, M. R. (2001). Serum 25-OH vitamin D3 in chronic rheumatic patients. *J. Bone Miner. Res.* **16**, S325.
31. Reichel, H., Deibert, B., Schmidt-Gayk, H., and Ritz, E. (1991). Calcium metabolism in early chronic renal failure: Implications for the pathogenesis of hyperparathyroidism. *Nephrol. Dial. Transplant.* **6**, 162–169.
32. Petri, M. (2001). Exogenous estrogen in systemic lupus erythematosus: Oral contraceptives and hormone replacement therapy. *Lupus* **10**, 222–226.

33. Mok, C. C., Lau, C. S., and Wong, R. W. (2001). Use of exogenous estrogens in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **30**, 426–435.
34. Cooper, G. S., Dooley, M. A., Treadwell, E. L., St. Clair, E. W., and Gil Keson, G. S. (2002). Hormonal and reproductive risk factors for development of systemic lupus erythematosus: Results of a population-based, case-control study. *Arthritis Rheum.* **46**, 1830–1839.
35. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1981). Increased 16-alpha hydroxylation of estradiol in systemic lupus erythematosus. *Clin. Endocrinol. Metab.* **53**, 174–178.
36. Van Vollenhaven, R. F. (2000). Dehydroepiandrosterone in systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **26**, 349–362.
37. Petri, M. A., Lahita, R. G., Van Vollenhaven, R. F., Merril, J. T., Schiff, M., *et al.* (2000, 2002). Effects of prasterone on corticosteroid requirements of women with systemic lupus erythematosus: A double-blind, randomized, placebo-controlled trial. *Arthritis Rheum.* **46**, 1820–1829; *Arthritis Rheum.* **43**, 271S.
38. Lahita, R. G., Bradlow, H. L., Fishmen, J., and Kunkel, H. G. (1982). Hormonal estrogen and androgen metabolism in the human with systemic lupus erythematosus. *Am. J. Kidney Dis.* **2**, 206–211.
39. Star, V. L., and Hochberg, M. C. (1994). Osteoporosis in patients with rheumatic disease. *Rheum. Dis. North Am.* **20**, 561–576.
40. Laan, R. F., van Riel, P. L., van Erning, *et al.* (1992). Vertebral osteoporosis in rheumatoid arthritis patients: Effect of low dose prednisone therapy. *Br. J. Rheumatol.* **31**, 91–96.
41. Gluck, O. S., Murphy, W. A., Hahn, T. J., and Hahn, B. H. (1981). Bone loss in adults receiving alternate day glucocorticoid therapy. *Arthritis Rheum.* **24**, 892–897.
42. Saag, K. G., Koehnke, R., Caldwell, J. R., Brasington, R., Burmeister, L. F., *et al.* (1994). Low dose long term corticosteroid therapy in rheumatoid arthritis: An analysis of serious adverse events. *Am. J. Med.* **96**, 115–123.
43. Adinoff, A. D., and Hollister, J. R. (1983). Steroid induced fractures and bone loss in patients with asthma. *N. Engl. J. Med.* **309**, 265–268.
44. Buckley, L. M., Leib, E., Cartularo, K. S., *et al.* (1996). Calcium and vitamin D3 supplementation prevents bone loss in the spine secondary to low dose corticosteroids in patients with rheumatoid arthritis. A randomized, double-blind, placebo controlled trial. *Ann. Intern. Med.* **125**, 961–968.
45. van Staa, T. P., Cooper, C., Barton, I., Cohen, S., Reid, D. M., *et al.* (2002). Predictors for vertebral fracture and fracture threshold inpatients using oral corticosteroids. *Arthritis Rheum.* **46**, S585.
46. Hofbauer, L. C., Gori, F., and Riggs, B. L. (1999). Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: Potential paracrine mechanisms of glucocorticoid-induced osteoporosis. *Endocrinology* **140**, 4382–4389.
47. Pearce, G., Tabensky, D. A., Delmas, P. D., and Seeman, E. (1998). Corticosteroid-induced osteoporosis in men. *J. Clin. Endocrinol. Metab.* **83**, 801–806.
48. Weinstein, R. S., and Manolagas, S. C. (2000). Apoptosis and osteoporosis. *Am. J. Med.* **108**, 153–164.
49. Lane, N. E., and Lukert, B. (1998). The science and therapy of glucocorticoid induced bone loss. *Endocrinol. Metab. Clin. North Am.* **27**, 465–483.
50. Preston, S. J., Diamond, T., Scott, A., and Lauren, M. R. (1993). Methotrexate osteopathy in rheumatoid arthritis. *Ann. Rheum. Dis.* **52**, 582–585.
51. Menon, R. K., Gill, D. S., Thomas, M., *et al.* (1987). Impaired carboxylation of osteocalcin in warfarin treated patients. *J. Clin. Endocrinol. Metab.* **64**, 59–61.
52. Weger, M., Deutschmann, H., Weger, W., Kotanko, P., and Skrabal, F. (1999). Incomplete renal tubular acidosis in "primary" osteoporosis. *Osteopor. Int.* **10**, 325–329.
53. Feldkamp, J., Becker, A., Witte, O. W., Scharff, D., and Scherbaum, W. A. (2000). Long term anticonvulsant therapy leads to low bone mineral density evidence for direct drug effects of phenytoin and carbamazepine on human osteoblast-like cells. *Exp. Clin. Endocrinol. Diabetes* **108**, 37–43.
54. Cummings, S. R., Nevitt, M. C., Browner, W. S., *et al.* (1995). Risk factors for hip fracture in White women. *N. Engl. J. Med.* **332**, 767–773.
55. Maricic, M., and Chen, Z. (2000). Bone densitometry. *Clin. Lab. Med.* **3**, 469–488.
56. Miller, P. D., Bonnick, S. L., and Rosen, C. J. (1996). Consensus of an international panel on the clinical utility of bone mass measurements in the detection of low bone mass in the adult population. *Calcif. Tissue Int.* **58**, 207–214.
57. Bonnick, S. L. (1998). "Bone Densitometry in Clinical Practice—Application and Interpretation." Humana Press, Totowa, New Jersey.
58. Looker, A. C., Wahner, H. W., Dunn, W. L., Calvo, M. S., Harris, T. B., Heyse, S. P., Johnston, C. C., and Lindsay, R. L. (1995). Proximal femur bone mineral levels of US adults. *Osteopor. Int.* **5**, 389–409.
59. Hamdy, R. C., Petak, S. M., and Lenchick, L. (2002). Which central dual x-ray absorptiometry skeletal sites and regions of the interest should be used to determine the diagnosis of osteoporosis? *J. Clin. Densitom.* **5**, S11–S17.
60. Grampp, S., Lang, P., Jergas, M., *et al.* (1995). Assessment of the skeletal status by quantitative peripheral computed tomography: Short-term precision in-vivo and comparison to dual x-ray absorptiometry. *J. Bone Miner. Res.* **10**, 1566–1576.
61. Lane, N. E., Sanchez, S., Modin, G. W., Genant, H. K., Pierini, E., and Arnaud, C. D. (2000). Bone mass continues to increase at the hip after parathyroid hormone treatment is discontinued in glucocorticoid induced osteoporosis: Results of a randomized controlled clinical trial. *J. Bone Miner. Res.* **15**, 944–951.
62. Njeh, C. F., Biovin, C. M., and Langton, C. M. (1997). The role of ultrasound in the assessment of osteoporosis: A review. *Osteopor. Int.* **7**, 7–22.

63. Falcini, F., Bindi, G., Ermini, M., Galluzzi, F., Poggi, G., Rossi, S., Masi, L., Cimaz, R., and Brandi, M. L. (2000). Comparison of quantitative calcaneal ultrasound and dual energy X-ray absorptiometry in the evaluation of osteoporotic risk in children with chronic rheumatic diseases. *Calcif. Tissue Int.* **67**, 19–23.
64. Amin, S., Lavalley, M. P., Simms, R. W., and Felson, D. T. (2002). The comparative efficacy of drug therapies used for the management of corticosteroid-induced osteoporosis: A meta-regression. *J. Bone Miner. Res.* **17**, 1512–1526.
65. Sanchez-Guerrero, J., Karlson, E. W., Liang, M. H., *et al.* (1997). Past use of oral contraceptives and the risk of developing systemic lupus erythematosus. *Arthritis Rheum.* **40**, 804–808.
66. Writing Group for the Women's Health Initiative Investigators. (2002). Risks and benefits of estrogen plus progestin in health postmenopausal women: Principal results from the Women's Health Initiative clinical trial. *JAMA* **288**, 321–333.
67. US Preventive Services Task Force. (2002). Postmenopausal hormone replacement therapy for primary prevention of chronic conditions: Recommendations and rationale. *Ann. Intern. Med.* **137**, 834–839.
68. Adachi, J. D., Saag, K. G., Delmas, P. D., Liberman, U. A., Emkey, R. D., *et al.* (2001). A. Two-year effects of alendronate on bone mineral density and vertebral fracture in patients receiving glucocorticoids: A randomized, double-blind, placebo-controlled extension trial. *Arthritis Rheum.* **44**, 202–211.
69. Reid, D. M., Hughes, R. A., Laan, R. F., *et al.* (2000). Efficacy and safety of daily risedronate in the treatment of corticosteroid induced osteoporosis in men and women: A randomized trial. European Corticosteroid Induced Osteoporosis Treatment study. *J. Bone Miner. Res.* **15**, 1006–1013.
70. Wallach, S., Cohen, S., Reid, D. M., Hughes, R. A., Hosking, D. J., *et al.* (2000). Effects of risedronate treatment on bone density and vertebral fracture in patients on corticosteroid therapy. *Calcif. Tissue Int.* **67**, 277–285.
71. Lane, N. E., Sanchez, S., Modin, G. W., Genant, H. K., Pierini, E., and Arnaud, C. D. (1998). Parathyroid hormone treatment can reverse corticosteroid induced osteoporosis. *J. Clin. Invest.* **102**, 1627–1633.
72. American College of Rheumatology Ad Hoc Committee. (2001). Recommendations for the prevention and treatment of glucocorticoid induced osteoporosis. *Arthritis Rheum.* **44**, 1496–1503.
73. Adachi, J. D., Bensen, W. G., Brown, J., Hanley, D., Hodsman, A., *et al.* (1997). Intermittent etidronate therapy to prevent corticosteroid-induced osteoporosis. *N. Engl. J. Med.* **337**, 382–387.
74. Boutsen, Y., Jamart, J., Esselinckx, W., Stoffel, M., and Devogelaer, J. P. (1997). Primary prevention of glucocorticoid-induced osteoporosis with intermittent intravenous pamidronate: A randomized trial. *Calcif. Tissue Int.* **61**, 266–271.
75. Lakshminarayanan, S., Walsh, S., Mohanraj, M., and Rothfield, N. (2001). Factors associated with low bone mineral density in female patients with systemic lupus erythematosus. *J. Rheumatol.* **28**, 102–108.
76. Mankin, H. J. (1992). Nontraumatic necrosis of bone. *N. Engl. J. Med.* **326**, 1473–1479.
77. Dumont, M., Danais, S., and Taillefer, R. (1983). Doughnut sign in avascular necrosis of bone. *Clin. Nucl. Med.* 9–44.
78. Chang, C. C., Greenspan, A., and Gershwin, M. E. (1993). Osteonecrosis. Current prospective on pathogenesis and treatment. *Semin. Arthritis Rheum.* 23–47.
79. Nagasawa, W. A., Tsukamoto, K., Tada, Y., *et al.* (1994). Imaging study of the mode of development and changes in avascular necrosis of the femoral head in systemic lupus erythematosus: Long term observations. *Br. J. Rheumatol.* 333–346.
80. Saito, S., Saito, M., Nishina, T., Ohzono, K., *et al.* (1989). Long-term results of total hip arthroplasty for osteonecrosis of the femoral head. *Clin. Orthop.* **44**, 198–206.
81. Nishimura, T., Matsumato, T., Nishiro, M., and Tomita, K. (1997). Histopathologic study of veins in steroid treated rabbits. *Clin. Orthop.* 334–337.
82. Jilka, R. L., Weinstein, R. S., Bellido, T., Parfitt, A. M., and Manolagas, S. C. (1998). Osteoblast programmed cell death (apoptosis): Modulation by growth factors and cytokines. *J. Bone Miner. Res.* **13**, 793–802.
83. Manolagas, S. C., and Weinstein, R. S. (1999). Perspective: New developments in the pathogenesis and treatment of steroid induced osteoporosis. *J. Bone Miner. Res.* **14**, 1061–1066.

39

IMMUNOLOGY OF ANTI-PHOSPHOLIPID ANTIBODIES AND COFACTORS

Tatsuya Atsumi
Eiji Matsuura
Takao Koike

HISTORICAL BACKGROUND

Anti-phospholipid antibodies (aPLs) are present in a wide range of infectious and autoimmune diseases. aPLs, in particular anti-cardiolipin antibodies (aCL) and lupus anticoagulants (LA), are of considerable clinical importance because of the close association with predominant clinical features of venous and arterial thrombosis and pregnancy morbidity. The term antiphospholipid syndrome (APS) has been used to define this set of pathologic features. Recognition of this syndrome is now better understood worldwide as related clinical implications are now more well defined [1–4].

Anti-phospholipid antibodies were first discovered by Wassermann *et al.* [5] as part of the spectrum of antibodies produced in response to syphilitic infection. They found that sera from syphilitic patients could agglutinate a lipoid tissue extract. In the early 1940s, Pangborn [6, 7] identified the antigenic component in tissue extracts used in these tests as a novel anionic phospholipid, named cardiolipin, and the antibodies reactive to cardiolipin were termed reagins. Reagins in serum samples can be detected with the antigen mixture of cardiolipin, cholesterol, and phosphatidylcholine (Venereal Disease Research Laboratory, or VDRL antigen), resulting in a visible flocculation in *in vitro* assays. Although reagins are found predominantly in association with syphilitic infection, they are not specific to antigens of *Treponema palladium*. With the development of tests for treponema-specific antigens (*Treponema palladium* hemagglutination assay; TPHA), in the 1950s, it was observed that individuals with chronically false-positive serologic tests for syphilis (STS), when followed for many years, often developed systemic lupus erythematosus (SLE) [8–11]. These reports were confirmed later in two additional studies [12, 13] and some individuals with SLE and a chronic false-positive STS had recurrent spontaneous fetal loss, thrombocytopenia, and thromboembolic problems, when compared with age-matched SLE patients with a negative STS [8].

False-positive STS results were also noted in early reports of SLE patients with acquired inhibitors of *in vitro* phospholipid-dependent coagulation tests, in particular, the activation of prothrombin [14]. Laurell and Nilsson [11] investigated this association and suggested that the anticoagulant effect and the positive reaction with phospholipid antigen could be ascribed to the same causal factor.

The name lupus anticoagulants, coined by Feinstein and Rapaport [15] represents the first of the major misnomers in this field. These antibodies, which were designated LA because they were originally detected in the plasma of patients with SLE, are now more frequently present in persons who do not have this condition. Although LA prolong coagulation reactions *in vitro*, clinically they can cause thrombotic diseases [16] and individuals with LA do not have a tendency to bleed unless they have a second coagulation defect such as

factor VIII deficiency or thrombocytopenia. Thus, the anticoagulant activity was thought to be linked to an antibody that reacted with a lipid antigen. Against this background, more recent evidence supports that these antibodies are specific for phospholipid complexes with plasma proteins, such as β2-glycoprotein I (β2-GPI).

In the early 1980s, solid phase immunoassays were devised for detection of aPLs and the use of cardiolipin as a solid phase antigen was prompted by observations relating to the presence of the LA to frequent findings of a biological false-positive STS in patients with SLE and other autoimmune disorders. Radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) which directly detect circulating aCL were originally devised by Harris *et al.* [1] and Koike *et al.* [17], respectively. Early studies on the aCL assay demonstrated the requirement of additive bovine serum in the blocking buffer, diluent, or both, to enhance aCL binding to the targeted phospholipid. In 1990, three groups independently reported the requirement of a cofactor (i.e., β2-GPI) for autoimmune aCL binding to the solid phase phospholipid [18–20]. Subsequently, it was shown that the epitope for aCL develops when β2-GPI is adsorbed on polyoxygenated polystyrene plates. It was also shown that β2-GPI interacts with a lipid membrane composed of negatively charged phospholipids [21].

The remarkable range of antigenic specificities of aPLs includes other proteins of protein–phospholipid complexes such as prothrombin, annexin V (placental anticoagulant protein-I), protein S or protein C, and high- and/or low-molecular-weight kininogens [22–25]. In particular, prothrombin has been recognized as the "second" major antigenic target of autoimmune aPLs, and anti-prothrombin antibodies are responsible for LA activity in many cases with APS [22]. The expression of epitopes by at least some of these phospholipid-binding proteins does not depend on the presence of phospholipids. The spectrurm of potential immunogenic targets facilitates subclassification of these heterogeneous antibodies.

In 1998, an international consensus preliminary criteria for the classification of the APS was stated in Sapporo, thus called Sapporo-Criteria (Table 1) [26]. In this criteria, IgG/M β2-GPI dependent aCL and LA detected according to the guidelines of the International Society on Thrombosis and Hemostasis are listed to categorize "definite" APS. As markers of APS, other aPLs are still on discussion.

TABLE 1 Preliminary Criteria for the Classification of the Antiphospholipid Syndrome (Sapporo-Criteria)

Clinical criteria

1. Vascular thrombosis:
   One or more clinical episodes of arterial, venous, or small vessel thrombosis, in any tissue or organ. Thrombosis must be confirmed by imaging or Doppler studies or histopathology, with the exception of superficial venous thrombosis. For histopathologic confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
2. Pregnancy morbidity
   (a) One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th weeks of gestation, with normal fetal morphology documented by ultrasound or by direct examination of the fetus, or
   (b) One or more premature births of a morphologically normal neonate at or before the 34th week of gestation because of severe pre-eclampsia or eclampsia, or severe placental insufficiency, or
   (c) Three more unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic, or hormonal abnormalities and paternal and maternal chromosomal causes excluded.

Laboratory criteria

1. Anti-cardiolipin antibody of IgG and/or IgM isotype in blood, present in medium or high titer, on two or more occasions, at least 6 weeks apart, measured by a standard enzyme linked immunosorbent assay for β-glycoprotein 1-dependent anti-cardiolipin antibodies.
2. Lupus anticoagulant present in plasma on two or more occasions at least 6 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis.

Definite APS is considered to be present if at least one of the clinical and one of the laboratory criteria are met.

PHOSPHOLIPIDS

Phospholipids are compounds composed of 1, 2-diacylglycerol and a phosphodiester bridge linking the glycerol backbone to the nitrogenous bases of choline, ethanolamine, the inositol derivatives, and glycerol [27]. Cardiolipin is made up of two phospholipid molecules joined by a central glycerol molecule attached to the phosphodiester group. Phospholipids are abundant in the brain, spinal cord, and in body fluids such as plasma, but do occur primarily in various organelle and cellular membranes, including mitochondria, endothelial cells, and aggregated and/or activated platelets. These cells are an essential source of anionic phospholipids such as phosphatidylserine, provide a catalytic surface for proteins, and function as modulators of coagulation pathways. The distribution of phospholipids over the two halves of the cellular membrane bilayer is extremely asymmetric for both phosphatidylserine and sphingomyelin, the first being almost exclusively present in the inner monolayer, whereas sphingomyelin is confined to the outer monolayer [28]. Phosphatidylcholine and phosphatidylethanolamine are distributed more

evenly although phosphatidylcholine seems to have a preference for the outer monolayer and phosphatidylethanolamine for the inner leaflet. The result of this distribution is that the outer surface of cells is in general about 80% composed of phosphatidylcholine, the remainder being occupied by phosphatidylethanolamine. The inner monolayer of the membrane contains virtually all the phosphatidylserine, phosphatidylinositol, phosphatidic acid, and a large fraction of phosphatidylethanolamine, resulting in an almost exclusive location of negatively charged phospholipids in the inner monolayer of the cellular membrane. However, in particular circumstances (i.e., ongoing apoptosis, cell activation, or cell injury) an enhanced loss of phospholipid asymmetry can occur. The phenomenon of scrambling of phospholipids was noted in blood platelets activated by the combined action of the physiologic activators collagen and thrombin.

ANTI-PHOSPHOLIPID ANTIBODIES (aPLs) AND THEIR COFACTORS

Numerous studies have elucidated the specificity of anti-phospholipid antibodies (aPLs). It is clear that the nomenclature of aPLs is a misnomer and that these autoantibodies react with phospholipid-binding plasma proteins (cofactors), such as β2-glycoprotein I (β2-GPI), prothrombin, annexin V, high-molecular-weight kininogen, protein S, and protein C.

Anti-cardiolipin Antibodies (aCLs) and β2-Glycoprotein I (β2-GPI)

Antibodies against β2-GPI

Anti-cardiolipin antibodies (aCLs) present in APS patients can be detected using immunoassays and solid phase cardiolipin as a putative antigen [4, 17]. However, antibodies directed against phospholipid-binding plasma or serum proteins (i.e., cofactors), in particular β2-GPI, are present in serum samples and can be components of a sample diluent and blocking buffer in various types of immunoassay systems. Many studies indicated that one of the predominant antibodies considered to be aCL in APS patients is that against β2-GPI, rather than any of the negatively charged phospholipids such as cardiolipin, phosphatidylserine, phosphatidic acid, and phosphatidylinositol [18–21, 29–35. In contrast, aPLs associated with infectious diseases bind directly to these negatively charged phospholipids [21, 33–35]. Anti-β2-GPI autoantibodies recognize a cryptic epitope on the β2-GPI molecule when β2-GPI interacts with a lipid membrane composed of negatively charged phospholipids or when β2-GPI is adsorbed on a polyoxygenated polystyrene plate treated with γ-irradiation or electrons [21, 29, 30]. With this treatment, oxygen atoms such as C—O and C═O are covalently introduced onto the surface of polystyrene plates, and epitopes recognized by autoimmune anti-β2-GPI antibodies appear [21]. Consistent with this evidence are reports showing that aPLs bind to β2-GPI adsorbed on various commercially available oxidatively modified polystyrene plates [36] or on a nitrocellulose membrane or on an experimentally γ-ray/UV-irradiated polystyrene plates [37] and that an epitope for aPLs is exposed by the β2-GPI molecule modified with glutaraldehyde [38].

Several groups timely reported that anti-β2-GPI autoantibodies in APS patients have a relatively low affinity, which was demonstrated by using IgG Fab′ of the autoantibodies, dimerized β2-GPI molecules which are formed via a disulfide bridge between two mutated domain Vs or between two apple 4 domains fused to the N-terminus of domain I [31, 32, 39].

The epitopic location on β2-GPI for anti-β2-GPI autoantibodies from APS patients is still controversial. In 1996, Igarashi *et al.* first reported that domain IV or I are candidates for epitopic location in the β2-GPI molecule, by using a series of deletion mutant proteins of β2-GPI [30]. Later, George *et al.* [40] demonstrated that domain IV of β2-GPI is one of the major epitopic location for aCL raised in APS patients, using the deletion mutant proteins. In contrast, Iverson *et al.* [41] and McNeeley *et al.* [42] reported that anti-β2-GPI autoantibodies in the major population of APS patients recognized a particular structure in domain I of β2-GPI and antibody binding was diminished by replacement of a related amino acid located in the domain [41, 42]. However, it was also reported that some particular mutations made in domain IV also affected the antibody binding to β2-GPI in anti-β2-GPI ELISA [43].

Nature of a Target Antigen (β2-GPI)

β2-GPI, a 50-kDa protein with a carbohydrate content of 17%, is present in normal human plasma at approximately 200 μg/ml and was first described by Schultze *et al.* in 1961 [44] as a perchloric acid soluble human plasma protein with an unknown function. Northern blots revealed that the polypeptide is mainly synthesized in the liver but also in various cells, as described later. After eliminating sialic acid from the molecule, multiple isoelectric subspecies were identified.

Because β2-GPI is present in the lipoprotein fractions, such as chyromicron, VLDL, and HDL, with ultra-

centrifugation and activated lipoprotein lipase *in vitro*, β2-GPI is also designated apolipoprotein H. β2-GPI binds to various negatively charged substances such as phospholipids, heparin, lipoproteins, and activated platelets and inhibits the intrinsic blood coagulation pathway, prothrombinase activity, and adenosine diphosphate (ADP)-dependent platelet aggregation [45–50].

The complete amino acid sequence of human β2-GPI, as determined by peptide sequencing, reveals a single polypeptide chain composed of 326 amino acid residues with five oligosaccharide attachment sites [51, 52]. The complete nucleotide sequence and the deduced amino acid sequence were defined by cDNA cloning from human liver-originated cells (e.g., a hepatoma cell line (HepG2)), and sequencing [53, 54]. β2-GPI is composed of five homologous motifs of approximately 60 amino acids and which contain highly conserved cysteins, prolines, and tryptophans (Fig. 1). The motif is characterized by a framework of four conserved half-cysteine residues related to the formation of two internal disulfide bridges. These repeating motifs were designated as short consensus repeats (SCR), complement control protein repeats (CCP), or sushi domains. However, the fifth domain (the carboxyl terminus, domain V) of β2-GPI is a modified form that contains 82 amino acid residues and six half cysteines. Table 2 lists proteins of this superfamily together with the number of SCRs units. Proteins listed include β2-GPI, C4 binding protein, complement receptor I (CD35), complement receptor 2 (CD21), decay accelerating factor (CD55), factor H, factor I, factor XIII, and haptoglobin. However, there are no reports as to whether or not β2-GPI is involved in the complement regulation [55].

TABLE 2 Short Consensus Repeat (SCR) Superfamily

Protein	Number of SCR units
β_2-glycoprotein I (apolipoprotein H)	5
C4 binding protein	8
Complement receptor 1 (CD35)	30
Complement receptor 2 (CD21)	16
Decay accelerating factor (CD55)	4
Factor H	20
Factor I	1
Factor XIII	10
Haptoglobin	2

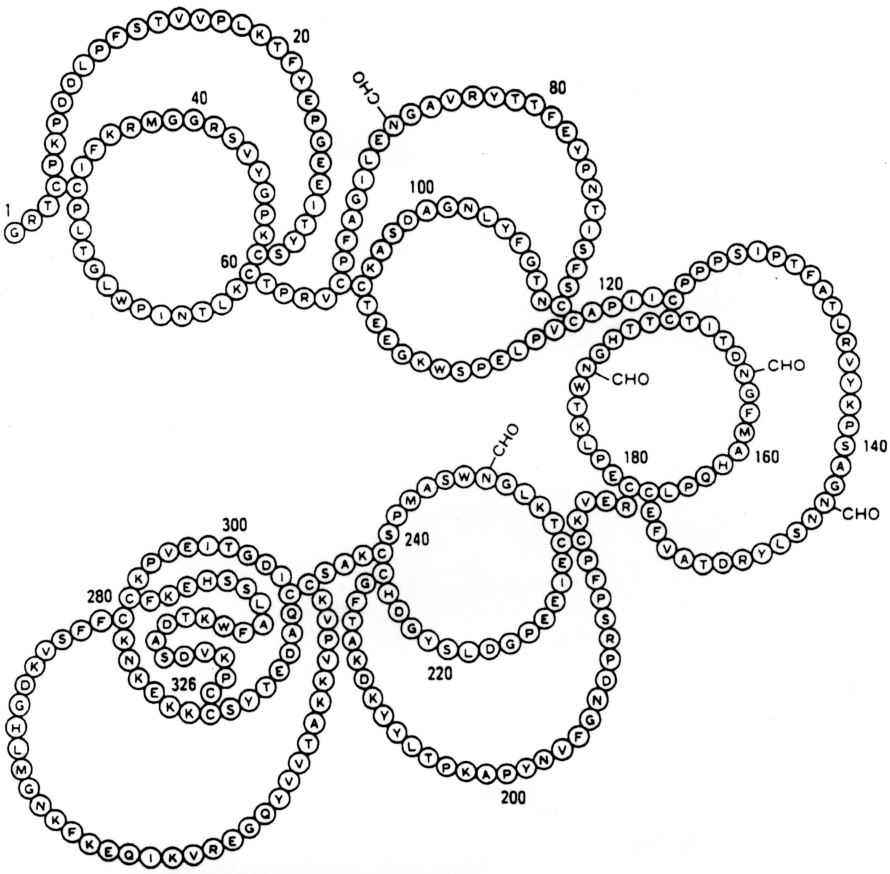


FIGURE 1 Amino acid sequence and location of disulfide bonds in human β2-GPI.

In 1999, two individual groups crystallized human β2-GPI and characterized its tertiary structure [56, 57]. The structure of β2-GPI, which is a highly glycosylated protein, reveals an elongated fishhook-like arrangement of the globular short consensus repeat domains (Figs. 2 and 3). Both of these crystal analyses consistently indicate that half of domain V deviates strongly from the standard fold, as observed in domain I to IV.

β2-GPI binds to solid phase phospholipids through a particular region in the fifth domain, C^{281}KNKEKKC^{288} and the phospholipid binding property is significantly diminished by cleavage of one particular site in the fifth domain of β2-GPI (i.e., K317 and T318) [35, 58–61]. These structural studies on the crystallized β2-GPI indicated that the aberrant half forms a specific phospholipid-binding site. A large patch of 14 positively charged amino acid residues provides electrostatic interactions with anionic phospholipid headgroups and an exposed membrane-insertion loop yields specificity for lipid layers. However, electron density corresponding to the loop, S^{311}-K^{317} is not visible due to high mobility (flexibility) in one of these two studies on the crystallized β2-GPI (Fig. 4) [56]. Further, Hoshino *et al.* [62] determined the tertiary structure of domain V and the result showed that the molecule is composed of well-defined four antiparallel β-strands and two short α-helices, as well as a long highly flexible loop. Thus, the positive charges on the domain interact with anionic phospholipid headgroups and the flexible loop of the sequence, S^{311}-K^{317}, specifically interacts with hydrophobic molecules [63] and putatively inserts into the lipid layer, thereby anchoring the protein molecule to the membrane [56]. Further, it is also known that the interaction of β2-GPI alters the fluidity and polarlization of the target membranes [64].

Hagihara *et al.* [65] reported that the cleavage is weakly produced by factor Xa. Later, the same research group showed that the site-specific cleavage was specifically produced by plasmin treatment [66]. Matsuura *et al*. attempted to predict tertiary structures of intact and nicked (cleaved) domain V of β2-GPI, through protein modeling and analysis on epitopic study on an anti-β2-GPI monoclonal antibody, which is specific to the intact β2-GPI using phage library technology [67]. These data suggested that the two flanking cysteines were important for peptide conformational presentation of the fifth domain, when binding to phospholipids. In contrast, the pathophysiologic implication of plasma/serum levels of the nicked β2-GPI has been discussed. Brighton *et al.* [68] first found that β2-GPI levels are significantly low in patients with thrombosis or disseminated intravascular coagulation syndrome (DIC). Horbach *et al.* [69] reported that plasma β2-GPI levels are lower in patients with DIC as compared with controls and the nicked β2-GPI levels were contrarily high. They suggested that the reduction of β2-GPI levels and the generation of the nicked β2-GPI occurred due to absorption by apoptotic cells and activation of fibrinolysis, respectively. Further, Itoh *et al.* [70] reported highly increased plasma levels of the nicked β2-GPI in patients with leukemia and with lupus anticoagulant. Cleavage of β2-GPI which leads to its reduced antigenicity may play a role in a natural self-defense against autoimmunity and the development of thrombosis in APS; however, the clinical significance of the cleavage should be elucidated in future studies.

β2-GPI is expressed in liver and hepatocyte cell lines, including HepG2 and PLC/PRF/5 [54, 71]. Transcription of β2-GPI apparently does not occur in kidney, liver, or the cell lines HeLa, Molt-4, Raji, and BSM, but the mRNA for β2-GPI has been identified in extracts of villous placental tissue, the choriocarcinoma cell lines, that is, Jeg-3, BeWo, and Jar, endothelial cells, astrocytes, and neurones [54, 72, 73]. β2-GPI has been immunohistochemically localized to the syncytiotrophoblasts and extravillous cytotrophoblasts of both normal term placenta and placenta from pregnancies affected by aPLs [73, 74].

Genetically determined structural variation in the β2-GPI molecule has been identified by isoelectric focusing and by immunoblotting [75]. APOH*2 is the most common allele followed by APOH*1 and APOH*3 alleles in all racial groups [76]. Further, the relationship between β2-GPI polymorphism and its plasma levels were reported [77]. The molecular basis of β2-GPI protein polymorphism (codons at 88, 247, 306, and 316) and its distribution in large U.S. populations, non-Hispanic Whites, Hispanics, Blacks, and/or Japanese has been reported [78–81]. In addition, complete β2-GPI deficient individuals were reported by our group. Analysis of their β2-GPI genes revealed that a thymine corresponding to position 379 of the β2-GPI cDNA was deleted (β2-GPI-Sapporo), hence, a frame shift would occur and this would make the gene code for an amino acid sequence unrelated to β2-GPI beyond this position [82].

Megalin/gp330, is an endocytic receptor that internalizes multiple ligands including apolipoprotein E and B100. Megalin is the main antigenic target in passive Heymann nephritis, where it binds circulating autoantibodies, leading to the formation of subepithelium immune deposits. In 1998, it was reported that megalin is a receptor for a β2-GPI molecule and a β2-GPI–phospholipid complex [83].

Animal Models of APS and β2-GPI-Knockout Mice

In NZW × BXSB (WB) F1 male mice with systemic lupus-like disease, several autoantibodies, circulating

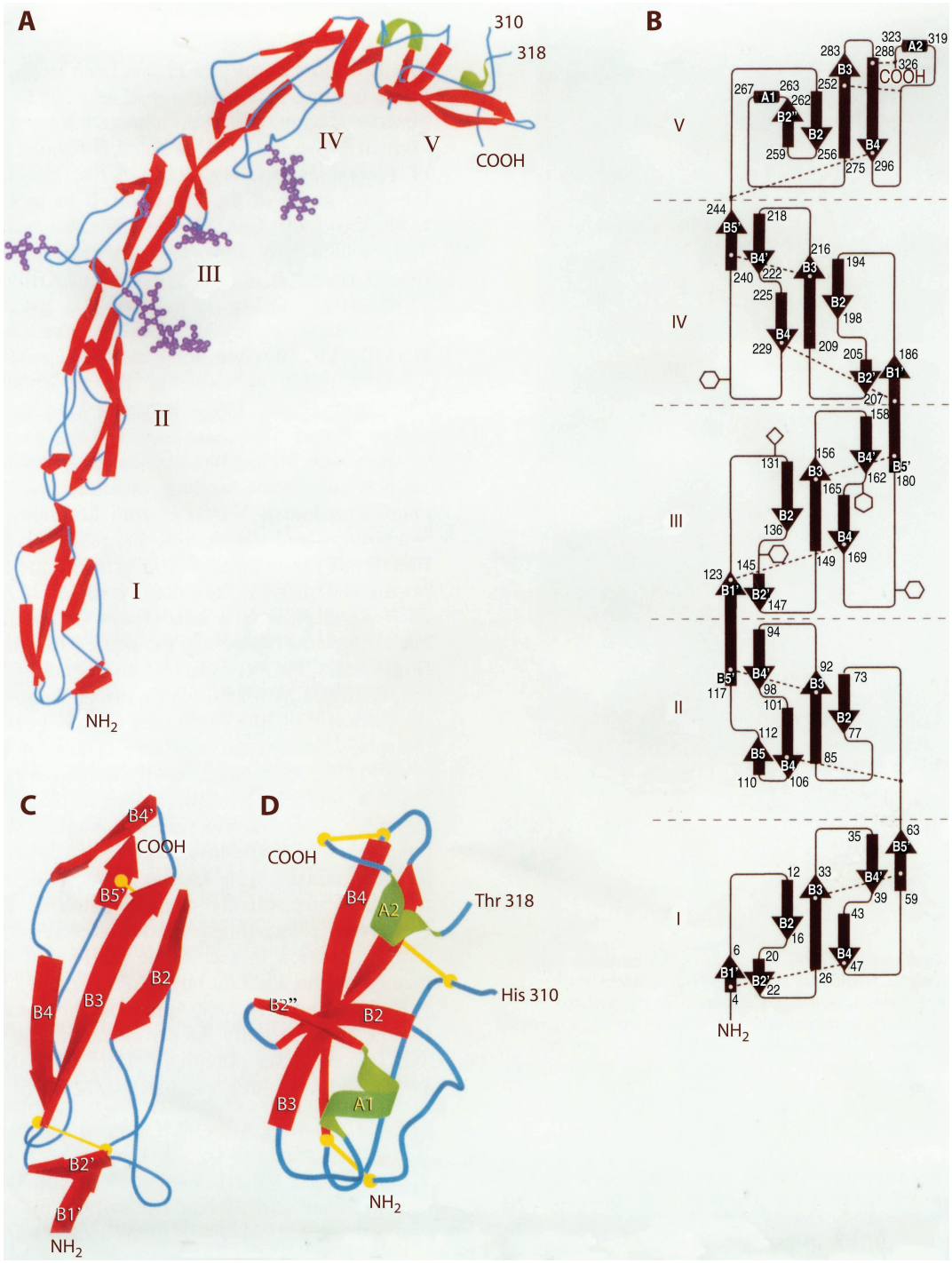


FIGURE 2 Structure representations of human blood plasma β2-GPI revealing the extended chain of the five SCR domains. (**A**) Ribbon drawing of β2-GPI with consecutive domains labeled I–V. (**B**) Topology gram of β2-GPI. (**C**) Ribbon representation of domain III of β2-GPI with labeled secondary structure elements. (**D**) Ribbon representation of domain V of β2-GPI with labeled secondary structure elements. (*EMBO J*. **18**, 5175, 1999.)

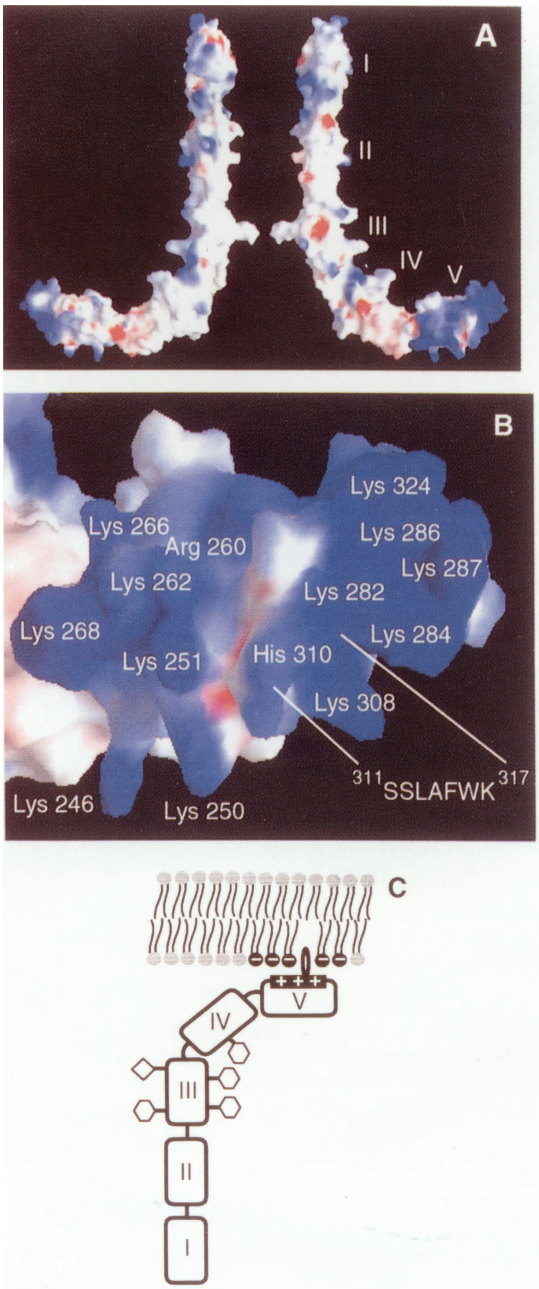


FIGURE 3 Structure of human β2-GPI and model of β2-GPI and phospholipids. (**A**) Two views, related by 180° rotation of the electrostatic potential surface of β2-GPI. (**B**) Positively charged patch on the aberrant half of domain V. (**C**) Diagram of the proposed model for binding of β2-GPI to acidic phospolipids. (*EMBO J*. **18**, 5175, 1999.)

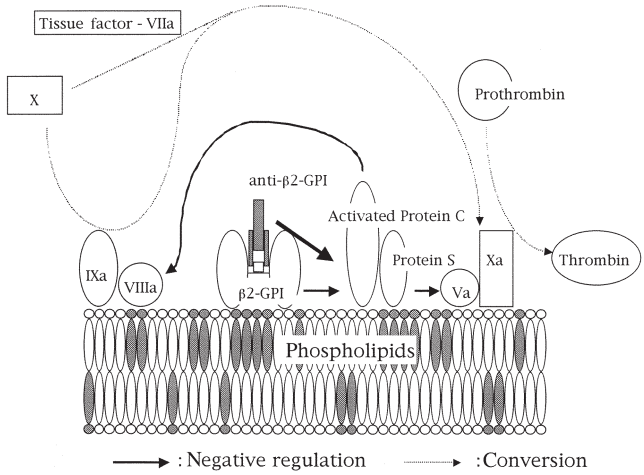


FIGURE 4 Regulation of thrombin generation by protein C system and its inhibition by anti-β2-GPI antibody.

immune complexes, and lupus nephritis are present [84, 85]. Myocardial infarction is the hallmark in the WB F1 male mice [84, 86]. Yoshida *et al.* [87] reported that the incidence of myocardial infarction in these mice increased with age and that over 80% of male WB F1 mice with myocardial infarction had small multiple infarctions in the right ventricular free wall and anterior, posterior, and septal ventricular walls. In the affected coronary arteries the intimate thickening consisted of cellular components with frequent recanalization or an eosinophil thrombus-like substance. Thrombocytopenia is a frequent finding in patients with aPLs. WB F1 mice also develop thrombocytopenia with age, and platelet-associated antibodies and circulating anti-platelet antibodies are present. The mechanism by which thrombocytopenia occurs is unknown.

Hashimoto *et al.* [88] were the first to report that WB F1 male mice produced autoantibodies against cardiolipin and that the titer of aCL increases with age. These antibodies were detected by conventional aCL ELISA [17], and alternatively could be detected by ELISA for anti-β2-GPI antibody detection, using β2-GPI complexed with solid phase cardiolipin [34] or using β2-GPI coated on a polyoxygenated polystyrene plate as an antigen [21].

As another approach to establish the animal model of human APS, active immunization either with aCL, β2-GPI, and a combination of anti-β2-GPI antibodies and β2-GPI were considered. It was suggested that immunization of animals with human β2-GPI induces antibodies that resemble aPLs from APS patients, regarding binding characteristics [89–91] and pathogenic properties [92]. However, the precise β2-GPI specificity of human aPLs requires more intensive investigation.

More recently, by using a homologous recombination approach, β2-GPI-knockout mice were generated [93]. When β2-GPI heterozygotes on a mixed genetic background were intercrossed, only 8.9% of the resulting 336 offspring possessed both disrupted alleles. They possessed an impaired *in vitro* ability to generate thrombin

relative to wild-type mice. Thus, β2-GPI appears to play an important role in thrombin-mediated coagulation.

Monoclonal Antibodies

Five human monoclonal antibodies (IgM) against β2-GPI have also been prepared from peripheral blood lymphocytes of three British patients with APS; sequential Epstein-Barr virus transformation and cell fusion techniques were used [94]. These antibodies bind both to the solid phase complexes of β2-GPI and negatively charged phospholipids and to β2-GPI adsorbed on a polyoxygenated polystyrene surface. All monoclonal antibodies obtained from WB F1 mice (namely, WB-CAL-1) and from APS patients have similar characteristics to those found in sera from APS patients. More recently, it was demonstrated that the WB-CAL-1 antibody or four out of six monoclonal anti-β2-GPI antibodies from patients with APS bind to a deletion mutant of human β2-GPI lacking domain V (DM I-IV) produced in a baculovirus/Sf9 expression system [30, 88, 94, 95]. However, the binding is significantly diminished by further removal of domain IV from DM I–IV. Further, as described previously, particular mutations made in domain IV also affected antibody binding to β2-GPI in anti-β2-GPI ELISA [43]. Thus, domain IV has an important role in expressing the epitope(s) recognized by many (if not most) anti-β2-GPI antibodies from APS patients. A cluster of discontinuous hydrophobic amino acids from domain IV was suggested to be a possible epitope recognized by anti-β2-GPI antibodies present in APS patients. The analyses were made using a phage displayed random peptide library and three-dimensional protein models [43]. In contrast, Iverson *et al.* [41] and McNeeley *et al.* [42] reported that anti-β2-GPI autoantibodies in the major population of APS patients recognized a particular structure in domain I of β2-GPI, and antibody binding was diminished by replacement of a related amino acid located in the domain.

In 1999, a chimeric antibody (namely, HCAL) with the human γ1 constant region as a putative standard for assays to detect IgG anti-β2-GPI antibodies was established [96]. The binding of HCAL was compared with the binding of standards for IgG aCL and anti-β2-GPI antibody assays done in 18 independent laboratories. The reactivity of HCAL is similar to the reactivity to standards for IgG aCL or anti-β2-GPI antibody assays done in collaborative laboratories.

T-Cell Clones Specific for β2-GPI

HLA class II-restricted β2-GPI-reactive $CD4^+$ T cells were identified in the peripheral blood of APS patients [97]. These β2-GPI-reactive T cells possess helper activity that induces the production of antibodies that specifically bind to β2-GPI immobilized on cardiolipin. Further, the same group has established β2-GPI-specific $CD4^+$ T-cell clones and investigated their antigen recognition profiles and helper activity involved in promoting anti-β2-GPI antibody production through IL-6 expression and CD40–CD40 ligand engagement [98]. Interestingly, in the study, the major T-cell clones which responded to a peptide encompassing amino acid residues 276 to 290 of β2-GPI, that contains the phospholipid binding site, were established.

Methodology for Antibody Detection

Detection of anti-β2-GPI antibodies in patients' sera is important for the diagnosis of APS. Such pathogenic anti-β2-GPI autoantibodies recognize an epitope of β2-GPI that appears only when β2-GPI binds to a lipid membrane composed of negatively charged phospholipids or when β2-GPI is adsorbed onto a polyoxygenated polystyrene plate. Anti-β2-GPI antibodies can be routinely detected by ELISA using two types of antigen solid phase: one is β2-GPI associated with a cardiolipin-coated polystyrene plate and the other β2-GPI adsorbed on a polyoxygenated polystyrene plate [21].

In assays using a cardiolipin-coated plate, a control assay without β2-GPI is of particular importance, and antibody binding must be compared in the absence and/or the presence of β2-GPI to evaluate the β2-GPI dependency of antibodies [20, 33, 34, 99, 100]. Antibodies specifically or nonspecifically reactive to phospholipids in sample fluids may directly bind to a cardiolipin-coated plate, even if the plate is first or simultaneously treated with β2-GPI. Direct binding of aPLs to a cardiolipin-coated plate is dose-dependently decreased by the addition of β2-GPI but is not completely diminished even when adding a large excess of β2-GPI [34]. The latter anti-β2-GPI ELISA, with use of a polyoxygenated plate, is much superior, procedures are simple and a false-positive result for aPLs is negligible. Monoclonal anti-β2-GPI antibodies, such as EY2C9 (human IgM) [94] and HCAL [96], have been proposed as international standards of calibration materials by different standardization committees.

Lupus Anticoagulant (LA) and Its Cofactors

History and Definition of LA

Circulating anticoagulants are defined as endogenously produced substances that may interfere with coagulation either *in vivo* or *in vitro* [101]. Most of such

inhibitors are immunoglobulins. Other inhibitors such as elevated levels of fibrin split products are rare. Lupus anticoagulants (LA) are those immunogloblins (IgG, IgM, IgA, or their combination) that interfere with *in vitro* phospholipid-dependent tests of coagulation (prothrombin time (PT), activated partial thromboplastin time (aPTT), kaolin clotting time (KCT), dilute Russell's viper venom time [dRVVT]) [102]. LA was first described (1954) in lupus patients with bleeding tendency [14], thus it was called LUPUS anticoagulant, but this term is a misnomer because the vast majority of patients with LA do not have SLE. The paradoxical relationship between LA and a predisposition to thrombosis was first identified in 1963 [16] and more recent studies clearly identified LA as a factor for both venous and arterial thromboembolic events [103]. In 1980, Thiagarajan *et al.* [104] reported that monoclonal IgM antibody with LA activity cross-reacted with anionic phospholipids, and they proposed that LA binds to anionic but not dipolar ionic phospholipids, thereby limiting the exposed surface required for binding the prothrombinase complex [105]. The *in vitro* action of LA appears to inhibit, albeit at a slow rate, the prothrombinase complex, which consists of factor Xa and Va (Xa/Va complerx) and prothrombin with Ca^{2+} and phospholipids [106].

Cofactors of LA

There are some subtypes of LA classified according to their clotting inhibitory behavior. In 1978, Exner *et al.* [107] reported that there are three types of LA mixing patterns by KCT. In 1990, β2-GPI was identified as a cofactor of solid-phase aCL assay, as described. After the finding, cofactors of LA have been also investigated. Oosting *et al.* [108] and Roubey *et al.* [109] demonstrated that many LA plasmas depended on the presence of β2-GPI in their anticoagulant activity, thus β2-GPI was identified as one of the major cofactors of LA. β2-GPI dependent LA is highly likely to comprise antibodies directed at β2-GPI (anti-β2-GPI antibodies), but many patients with anti-β2-GPI antibodies did not have LA. Takeya *et al.* [110] showed the heterogeneity of LA activity in a series of human/murine monoclonal anti-β2-GPI antibodies. Thus anti-β2-GPI antibodies can be either antibodies with LA activity or those without, and anti-β2-GPI antibodies with LA activity were assumed to be more pathogenic than anti-β2-GPI antibodies without LA [111]. On the other hand, murine monoclonal anti-human β2-GPI antibodies behaved as LA [112], suggesting that not only autoantibodies but also immunized antibodies can have LA activity and that antibody binding to β2-GPI is not necessarily related to the presentation of cryptic/neoepitopes on the molecule.

β2-GPI has only low affinity for phospholipid surfaces, compared with clotting factors [113]. As LA and/or its cofactors compete with coagulation factors for the available catalytic phospholipid surface, it is assumed that anti-β2-GPI antibodies with LA activity enhance the affinity of β2-GPI for phospholipids that have potent competitive reactivity with clotting factors for the catalytic surface. Williams *et al.* [114] reported, using ellipsometry, that high-affinity binding of aCL–β2GPI complex (purified from a single patient's serum) to phospholipids was due to the consequence of bivalent interactions between them and lipid bound β2-GPI. By the solid phase ELISA technique, Takeya *et al.* [110] showed that murine monoclonal anti-β2-GPI antibodies enhanced the β2-GPI binding to fixed phospholipids on ELISA plates. They demonstrated that the Fab′2 fragment of the monoclonal anti-β2-GPI antibody had also promoted the enhancement while the Fab fragment did not, confirming the significance of the bivalency. Using real-time biospecific interaction analysis based on plasmon surface resonance technology, Arnout *et al.* [115] found a high affinity interaction between β2-GPI and phospholipids in the presence of murine monoclonal anti-β2-GPI antibody with LA activity, but not in the presence of monoclonal anti-β2-GPI antibodies without LA activity. Fab' fragments inhibited the formation of stable high affinity complexes on phospholipid surfaces. Arnout *et al.* noted that bivalency may be essential to cross-link two β2-GPI molecules and induce a correct spatial orientation of the phospholipid binding domains of both β2-GPI molecules, thus markedly increasing their affinity for phospholipid surface.

Bevers *et al.* [22] highlighted the importance of prothrombin in causing LA activity when they studied 16 patients with both aCL and LA. After incubation with cardiolipin-containing liposomes and removal of aCL (anti-β2-GPI antibodies), the LA activity remained in the supernatant in 11/16 patients. These 11 samples demonstrated LA activity in a phospholipid-bound prothrombin dependent fashion. Therefore, prothrombin was considered as a second cofactor of LA activity. Unlike β2-GPI, the cofactor effect of prothrombin was highly species-specific and bovine prothrombin failed to support the inhibitory activity of LA [116].

It was suggested that anti-prothrombin antibodies prolong *in vitro* clotting time by inhibiting the conversion from prothrombin into thrombin [117]. One can assume that anti-prothrombin antibodies bind near the cleavage site on the prothrombin molecule by the prothrombinase complex and hamper the thrombin generation. It is not very likely, however, as the phenomena do not explain the correction of the clotting time prolongation by the excess of phospholipids.

Horbach and co-workers [118] reported that purified anti-prothrombin antibodies not only inhibited the prothrombinase activity but also the tenase complex and the inactivation of factor Va by activated protein C. The results may suggest that anti-prothrombin antibodies increase the affinity of prothrombin for phospholipids so that prothrombin–anti-prothrombin antibody complexes compete with the binding of other coagulation factors on phospholipids surface [119], the mechanism being similar to that described for complexes of β2-GPI and anti-β2-GPI antibodies. However, Galli *et al.* [120] demonstrated the dominant inhibitory effect on activated protein C of the anti-β2-GPI antibodies, compared with that of anti-prothrombin antibodies, thus the phenomena may depend on the specificity of antibodies used in the experiments.

Apart from those two major LA cofactors, some other phospholipid binding proteins, such as high- and/or low-molecular-weight kininogen, annexin V, protein C, and protein S, have been proposed as cofactors of LA [121]. However, the roles of these potential cofactors in LA activity are obscure.

Methods for LA Detection

The Scientific and Standardisation Committee of the International Society of Thrombosis and Haemostasis established a subcommittee of lupus anticoagulants/anti-phospholipid antibodies. In 1995, the subcommittee recommended a diagnosis of LA [122] (Table 3). The steps seem relatively simple, but the identification of LA is often difficult because of its highly heterogeneous properties that are due to the difference of isotypes, avidity, or properties of directed antigens. Also there are a number of technical issues which may significantly compromise the diagnosis [123]. Platelets in frozen/thawed samples lead to false-negative LA result, known as platelet neutralization phenomenon [124], thus platelet count in plasma samples should be less than 10,000/μl by double-centrifuging and/or 0.22-μm filtrating [123].

The screening stage of LA detection must be as sensitive as possible. Unfortunately, none of the LA tests have 100% sensitivity, thus a combination of phospholipid-dependent coagulation tests should be performed as the frontline investigation. The choice of the coagulation tests and of the reagents are crucial for sensitivity to detect LA. Lesperance *et al.* [125] ranked tests in order of sensitivity, KCT > tissue thromboplastin inhibition test (TTI) > APTT > dRVVT, whereas Forastiero *et al.* [126] used APTT = dRVVT > KCT. Pengo *et al.* [127] demonstrated that dRVVT was more sensitive, to detect β2-GPI associated LA, than KCT or TTI. These confusing results can be simply attributed to differences in methodology [128]. For the standardization of LA, Arnout *et al.* [112, 129] distributed monoclonal anti-human β2-GPI antibody with LA activity to several laboratories and compared the LA results, showing that LA responsiveness varied largely according to the reagents used. A population of tested samples can also result in the discrepant results found in LA sensitivity. Galli *et al.* [130] showed that KCT is more sensitive for β2-GPI-dependent LA whereas dRVVT is more sensitive for prothrombin-dependent LA. Thus, when LA test sensitivity is investigated using a β2-GPI-dependent LA dominant population, KCT would be more sensitive than dRVVT, and vice versa.

The performance of mixing tests usually provides evidence of an inhibitor. Normal plasma is added to the patient's plasma and will correct coagulation deficiencies so that the prolonged clotting time will return to normal. If an inhibitor is present, the clotting time will remain prolonged or even may be further prolonged. In case of weak or low titer LA, however, the immunoglobulins responsible for LA are diluted in this procedure, leading to the loss of detectable LA activity and a false-negative result. Confirmation of the phospholipid-dependent nature of the inhibitor is obtained using activated platetets, which have negatively charged phospholipids on their surface, or high concentration of phospholipids, demonstrating correction of the coagulation time. A variety of screening tests and correction tests are listed in Table 3 [131].

There currently is no consensus about which test is the "ideal LA test." Scientists need to establish specific LA tests in their laboratories using quality control plasma and an appropriate set of healthy samples to determine the normal limits of clotting time. Some kits for LA determination are now commercially available and they have gained popularity worldwide because of their convenience.

TABLE 3 Diagnosis of Lupus Anticoagulant (SSC of ISTH)

Step 1: Demonstration of a prolonged phospholipid-dependent coagulation screening test(s) (e.g., APTT, KCT);
Step 2: Failure to correct the prolonged screening coagulation test upon mixing with normal platelet poor plasma;
Step 3: Shortening or correction of the prolonged screening test value upon the addition of excess/or reconfigured phospholipid; and
Step 4: Rule out other coagulopathies (such as factor VIII inhibitor, heparin)

Other APS-Related Autoantibodies

Anti-Prothrombin Antibodies

As described earlier, prothrombin was identified as one of the cofactors of LA, thus autoantibodies against prothrombin should be present in patients with APS.

The first techniques used for screening anti-prothrombin antibodies were double diffusion and cross-immunoelectrophoresis [132–135]. Other assays are based on the impairment of prothrombin activation by anti-prothrombin antibodies [24, 136]. However, none of these techniques is suitable for the routine evaluation of large numbers of patients with APS. In 1995, Arvieux *et al.* [137] showed that anti-prothrombin antibodies could be detected using a standard ELISA, prothrombin coated onto irradiated plates (the aPT system). Interestingly, the mode of presentation of prothrombin in solid phase seems to influence its recognition by anti-prothrombin antibodies. Anti-prothrombin antibodies cannot be detected when prothrombin is immobilized onto nonirradiated plates [137] but binding is observed to prothrombin on a suitable anionic surface, adsorbed on γ-irradiated plates or exposed to immobilized anionic phospholipids.

Galli *et al.* [138] showed that prothrombin was recognized more efficiently when the prothrombin was bound to phosphatidylserine-coated ELISA plates using calcium ions, thus anti-prothrombin antibodies could be detected by the phospholipid-bound system in which antibodies were directed against phosphatidylserine–prothrombin complex (the aPS/PT system). Analogy between the behavior of anti-prothrombin antibodies and that of anti-β2-GPI antibodies has been hypothesized. Anti-prothrombin autoantibodies may be directed against cryptic epitopes or neoepitopes that are exposed when prothrombin binds to anionic phospholipds and/or may act as low-affinity antibodies that bind bivalently to immobilized prothrombin. Unlike anti-β2-GPI antibodies or other autoantibodies, however, a high percentage of anti-prothrombin antibodies has species-specificity for the human protein [22] and a minority of anti-prothrombin antibodies reacts with bovine prothrombin [116]. The epitope(s) recognized by anti-prothrombin antibodies have not yet been defined. Some studies suggested that dominant epitopes are likely to be located near the phospholipid-binding site of the prothrombin molecule, although they may have heterogeneous distribution [119, 139].

Studies were done to investigate the clinical significance of anti-prothrombin antibodies detected by ELISA using the aPT system. Some studies have shown their positive correlations with the clinical manifestations of APS [140–142]. In a population of nonautoimmune setting, it has been reported that high levels of anti-prothrombin antibodies confer high risk of myocardial infarction or cardiac death in middle-aged men [143] and thrombotic events [144]. Conversely, other investigators have found no statistically significant correlations between the presence of anti-prothrombin antibodies and manifestations of APS [138, 145–149]. Therefore, the value of anti-prothrombin antibodies as markers of APS has been controversial.

In contrast, anti-prothrombin antibodies detected by the aPS/PT system correlated strongly with the presence of LA and with the clinical manifestations of APS [150]. Most patients with aPS/PT were positive for LA. Many investigators have tested only anti-prothrombin antibodies using the aPT system, presuming that aPT and aPS/PT systems detect the same antibodies. However, no correlation of the titers was found between aPS/PT and anti-prothrombin antibodies in patients with APS or other autoimmune diseases [150]. Thus, aPS/PT represents one of the markers of APS, and testing aPS/PT may help to confirm the presence of LA.

Anti-Annexin V Antibodies

Annexin V (previously known as placental anticoagulant protein I and vascular anticoagulant α) is found in the placenta and in the vascular endothelium, among other tissues [151]. This protein, whose physiologic function remains to be established, has potent anticoagulant properties that are based on its capacity to displace coagulation factors from phospholipid surfaces [152]. It was reported that annexin V is present on the apical surface in placental syncytiotrophoblasts and that levels of this protein are markedly reduced on placental villi in patients with APS [153, 154]. Therefore, it has been thought that annexin V has an antithrombotic role *in vivo* and that the thrombosis in APS patients may be due to reduced levels of the protein at sites where circulating blood contacts cells lining the vasculature. Some reports show the presence of anti-annexin V autoantibodies [23, 155–158], but other aPLs may also affect the annexin-V function via their antigens [159, 160].

Anti-phosphatidylethanolamine Antibodies and Their Cofactors

Phosphatidylethanolamine (PE) is a zwitterionic phospholipid present in the outer leaflet of cell membranes, which has an important role in phospholipid-dependent reactions of protein C system [161]. Although autoantibodies against PE were detectable in some populations of patients [162, 163], their association with thrombosis or LA were not confirmed [164, 165].

Sugi and McIntyre [25, 166] demonstrated that the PE-binding proteins responsible for anti-PE antibodies were high-molecular-weight kininogen (HMWK) and low-molecular-weight kininogen (LMWK) and that anti-PE antibodies recognized HMWK and LMWK

only when presented on PE substrate. Therefore, kininogen was recognized as one of cofactors of aPLs. McIntyre and Wagenknecht [167] stated that fetal and newborn bovine serum should not be used as a blocking agent or in a patient serum diluent because kininogen is present in significantly lower concentration in fetal/new born bovine serum compared with adult bovine serum [168]. More recent studies have shown a preliminary clinical significance of kininogen-dependent anti-PE antibodies [169, 170], in particular, in the obstetric field [155, 171]. However, the clinical utility of anti-PE antibodies is still under discussion.

Other Autoantibodies Associated with APS

Protein C, a vitamin K-dependent plasma glycoprotein, can be activated by the thrombin–thrombomodulin complex. The activated protein C subsequently functions as an anticoagulant, as related to proteolytically inactivating factor Va and VIIa, thereby limiting thrombin generation. Inactivation of these factors is most efficient together with another vitamin K-dependent cofactor protein, protein S. Oosting *et al.* [24] demonstrated that antibodies responsible for inhibiting factor Va degeneration are directed against phospholipid-bound protein C or protein S.

Antibodies against factor XII, which plays a role in initiating intrinsic coagulation and/or intrinsic fibrinolysis, have been reported [172].

Those antibodies may be considered as evidence for established-aPLs negative APS in the future.

PATHOGENESIS OF ANTI-PHOSPHOLIPID ANTIBODIES

Many varieties of pathophysiologic mechanisms have been explored in order to understand the wide spectrum of antigenic specificities of aPLs. Numerous observations will be reviewed here concerning putative mechanisms related to anti-β2-GPI antibodies or other aPLs predisposing to thrombosis and to atherosclerosis.

Platelet Interaction

It is now well appreciated that membranes of activated platelets are an important source of negatively charged phospholipids (e.g., phosphatidylserine, which provides a catalytic surface for blood coagulation). Factor Xa and thrombin are generated by the tenase and prothrombinase complexes, respectively, via the catalytic surface of activated platelets and procoagulant microparticles shed by activation of platelets. Shi *et al.* [173] reported that β2-GPI inhibited the generation of factor Xa by activated platelets and that aCLs (i.e., anti-β2-GPI antibodies) interfered with this inhibition. It was also indicated that β2-GPI affected the generation of factor Xa rather than its binding to platelet-derived microparticles, and that aCLs (i.e., anti-β2-GPI antibodies) interfered with this inhibition [174]. Thus, activated platelets may be a predominant immunotarget of anti-β2-GPI antibodies. Another mechanism by which anti-β2-GPI antibodies may inhibit phospholipid-dependent coagulation reactions has been reported [110, 114]. β2-GPI alone binds weakly to membranes containing physiologic concentrations of anionic phospholipids and does not inhibit the binding of coagulation factors or anticoagulant proteins. Autoantibodies' cross-linking of membrane-bound β2-GPI greatly decreases the rate at which β2-GPI dissociates from the membrane. Thus cross-linked antibody–β2-GPI complexes binding tightly to membranes results in inhibition of binding of other phospholipid-binding proteins.

Endothelial Cell Interaction

Cellular interactions of APS associated autoantibodies have also gained increasing attention. Endothelial cells may be a predominant target leading to a procoagulant state in APS.

Thrombin–thrombomodulin complex activates protein C [175–177], leading to the inhibition of clotting by the proteolytic cleavage of factors Va and VIIIa in the presence of protein S that is synthesized by endothelial cells as a cofactor [178, 179]. Therefore, thrombomodulin, is an anticoagulant receptor that plays a crucial role in the regulation of thrombin generation. Many data have supported the suppressed thrombomodulin–protein C system by aPLs (Fig. 4). The fact that protein C and its cofactor protein S are phospholipid binding plasma proteins, has made this system one of the most likely to be involved in the pathophysiology of thrombosis in APS. APLs might inhibit phospholipid-dependent reactions of the protein C pathway in different ways. First, they can interfere with the activation of protein C by the thrombin–thrombomodulin complex. Thrombin formation inhibited by aPLs could paradoxically cause a prothrombotic tendency due to insufficient protein C activation [180]. It has also been shown that LA positive IgGs inhibit the activation of purified protein C by thrombin on endothelial cells [181, 182] or by thrombin/purified thrombomodulin [183]. Second, the proteolytic effect of activated protein C (APC) on factor Va/factor VIIIa can be inhibited by aPLs. Marciniak and Romond [184] reported that factor

Va degradation was reduced in plasma from patients with LA. Malia *et al.* [185] confirmed the inhibitory effect of IgGs purified from aPL positive patients on factor Va degradation by APC. We [186] showed that human monoclonal anti-β2-GPI antibodies inhibit APC function, definitely confirming the autoimmune aPLs' effect on this system. Finally, the cofactor effect of protein S in the protein C pathway can be affected by aPLs [24, 187, 188].

Bacterial endotoxin or cytokines (tumor necrosis factor or interleukin 1) induce tissue factor (TF), activating the extrinsic clotting cascade [189, 190]. TF is a single chain transmembrane protein composed of 263 amino acid residues [191]. Of the many factors involved in the activation of the intrinsic and extrinsic pathways of coagulation, TF is the only factor that is fully actived in its native form, not dependent on proteolysis, therefore procoagulant activity generation merely requires TF exposure on cell surfaces [192]. Numerous investigators have focused on the upregulation of TF expression by aPLs. We [193] and others [194, 195] showed that aPLs induce TF activity, antigen, or mRNA on endothelial cells as well as on monocytes. As described earlier, TF does not require activation by other enzymes to function, thus its expression per se represents hypercoagulation in patients with aPLs.

Endothelin-1 (ET-1) is the most potent endothelium-derived contracting factor modulating vascular smooth muscle tone [196, 197]. ET-1 is extremely labile, thus the mature peptide is not found within endothelial cells [198]; however, the levels of prepro ET-1 (a precursor of ET-1) mRNA are induced by a range of stimuli, such as thrombin [199] or cytokines. Production and secretion of mature ET-1 peptide occurs within minutes of exposure to these stimuli [200]. Vascular cells are, therefore, able to adjust rapidly ET-1 production as required for the regulation of vasomotor tone [197]. We [201] also showed that markedly increased plasma ET-1 levels were found in APS patients with arterial thrombosis but not in those with venous thrombosis. It is so far the only specific marker for arterial thrombosis in APS. Human monoclonal aCL induced prepro ET-1 mRNA levels, confirming aPLs' direct effect on endothelium regarding ET-1 production. These data suggest that the production of ET-1 induced by aPLs may play an important role in altering arterial tone and contributing to arterial occlusion.

Adhesion receptors are proteins in cell membranes that facilitate the interaction of cells with matrix. Intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin are typical molecules found on activated endothelial cells. Leukocytes stick on endothelial cell via those molecules, further activating endothelium. Some proteases derived from such leukocytes also cause endothelium injury. Cellular interaction of aPLs have attracted great interest. Purified aPLs or monoclonal anti-β2-GPI antibodies induce expression of the adhesion molecules E-selectin, VCAM-1, and ICAM-1. Pierangeli *et al.* [202] showed an *in vivo* model to present the increased leukocyte adhesion by aPLs infusion to mice, in which adhered leukocytes are visualized using scrotum under the microscope.

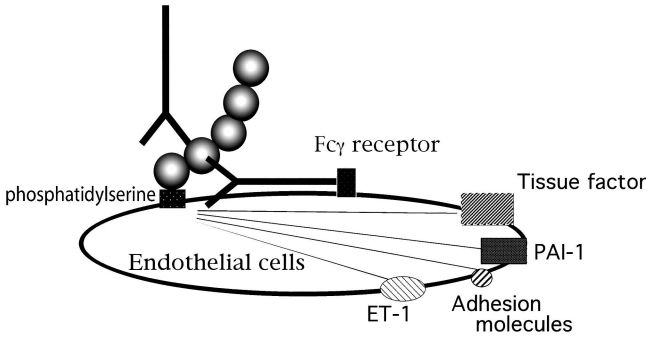


FIGURE 5 Activation of endothelial cells by anti-β2-GPI antibodies. Anti-β2-GPI antibody recognizes β2-GPI bound endothelial cells and induces tissue factor, plasminogen activator inhibitor-1 (PAI-1), adhesion molecules (ICAM-1, VCAM-1, E-selectin), endothelin-1 (ET-1), etc. Consequently, activated endothelial cells express procoagulant activity.

Endothelial cells secrete the inhibitor of plasminogen activator-1 (PAI-1) to depress fibrinolysis by blocking tissue plasminogen activator (t-PA) activity, when activated. Impaired fibrinolysis in APS has been shown in some reports [203–205], but no direct evidence has been reported in the induction of PAI-1 by aPLs.

In any case, aPLs activate endothelium, leading to its perturbation (Fig. 5). The activation may depend on the immune complex formation on cell surface and/or Fcγ receptor stimulation and/or thrombin receptor that delivers a stimulatory signal by thrombin [206].

Gharavi *et al.* reported that monoclonal antibodies against a synthetic peptide consisting of 15 amino acids, which contains a sequence of the phospholipid binding site located in domain V of β2-GPI, enhanced thrombosis and activated endothelial cells [207]. More recently, Dunoyer-Geindre *et al.* demonstrated that β2-GPI localized in late endosomes of human umbilical vein endothelial cells and incubation of anti-β2-GPI antibodies resulted in the antibodies coaccumulation [208]. These findings may lend support to the notion of anti-β2-GPI antibodies inducing thrombotic diathesis but the nature of the β2-GPI receptor on endothelial cells and the mechanism by which antibody binding to β2-GPI on the cell surface transduces a signal have yet to be defined.

Involvement of Apoptosis

Phospholipids are distributed in an asymmetric manner throughout the cell membrane [209]. Induction of apoptosis in murine thymocytes and human neutrophils leads to redistribution of anionic phospholipids to the outer leaflet of cell membranes [210, 211]. It was demonstrated that apoptotic thymocytes are targeted by anti-β2-GPI antibodies in the presence of β2-GPI [212]. Pittoni *et al.* [213] demonstrated that human monoclonal aPL and anti-β2-GPI antibody bind to "early" apoptotic cells. These investigators proposed that apoptotic cells may be a natural immunogen, target, or both for aPLs and the interaction of circulating β2-GPI with redistributed anionic phospholipid may itself generate a novel ligand by which apoptotic cells are recognized directly for phagocytic clearance.

Arterial Diseases and Atherosclerosis

In SLE, myocardial infarction is a major cause of mortality [214–219] and although *in situ* thrombosis in major arteries could be the pathology, coronary atherosclerosis has been demonstrated in a number of postmortem studies despite the relative youth of most of these patients [220]. In 1982, Homcy *et al.* [215] described six cases of ischemic heart disease in patients with SLE aged 15 to 29 years. Of these, 3 patients had atherosclerotic lesions. Doherty and Siegel [220] analyzed the features of 33 patients younger than 35 years of age with SLE and coronary artery disease. The vessel lesion was atherosclerosis in 12 patients, arteritis in 7, and stenosis in 6. It is unclear whether the latter resulted from atherosclerosis or vasculitis.

Epidemiological studies have established that an elevated plasma level of low-density lipoprotein (LDL) represents one of the most important risk factors for the development of atherosclerosis [221]. A subset of chemically modified LDLs such as oxidized LDL (oxLDL) has attracted the interest of researchers as one of the causative factors underlying atherosclerosis [222, 223]. The *in vivo* oxidative modification of LDL has been proposed to play a central role in atherosclerosis [224] as suggested by the presence of oxLDL particles in the early phase of atherosclerotic plaque formation.

It was reported that antibodies against epitopes of oxLDL recognize materials in atherosclerotic lesions but not in normal arteries, and the major antigenic epitopes are induced in apolipoprotein B (apoB) during oxidative modification of LDL [225]. Malondialdehyde (MDA) is an end-product of lipid peroxidation, and conjugation to the lysine residues of apoB induces the epitope for antibodies from human sera [226]. However, autoantibodies induced to oxidative modified lipoproteins occur not only in apoB but also in phospholipids composing of LDL [227].

Vaarala *et al.* [228] reported that aCL raised in SLE patients might cross-react with MDA-modified LDL. Later, others failed to show any cross-reaction between anti-β2-GPI antibodies and antibodies against ox-LDL (anti-oxLDL) in APS patients [229, 230]. It was also shown that anti-β2-GPI antibodies could be a marker for arterial thrombosis in SLE patients, whereas IgG anti-oxLDL is not associated with arterial thrombosis [231]. Hasunuma *et al.* [232] reported a specific interaction among Cu^{2+}-oxidized plasma lipoproteins, β2-GPI and anti-β2-GPI antibodies and an involvement of both β2-GPI and an anti-β2-GPI antibody in oxLDL uptake by macrophages.

There was another set of reports suggesting that some aPLs in APS patients are directed to neoepitopes of oxidized PLs or those generated by adduct formation between oxidised PLs and associated proteins [233, 234]. They also hypothesized that one candidate of oxidized phospholipids, that is, 1-palmitoyl-2-(5-oxovaleroyl)-phosphatidylcholine (POVPC), which is one of the oxidized form of 1-palmitoyl-2-arachidonoyl-phosphatidylcholine (PAPC), comes from oxidation of LDL [235, 236].

Against this background, a ligand specific for β2-GPI, namely, oxLig-1, was isolated from Cu^{2+}-oxLDL and its structure was characterized as 7-ketocholesteryl-esterified fatty acid esters which are carboxylated at the ω-position of fatty acid residues, such as oxLig-1 (7-ketocholesteryl-9-carboxynonanoate, i.e., 9-oxo-9-(7-ketocholest-5-en-3b-yloxy)nonanoic acid) [237]. In this study, it was clearly shown that epitopes for anti-β2-GPI autoantibodies expressed on a complex of β2-GPI and oxLig-1, formed by electrostatic interactions.

It was also shown that liposomes containing oxLig-1, a model of oxLDL, are avidly taken up by macrophages, by a process dependent on both β2-GPI and anti-β2-GPI antibodies [237]. This binding might be mediated by the Fcγ receptor because it was reported that the uptake by macrophages of immune complexes containing oxLDL through the Fcγ type I receptor transformed macrophages into foam cells [238, 239], and could accelerate the atherogenic process (Fig. 6) [240–242]. In addition, anti-β2-GPI autoantibodies derived from APS patients, with episodes of arterial thrombosis, were detected in ELISA, using a solid phase β2-GPI complexed with oxLig-1.

Several interesting reports have been published concerning a possible association with β2-GPI, anti-β2-GPI antibodies, and atherosclerosis. George *et al.* [243] showed that oxLDL, but not native LDL, aggravates the manifestations of experimental APS. These findings strongly suggest that oxLDL is sequentially targeted not

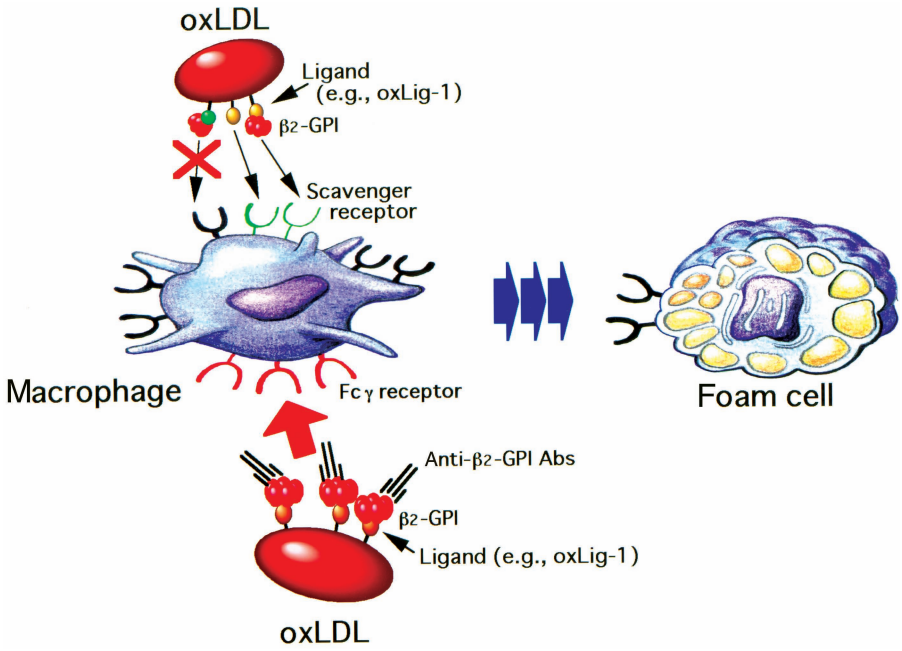


FIGURE 6 Possible mechanisms of anti-β2-GPI antibody-mediated formation of foam cells.

only by antibodies directly reactive for negatively charged phospholipids but also by anti-β2-GPI antibodies via β2-GPI adhesion. It was shown that LDL-receptor-deficient mice fed a chow diet and immunized with β2-GPI have accelerated atherosclerosis [243]. They also found that β2-GPI is abundantly expressed within subendothelial regions and intimal–medial borders of human atherosclerotic plaques, and that it colocalizes with monocytes and with CD4-positive lymphocytes [244]. Thus, there is increasing circumstantial evidence of an autoimmune mechanism involving β2-GPI and oxLDL in the atherogenesis in APS.

Pregnancy Morbidity

The clinical manifestations related with aPLs, such as fetal death, growth impairment, and preeclampsia, are consequences of impaired placental function. It is likely that those placental dysfunctions are due to the uteroplacental circulation disorder related with thrombosis. Many early reports showed thrombosis and necrosis in placentas from patients with APS [245–247]. In fact, 82% of placentas from individuals with aPLs had thrombosis or infaction [248].

A common finding in the placental bed of pregnancies complicated by aPLs is an increase in atherosclerotic lesions, described as "acute atherosis." The uptake of oxLDL by macrophages is recognized to be an important step in atherogenesis. Thus, aPLs and β2-GPI may combine *in vivo* to enhance atherogenesis in spiral arteries, thereby contributing to complications of pregnancy.

However, the histological findings of placenta in APS cases are nonspecific and not all of APS placentas have thrombotic change [249]. Placental thrombosis may be only one of the mechanisms of pregnancy morbidity in APS. The nonthrombotic functional problems in gestational tissues may be also involved. di Somone *et al.* [250] demonstrated that anti-β2-GPI antibodies bound to trophoblast in the presence of β2-GPI, leading to the impairment of gonadotropin secretion.

Acknowledgments

We thank Professor Masahiro Ieko, Department of Medicine, Health Science University of Hokkaido, for helpful discussions, and Dr. Olga Amengual for her fruitful suggestions and comments on the manuscript.

References

1. Harris, E. N., Gharavi, A. E., and Hughes, G. R. V. (1985). Anti-phospholipid antibodies. *Clin. Rheum. Dis.* **11**, 591.
2. Hughes, G. R. V., Harris, E. N., and Gharavi, A. E. (1986). The anticardiolipin syndrome. *J. Rheumatol.* **13**, 486.
3. McNeil, H. P., Chesterman, C. N., and Krilis, S. A. (1991). Immunology and clinical importance of antiphospholipid antibodies. *Adv. Immunol.* **49**, 193.
4. Harris, E. N., Gharavi, A. E., Boey, M. L., Patel, B. M., Mackworth-Young, C. G., Loizou, S., and Hughes, G. R. V. (1983). Anti-cardiolipin antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* **2**, 1211.
5. Wassermann, A., Neisser, A., and Bruck, C. (1906). Eine serodiagnostishe reaction bei syphilis. *Dtsch. Med. Wochenschr.* **32**, 745.

6. Pangborn, M. C. (1941). A new serologically active phospholipid from beef heart. *Proc. Soc. Exp. Biol. Med.* **48**, 745.
7. Pangborn, M. C. (1942). Isolation and purification of a serologically active phospholipid from beef heart. *J. Biol. Chem.* **143**, 247.
8. Moore, J. E., and Lutz, W. B. (1955). The natural history of systemic lupus erythematosus: An approach to its study through chronic biological false positive reactors. *J. Chronic Dis.* **1**, 297.
9. Moore, J. E., and Mohr, C. F. (1952). Biologically false positive serologic test for syphilis. *JAMA* **150**, 467.
10. Mueller, J. F., Ratnoff, O., and Henile, R. W. (1951). Observations on the characteristics of an unusal circulating anticoagulant. *J. Lab. Clin. Med.* **38**, 254.
11. Laurell, A. B., and Nilsson, I. M. (1957). Hypergammaglobulinaemia, circulating anticoagulant, and biological false positive Wassermann reaction: A study of two cases. *J. Lab. Clin. Med.* **49**, 694.
12. Haserick, J. R., and Long, R. (1951). Systemic lupus erythematosus preceded by false positive fest for syphilis: Presentation of five cases. *Ann. Intern. Med.* **37**, 559.
13. Harvey, A. M., and Shulman, L. E. (1966). Connective tissue disease and the chronic biologic false-positive test for syphilis (BFP reaction). *Med. Clin. North Am.* **50**, 1271.
14. Conley, C. L., and Hartmann, R. C. (1952). A hemorrhogic disorder caused by circulating anticoagulants in patients with disseminated lupus erythematosus. *J. Lab. Clin. Med.* **31**, 621.
15. Feinstein, D. I., and Rapaport, S. I. (1972). Acquired inhibitors of blood coagulation. *Prog. Hemost. Thromb.* **1**, 75.
16. Bowie, E. J. W., Thompson, J. H., and Pascuzzi, C. A. (1963). Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. *J. Lab. Clin. Med.* **62**, 416.
17. Koike, T., Sueishi, M., Funaki, H., Tomioka, H., and Yoshida, S. (1984). Antiphospholipid antibodies and biological false positive serological test for syphilis in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **56**, 193.
18. McNeil, H. P., Simpson, R. J., Chesterman, C. N., and Krilis, S. A. (1990). Anti-phospholipid antibodies are directed against a complex antigen that induces a lipid-binding inhibitor of coagulation: β2-glycoprotein I (apolipoprotein H). *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4120.
19. Galli, M., Comfurius, P., Maassen, C., Hemker, H. C., de Baets, M. H., van Breda-Vriesman, P. J. C., Barbui, T., Zwaal, R. F. A. Z., and Bevers, E. M. (1990). Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* **335**, 952.
20. Matsuura, E., Igarashi, Y., Fujimoto, M., Ichikawa, K., and Koike, T. (1990). Anticardiolipin cofactor(s) and differential diagnosis of autoimmune disease. *Lancet* **336**, 177.
21. Matsuura, E., Igarashi, Y., Yasuda, T., Triplett, D. A., and Koike, T. (1994). Anticardiolipin antibodies recognize β2-glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. *J. Exp. Med.* **179**, 457.
22. Bevers, E. M., Galli, M., Barbui, T., Comfurius, P., and Zwaal, R. F. (1991). Lupus anticoagulant IgG's (LA) are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb. Haemost.* **66**, 629.
23. Matsuda, J., Saitoh, N., Gohchi, K., Gotoh, M., and Tsukamoto, M. (1994). Anti-annexin V antibody in systemic lupus erythematosus patients with lupus anticoagulant and/or anticardiolipin antibody. *Am. J. Hematol.* **47**, 56.
24. Oosting, J. D., Derksen, R. H. W. M, Bobbink, I. W. G., Hackeng, T. M., Bouma, B. N., and de Groot, P. G. (1993). Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C, or protein S: An explanation for their pathogenic mechanism? *Blood* **81**, 2618.
25. Sugi, T., and McIntyre, J. A. (1995). Autoantibodies to phosphatidylethanolamine (PE) recognize a kininogen-PE complex. *Blood* **86**, 3083.
26. Wilson, W. A., Gharavi, A. E., Koike, T., Lockshin, M. D., Branch, D. W., Piette, J. C., Brey, R., Derksen, R., Harris, E. N., Hughes, G. R., Triplett, D. A., and Khamashta, M. A. (1999). International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. *Arthritis Rheum.* **42**, 1309.
27. Stryer, L. (1988). Introduction to biological membranes. In "Biochemistry" 3rd ed. Freeman, New York.
28. Op dem Kamp, J. A. F. (1979). Lipids asymmetry in membranes. *Annu. Rev. Biochem.* **48**, 47.
29. Koike, T., Tsutsumi, A., Ichikawa, K., and Matsuura, E. (1995). Antigenic specificity of the "anticardiolipin" antibodies. *Blood* **85**, 2277.
30. Igarashi, M., Matsuura, E., Igarashi, Y., Nagae, H., Ichikawa, K., Triplett, D. A., and Koike, T. (1996). Human β2-glycoprotein I as an anticardiolipin cofactor determined using deleted mutants expressed by a Baculovirus system. *Blood* **87**, 3262.
31. Roubey, R. A. S., Eisenberg, R. A., Harper, M. F., and Winfield, J. B. (1995). "Anticardiolipin" autoantibodies recognize β2-glycoprotein I in the absence of phospholipid. *J. Immunol.* **154**, 954.
32. Tincani, A., Spatola, L., Prati, E., Allegri, F., Ferremi, P., Cattaneo, R., Meroni, P., and Balestrieri, G. (1996). The anti-β2-glycoprotein I activity in human anti-phospholipid syndrome sera is due to monoreactive low-affinity autoantibodies directed to epitopes located on native β2-glycoprotein I and preserved during species' evolution. *J. Immunol.* **157**, 5732.
33. Koike, T., and Matsuura, E. (1991). What is the "true" antigen for anticardiolipin antibodies? *Lancet* **337**, 671.
34. Matsuura, E., Igarashi, Y., Fujimoto, M., Ichikawa, K., Suzuki, T., Yoshida, S., and Koike, T. (1992). Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. *J. Immunol.* **148**, 3885.

35. Hunt, J., and Krilis, S. (1994). The fifth domain of β2-glycoprotein I contains a phospholipid binding site (Cys281-Cys288) and a region recognized by anticardiolipin antibodies. *J. Immunol.* **152**, 653.
36. Tsutsumi, A., Ichikawa, K., Matsuura, E., Sawada, K., and Koike, T. (2000). Heterogeneous behavior of anti-β2-glycoprotein I antibodies on various commercially available enzyme immunoassay plates coated with β2-glycoprotein I. *J. Rheumatol.* **27**, 391.
37. Chamley, L. W., Duncalf, A. M., Konarkowska, B., Mitchell, M. D., and Johnson, P. M. (1999). Conformationally altered β2-glycoprotein I is the antigen for anti-cardiolipin autoantibodies. *Clin. Exp. Immunol.* **115**, 571.
38. Galazka, M., Tang, M., DeBari, V. A., Kohles, J. D., Lee, J. K., Keil, L. B., and Petersheim, M. (1999). Modification of β2-glycoprotein I by glutardialdehyde. Conformational changes and aggregation accompany exposure of the cryptic autoepitope. *Appl. Biochem. Biotechnol.* **76**, 1.
39. Sheng, Y., Kandiah, D. A., and Krilis, S. A. (1998). Anti-β2-glycoprotein I autoantibodies from patients with the "antiphospholipid" syndrome bind to β2-glycoprotein I with low affinity: Dimerization of β2-glycoprotein I induces a significant increase in anti-β2-glycoprotein I antibody affinity. *J. Immunol.* **161**, 2038.
40. George, J., Gilburd, B., Hojnik, M., Levy, Y., Langevitz, P., Matsuura, E., Koike, T., and Shoenfeld, Y. (1998). Target recognition of β2-glycoprotein I (β2GPI)-dependent anticardiolipin antibodies: Evidence for involvement of the fourth domain of β2GPI in antibody binding. *J. Immunol.* **150**, 3917.
41. Iverson, G. M., Victoria, E. J., and Marquis, D. M. (1998). Anti-β2 glycoprotein I (β2GPI) autoantibodies recognize an epitope on the first domain of β2GPI. *Proc. Natl. Acad. Sci. U.S.A.* **95**, 15542.
42. McNeeley, P. A., Dlott, J. S., Furie, R. A., Jack, R. M., Ortel, T. L., Triplett, D. A., Victoria, E. J., and Linnik, M. D. (2001). β2-glycoprotein I-dependent anticardiolipin antibodies preferentially bind the amino terminal domain of β2-glycoprotein I. *Thromb. Haemost.* **86**, 590.
43. Koike, T., Ichikawa, K., Atsumi, T., Kasahara, H., and Matsuura, E. (2000). β2-glycoprotein I-anti-β2-glycoprotein I interaction. *J. Autoimmun.* **15**, 97.
44. Schultze, H. E., Heide, K., and Haput, H. (1961). Uber ein bisher ubekanntes niedermolekulars β2-Globulin des Humanserums. *Naturwissenschaften* **48**, 719.
45. Wurm, H. (1984). β2-glycoprotein (apolipoprotein H) interactions with phospholipid vesicles. *Int. J. Biochem.* **16**, 511.
46. Polz, E. (1979). "Isolation of a specific lipid-binding protein from human serum by affinity chromatography using heparinSepharose" (H. Peeters Ed.), Pergamon, Oxford.
47. Polz, E., and Kostner, G. M. (1979). The binding of β2-glycoprotein-I to human serum lipoproteins: Distribution among density fractions. *FEBS Lett.* **102**, 183.
48. Nimpf, J., Bevers, E. M., Bomans, P. H., Till, U., Wurm, H., Kostner, G. M., and Zwaal, R. F. (1986). Prothrombinase activity of human platelets is inhibited by beta 2-glycoprotein-I. Biochim. *Biophys. Acta* **884**, 142.
49. Schousboe, I. (1985). β2-glycoprotein I: Plasma inhibitor of contact activation of the intrinsic blood coagulation pathway. *Blood* **66**, 1086.
50. Nimpf, J., Wurm, H., and Kostner, G. M. (1987). β2-glycoprotein-I (apo-H) inhibits the release reaction of human plateletsduring ADP-induced aggregation. *Atherosclerosis* **63**, 109.
51. Lozier, J., Takahashi, N., and Putnam, F. W. (1984). Complete amino acid sequence of human plasma β2-glycoprotein I. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3640.
52. Kato, H., and Enjyoji, K. (1991). Amino acid sequence and location of the disulfide bonds in bovine β2-glycoprotein I: The presence of five Sushi domains. *Biochemistry* **30**, 11687.
53. Matsuura, E., Igarashi, M., Igarashi, Y., Nagae, H., Ichikawa, K., Yasuda, T., and Koike, T. (1991). Molecular definition of human β2-glycoprotein I (β2-GPI) by cDNA cloning and inter species differences of β2-GPI in alternation anticardiolipin binding. *Int. Immunol.* **3**, 1217.
54. Steinkasserer, A., Estaller, C., Weiss, E. H., Sim, R. B., and Day, A. J. (1991). Complete nucleotide and deduced amino acid sequence of human beta 2-glycoprotein I. *Biochem. J.* **277**, 387.
55. Reid, K. B., and Day, A. J. (1989). Structure–function relationships of the complement components. *Immunol. Today* **10**, 177.
56. Bouma, B., de Groot, P. G., van den Elsen, J. M., Ravelli, R. B., Schouten, A., Simmelink, M. J., Derksen, R. H., Kroon, J., and Gros, P. (1999). Adhesion mechanism of human β(2)-glycoprotein I to phospholipids based on its crystal structure. *EMBO J.* **18**, 5166.
57. Schwarzenbacher, R., Zeth, K., Diederichs, K., Gries, A., Kostner, G. M., Laggner, P., and Prassl, R. (1999). Crystal structure of human beta2-glycoprotein I: Implications for phospholipid binding and the antiphospholipid syndrome. *EMBO J.* **18**, 6228.
58. Steinkasserer, A., Barlow, P. N., Willis, A. C., Kertesz, Z., Campbell, I. D., Sim, R. B., and Norman, D. G. (1992). Activity, disulphide mapping and structural modelling of the fifth domain of human β2-glycoprotein I. *FEBS Lett.* **313**, 193.
59. Matsuura, E., Igarashi, M., Igarashi, Y., Katahira, T., Nagae, H., Ichikawa, K., Triplett, D. A., and Koike, T. (1995). Molecular studies on phospholipid-binding sites and cryptic epitopes appering on β2-glycoprotein I structure recognized by anticardiolipin antibodies. *Lupus* **4**(Suppl. 1), S13.
60. Sheng, Y., Sali, A., Herzog, H., Lahnstein, J., and Krilis, S. A. (1996). Site-directed mutagenesis of recombinant human beta 2-glycoprotein I identifies a cluster of lysine residues that are critical for phospholipid binding and anti-cardiolipin antibody activity. *J. Immunol.* **157**, 3744.
61. Hunt, J. E., Simpson, R. J., and Krilis, S. A. (1993). Identification of a region of β2-glycoprotein I critical for lipid binding and anti-cardiolipin antibody cofactor activity. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2141.

62. Hoshino, M., Hagihara, Y., Nishii, I., Yamazaki, T., Kato, H., and Goto, Y. (2000). Identification of the phospholipid-binding site of human β2-glycoprotein I domain V by heteronuclear magnetic resonance. *J. Mol. Biol.* **304**, 927.
63. Hong, D. P., Hagihara, Y., Kato, H., and Goto, Y. (2001). Flexible loop of β2-glycoprotein I domain V specifically interacts with hydrophobic ligands. *Biochemistry* **40**, 8092.
64. Lee, A. T., Balasubramanian, K., and Schroit, A. J. (2000). β2-glycoprotein I-dependent alterations in membrane properties. *Biochim. Biophys. Acta* **1509**, 475.
65. Hagihara, Y., Enjyoji, K., Omasa, T., Katakura, Y., Suga, K., Igarashi, M., Matsuura, E., Kato, H., Yoshimura, T., and Goto, Y. (1997). Structure and function of the recombinant fifth domain of human β2-glycoprotein I: Effects of specific cleavage between Lys77 and Thr78. *J. Biochem. (Tokyo)* **121**, 128.
66. Ohkura, N., Hagihara, Y., Yoshimura, T., Goto, Y., and Kato, H. (1998). Plasmin can reduce the function of human beta2 glycoprotein I by cleaving domain V into a nicked form. *Blood* **91**, 4173.
67. Matsuura, E., Inagaki, J., Kasahara, H., Yamamoto, D., Atsumi, T., Kobayashi, K., Kaihara, K., Zhao, D., Ichikawa, K., Tsutsumi, A., Yasuda, T., Triplett, D. A., and Koike, T. (2000). Proteolytic cleavage of β2-glycoprotein I: Reduction of antigenicity and the structural relationship. *Int. Immunol.* **12**, 1183.
68. Brighton, T. A., Hogg, P. J., Dai, Y. P., Murray, B. H., Chong, B. H., and Chesterman, C. N. (1996). Beta2-glycoprotein I in thrombosis: Evidence for a role as a natural anticoagulant. *Br. J. Haematol.* **93**, 185.
69. Horbach, D. A., van Oort, E., Lisman, T., Meijers, J. C., Derksen, R. H., and de Groot, P. G. (1999). β2-glycoprotein I is proteolytically cleaved in vivo upon activation of fibrinolysis. *Thromb. Haemost.* **81**, 87.
70. Itoh, Y., Inuzuka, K., Kohno, I., Wada, H., Shiku, H., Ohkura, N., and Kato, H. (2000). Highly increased plasma concentrations of the nicked form of β2 glycoprotein I in patients with leukemia and with lupus anticoagulant: Measurement with a monoclonal antibody specific for a nicked form of domain V. *J. Biochem. (Tokyo)* **128**, 1017.
71. Steinkasserer, A., Cockburn, D. J., Black, D. M., Boyd, Y., Solomon, E., and Sim, R. B. (1992). Assignment of apolipoprotein H (APOH: β-2-glycoprotein I) to human chromosome 17q23-qter; determination of the major expression site. *Cytogenet. Cell Genet.* **60**, 31.
72. Chamley, L. W., Allen, J. L., and Johnson, P. M. (1997). Synthesis of β2 glycoprotein 1 by the human placenta. *Placenta* **18**, 403.
73. Caronti, B., Calderaro, C., Alessandri, C., Conti, F., Tinghino, R., Palladini, G., and Valesini, G. (1999). β2-glycoprotein I (β2-GPI) mRNA is expressed by several cell types involved in anti-phospholipid syndrome-related tissue damage. *Clin. Exp. Immunol.* **115**, 214.
74. Chamley, L. W., Pattison, N. S., and McKay, E. J. (1993). Elution of anticardiolipin antibodies and their cofactor β2-glycoprotein 1 from the placentae of patients with a poor obstetric history. *J. Reprod. Immunol*. **25**, 209.
75. Kamboh, M. I., Ferrell, R. E., and Sepehrnia, B. (1988). Genetic studies of human apolipoprotein. IV. Structural heterogeneity of apolipoprotein H (β2-glycoprotein I). *Am. J. Hum. Genet.* **42**, 452.
76. Kamboh, M. I., and Ferrell, R. E. (1991). Apolipoprotein H polymorphism and its role in lipid metabolism. *Adv. Lipid Res.* **1**, 9.
77. Mehdi, H., Aston, C. E., Sanghera, D. K., Hamman, R. F., and Kamboh, M. I. (1999). Genetic variation in the apolipoprotein H (β2-glycoprotein I) gene affects plasma apolipoprotein H concentrations. *Hum. Genet.* **105**, 63.
78. Sanghera, D. K., Kristensen, T., Hamman, R. F., and Kamboh, M. I. (1997). Molecular basis of the apolipoprotein H (β2-glycoprotein I) protein polymorphism. *Hum. Genet.* **100**, 57.
79. Gushiken, F. C., Arnett, F. C., Ahn, C., and Thiagarajan, P. (1999). Polymorphism of beta2-glycoprotein I at codons 306 and 316 in patients with systemic lupus erythematosus and antiphospholipid syndrome. *Arthritis Rheum.* **42**, 1189.
80. Hirose, N., Williams, R., Alberts, A. R., Furie, R. A., Chartash, E. K., Jain, R. I., Sison, C., Lahita, R. G., Merrill, J. T., Cucurull, E., Gharavi, A. E., Sammaritano, L. R., Salmon, J. E., Hashimoto, S., Sawada, T., Chu, C. C., Gregersen, P. K., and Chiorazzi, N. (1999). A role for the polymorphism at position 247 of the beta2-glycoprotein I gene in the generation of anti-beta2-glycoprotein I antibodies in the antiphospholipid syndrome. *Arthritis Rheum.* **42**, 1655.
81. Atsumi, T., Tsutsumi, A., Amengual, O., Khamashta, M. A., Hughes, G. R., Miyoshi, Y., Ichikawa, K., and Koike, T. (1999). Correlation between beta2-glycoprotein I valine/leucine247 polymorphism and anti-beta2-glycoprotein I antibodies in patients with primary antiphospholipid syndrome. *Rheumatology* **38**, 721.
82. Yasuda, S., Tustsumi, A., Chiba, H., Yanai, H., Miyoshi, Y., Takeuchi, R., Horita, T., Atsumi, T., Ichikawa, K., Matsuura, E., and Koike, T. (2000). Beta(2)-glycoprotein I deficiency: Prevalence, genetic background and effects on plasma lipoprotein metabolism and hemostasis. *Atherosclerosis* **152**, 337.
83. Moestrup, S. K., Schousboe, I., Jacobsen, C., Leheste, J. R., Christensen, E. I., and Willnow, T. E. (1998). β2-glycoprotein-I (apolipoprotein H) and β2-glycoprotein-I-phospholipid complex harbor a recognition site for the endocytic receptor megalin. *J. Clin. Invest.* **102**, 902.
84. Berden, J. H., Hang, L., McConahey, P. J., and Dixon, F. J. (1983). Analysis of vascular lesions in murine SLE. I. Association with serologic abnormalities. *J. Immonol.* **130**, 1699.
85. Hang, L. M., Izui, S., and Dixon, F. J. (1981). (NZW × BXSB)F1 hybrid. A model of acute lupus and coronary vascular disease with myocardial infarction. *J. Exp. Med.* **154**, 216.
86. Hang, L., Stephens-Larson, P., Henry, J. P., and Dixon, F. J. (1983). The role of hypertension in the vascular disease and myocardial infarcts associated with murine systemic lupus erythematosus. *Arthritis Rheum.* **26**, 1340.

87. Yoshida, H., Fujiwara, H., Fujiwara, T., Ikehara, S., and Hamashima, Y. (1987). Quantitative analysis of myocardial infarction in (NZW × BXSB)F1 hybrid mice with systemic lupus erythematosus and small coronary artery disease. *Am. J. Pathol.* **129**, 477.
88. Hashimoto, Y., Kawamura, M., Ichikawa, K., Suzuki, T., Sumida, T., Yoshida, S., Matsuura, E., Ikehara, S., and Koike, T. (1992). Anticardiolipin antibodies in NZW × BXSB F1 mice. A model of antiphospholipid syndrome. *J. Immonol.* **149**, 1063.
89. Blank, M., Faden, D., Tincani, A., Kopolovic, J., Goldberg, I., Gilburd, B., Allegri, F., Balestrieri, G., Valesini, G., and Shoenfeld, Y. (1994). Immunization with anticardiolipin cofactor (β-2-glycoprotein I) induces experimental antiphospholipid syndrome in naive mice. *J. Autoimmun.* **7**, 441.
90. Kouts, S., Wang, M. X., Adelstein, S., Krilis, S. A. (1995). Immunization of a rabbit with β2-glycoprotein I induces charge-dependent crossreactive antibodies that bind anionic phospholipids and have similar reactivity as autoimmune anti-phospholipid antibodies. *J. Immunol.* **155**, 958.
91. George, J., Blank, M., Gilburd, B., Hojnik, M., Shenkman, B., Tamarin, I., Varon, D., Matsuura, E., Koike, T., and Shoenfeld, Y. (1997). Immunologic characterization and functional properties of murine antibodies raised against deleted mutants of human β2-glycoprotein I. *Int. Immunol.* **9**, 913.
92. Pierangeli, S. S., Liu, X. W., Anderson, G., Barker, J. H., and Harris, E. N. (1996). Thrombogenic properties of murine anti-cardiolipin antibodies induced by β2 glycoprotein I and human immunoglobulin G antiphospholipid antibodies. *Circulation* **94**, 1746.
93. Sheng, Y., Reddel, S. W., Herzog, H., Wang, Y. X., Brighton, T., France, M. P., Robertson, S. A., and Krilis, S. A. (2001). Impaired thrombin generation in β2-glycoprotein I null mice. *J. Biol. Chem.* **276**, 13817.
94. Ichikawa, K., Khamashta, M., Koike, T., Matsuura, E., and Hughes, G. R. V. (1994). Reactivity of monoclonal anticardiolipin antibodies from patients with the antiphospholipid syndrome to β2-glycoprotein I. *Arthritis Rheum*. **37**, 1453.
95. Igarashi, M., Matsuura, E., Igarashi, Y., Nagae, H., Matsuura, Y., Ichikawa, K., Yasuda, T., Voelker, D. R., and Koike, T. (1993). Expression of anticardiolipin cofactor, human β2-glycoprotein I, by a recombinant baculovirus/insect cell system. *Clin. Exp. Immunol.* **93**, 19.
96. Ichikawa, K., Tsutsumi, A., Atsumi, T., Matsuura, E., Kobayashi, S., Hughes, G. R. V., Khamashta, M. A., and Koike, T. (1999). A chimeric antibody with the human gamma1 constant region as a putative standard for assays to detect IgG β2-glycoprotein I-dependent anticardiolipin and anti-β2-glycoprotein I antibodies. *Arthritis Rheum.* **42**, 2461.
97. Hattori, N., Kuwana, M., Kaburaki, J., Mimori, T., Ikeda, Y., and Kawakami, Y. (2000). T cells that are autoreactive to β2-glycoprotein I in patients with antiphospholipid syndrome and healthy individuals. *Arthritis Rheum.* **43**, 65.
98. Arai, T., Yoshida, K., Kaburaki, J., Inoko, H., Ikeda, Y., Kawakami, Y., and Kuwana, M. (2001). Autoreactive CD4(+) T-cell clones to β2-glycoprotein I in patients with antiphospholipid syndrome: Preferential recognition of the major phospholipid-binding site. *Blood* **98**, 1889.
99. Hunt, J. E., McNeil, H. P., Morgan, G. J., Crameri, R. M., and Krilis, S. A. (1992). A phospholipid–β2-glycoprotein I complex is an antigen for anticardiolipin antibodies occurring in autoimmune disease but not with infection. *Lupus* **1**, 75.
100. Tsutsumi, A., Matsuura, E., Ichikawa, K., Fujisaku, A., Mukai, M., Kobayashi, S., and Koike, T. (1996). Antibodies to β2-glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus. *Arthritis. Rheum.* **39**, 1466.
101. Triplett, D. A., and Brandt, J. T. (1988). Lupus anticoagulants: Misnomer, paradox, riddle, epiphenomenon. *Hematol. Pathol.* **2**, 121.
102. Triplett, D. A. (1994). Assays for detection of antiphospholipid antibodies. *Lupus* **3**, 281.
103. Rosove, M. H., and Brewer, P. M. (1992). Antiphospholipid thrombosis: Clinical course after the first thrombotic event in 70 patients. *Ann. Intern. Med.* **117**, 303.
104. Thiagarajan, P., Shapiro, S. S., and De Marco, L. (1980). Monoclonal immunoglobulin M lambda coagulation inhibitor with phospholipid specificity. Mechanism of a lupus anticoagulant. *J. Clin. Invest.* **66**, 397.
105. Galli, M., Beguin, S., Lindhout, T., and Hemker, C. H. (1989). Inhibition of phospholipid and platelet-dependent prothrombinase activity in the plasma of patients with lupus anticoagulants. *Br. J. Haematol.* **72**, 549.
106. Yin, E. T., and W, G. L. (1965). Purification and kinetic studies on a circulating anticoagulant in a suspected case of lupus erythematosus. *Thromb. Haemost.* **14**, 88.
107. Exner, T., Rickard, K. A., and Kronenberg, H. (1978). A sensitive test demonstrating lupus anticoagulant and its behavioural patterns. *Br. J. Haematol.* **40**, 143.
108. Oosting, J. D., Derksen, R. H. W. M., Entjes, H. T., Bouma, B. N., and de Groot, P. G. (1992). Lupus anticoagulant activity is frequently dependent on the presence of beta 2-glycoprotein I. *Thromb. Haemost.* **67**, 499.
109. Roubey, R. A. S., Pratt, C. W., Buyon, J. P., and Winfield, J. B. (1992). Lupus anticoagulant activity of autoimmune antiphospholipid antibodies is dependent upon β2-glycoprotein I. *J. Clin. Invest.* **90**, 1100.
110. Takeya, H., Mori, T., Gabazza, E. C., Kuroda, K., Deguchi, H., Matsuura, E., Ichikawa, K., Koike, T., and Suzuki, K. (1997). Anti-beta2-glycoprotein I (beta2GPI) monoclonal antibodies with lupus anticoagulant-like activity enhance the beta2GPI binding to phospholipids. *J. Clin. Invest.* **99**, 2260.
111. Arnout, J., and Vermylen, J. (1998) Mechanism of action of β2-glycoprotein I-dependent lupus anticoagulants. *Lupus* **7**(Suppl. 1), S23.
112. Arnout, J., Vanrusselt, M., Wittevrongel, C., and Vermylen, J. (1998). Monoclonal antibodies against beta-2-glycoprotein I: Use as reference material for lupus anticoagulant tests. *Thromb. Haemost.* **79**, 955.

113. Harper, M. F., Hayes, P. M., Lentz, B. R., and Roubey, R. A. S. (1998). Characterization of β2-glycoprotein I binding to phospholipid membranes. *Thromb. Haemost.* **80**, 610.
114. Willems, G. M., Janssen, M. P., Pelsers, M. M., Comfurius, P., Galli, M., Zwaal, R. F., and Bevers, E. M. (1996). Role of divalency in the high-affinity binding of anticardiolipin antibody–β2-glycoprotein I complexes to lipid membranes. *Biochemistry* **35**, 13833.
115. Arnout, J., Wittevrongel, C., Vanrusselt, M., Hoylaerts, M., and Vermylen, J. (1998). β-2-glycoprotein I dependent lupus anticoagulants form stable bivalent antibody β-2-glycoprotein I complexes on phospholipid surfaces. *Thromb. Haemost.* **79**, 79.
116. Rao, L. V. M., Hoang, A. D., and Rapaport, S. I. (1995). Differences in the interactions of lupus anticoagulant IgG with human prothrombin and bovine prothrombin. *Thromb. Haemost.* **73**, 668.
117. Pierangeli, S. S., Goldsmith, G. H., Branch, D. W., and Harris, E. N. (1997). Antiphospholipid antibody: Functional specificity for inhibition of prothrombin activation by the prothrombinase complex. *Br. J. Haematol.* **97**, 768.
118. Simmelink, M. J., Horbach, D. A., Derksen, R. H., Meijers, J. C., Bevers, E. M., Willems, G. M., and de Groot, P. G. (2001). Complexes of anti-prothrombin antibodies and prothrombin cause lupus anticoagulant activity by competing with the binding of clotting factors for catalytic phospholipid surfaces. *Br. J. Haematol.* **113**, 621.
119. Rao, L. V., Hoang, A. D., and Rapaport, S. I. (1996). Mechanism and effects of the binding of lupus anticoagulant IgG and prothrombin to surface phospholipid. *Blood* **88**, 4173.
120. Galli, M., Ruggeri, L., and Barbui, T. (1998). Differential effects of anti-beta2-glycoprotein I and antiprothrombin antibodies on the anticoagulant activity of activated protein C. *Blood* **91**, 1999.
121. Triplett, D. A. (1998). Many faces of lupus anticoagulants. *Lupus* **7**(Suppl. 2), S18.
122. Brandt, J. T., Triplett, D. A., Alving, B., and Scharrer, I. (1995). Criteria for the diagnosis of lupus anticoagulants: An update. On behalf of the Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the ISTH. *Thromb. Haemost.* **74**, 1185.
123. Triplett, D. A. (2000). Use of the dilute Russell viper venom time (dRVVT): Its importance and pitfalls. *J. Autoimmun.* **15**, 173.
124. Triplett, D. A., Brandt, J. T., Kaczor, D., and Schaeffer, J. (1983). Laboratory diagnosis of lupus inhibitors: A comparison of the tissue thromboplastin inhibition procedure with a new platelet neutralization procedure. *Am. J. Clin. Pathol.* **79**, 678.
125. Lesperance, B., David, M., Rauch, J., Infante-Rivard, C., and Rivard, G. E. (1988). Relative sensitivity of different tests in the detection of low titer lupus anticoagulants. *Thromb. Haemost.* **60**, 217.
126. Forastiero, R. R., Falcon, C. R., and Carreras, L. O. (1990). Comparison of various screening and confirmatory tests for the detection of the lupus anticoagulant. *Haemostasis* **20**, 208.
127. Pengo, V., Biasiolo, A., Rampazzo, P., and Brocco, T. (1999). dRVVT is more sensitive than KCT or TTI for detecting lupus anticoagulant activity of anti-β2-glycoprotein I autoantibodies. *Thromb. Haemost.* **81**, 256.
128. Exner, T. (2000). Conceptions and misconceptions in testing for lupus anticoagulants. *J. Autoimmun.* **15**, 179.
129. Arnout, J., Meijer, P., and Vermylen, J. (1999). Lupus anticoagulant testing in Europe: An analysis of results from the first European Concerted Action on Thrombophilia (ECAT) survey using plasmas spiked with monoclonal antibodies against human beta2-glycoprotein I. *Thromb. Haemost.* **81**, 929.
130. Galli, M., Finazzi, G., Bevers, E. M., and Barbui, T. (1995). Kaolin clotting time and dilute Russell's viper venom time distinguish between prothrombin-dependent and β2-glycoprotein I-dependent antiphospholipid antibodies. *Blood* **86**, 617.
131. Mackie, I. J., Donohoe, S., and Machin, S. I. (2000). Lupus anticoagulant measurement. In "Hughes Syndrome" (M. A. Khamashta, Ed.), Springer-Verlag, London.
132. Bajaj, S. P., Rapaport, S. I., Fierer, D. S., Herbst, K. D., and Schwartz, D. B. (1983). A mechanism for the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome. *Blood* **61**, 684.
133. Edson, J. R., Vogt, J. M., and Hasegawa, D. K. (1984). Abnormal prothrombin crossed-immunoelectrophoresis in patients with lupus inhibitors. *Blood* **64**, 807.
134. Fleck, R. A., Rapaport, S. I., and Rao, L. V. M. (1988). Anti-prothrombin antibodies and the lupus anticoagulant. *Blood* **72**, 512.
135. Bajaj, S. P., Rapaport, S. I., Barclay, S., and Herbst, K. D. (1985). Acquired hypoprothrombinemia due to non-neutralizing antibodies to prothrombin: Mechanism and management. *Blood* **65**, 1538.
136. Galli, M., Bevers, E. M., Comfurius, P., Barbui, T., and Zwaal, R. F. (1993). Effect of antiphospholipid antibodies on procoagulant activity of activated platelets and platelet-derived microvesicles. *Br. J. Haematol.* **83**, 466.
137. Arvieux, J., Darnige, L., Caron, C., Reber, G., Bensa, J. C., and Colomb, M. G. (1995). Development of an ELISA for autoantibodies to prothrombin showing their prevalence in patients with lupus anticoagulant. *Thromb. Haemost.* **74**, 1120.
138. Galli, M., Beretta, G., Daldossi, M., Bevers, E. M., and Barbui, T. (1997). Different anticoagulant and immunological properties of anti-prothrombin antibodies in patients with antiphospholipid antibodies. *Thromb. Haemost.* **77**, 486.
139. Malia, R. G., Brooksfield, C., Bulman, I., and Greaves, M. (1997). Prothrombin fragment F1-2: The epitope for antiphospholipid antibody expression. *Thromb. Haemost.* **171**(Suppl.), 689.
140. Puurunen, M., Vaarala, O., Julkunen, H., Aho, K., and Palosuo, T. (1996). Antibodies to phospholipid-binding plasma proteins and occurrence of thrombosis in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **80**, 16.

141. Bertolaccini, M. L., Atsumi, T., Khamashta, M. A., Amengual, O., and Hughes, G. R. V. (1998). Autoantibodies to human prothrombin and clinical manifestations in 207 patients with systemic lupus erythematosus. *J. Rheumatol.* **25**, 1104.
142. Sorice, M., Pittoni, V., Circella, A., Misasi, R., Conti, F., Longo, A., Pontieri, G. M., and Valesini, G. (1998). Antiprothrombin but not "pure" anti-cardiolipin antibodies are associated with the clinical features of the antiphospholipid antibody syndrome. *Thromb. Haemost.* **80**, 713.
143. Vaarala, O., Puurunen, M., Manttari, M., Manninen, V., Aho, K., and Palosuo, T. (1996). Antibodies to prothrombin imply a risk of myocardial infarction in middle-aged men. *Thromb. Haemost.* **75**, 456.
144. Palosuo, T., Virtamo, J., Haukka, J., Taylor, P. R., Aho, K., Puurunen, M., and Vaarala, O. (1997). High antibody levels to prothrombin imply a risk of deep venous thrombosis and pulmonary embolism in middle-aged men—a nested case-control study. *Thromb. Haemost.* **78**, 1178.
145. Pengo, V., Biasiolo, A., Brocco, T., Tonetto, S., and Ruffatti, A. (1996). Autoantibodies to phospholipid-binding plasma proteins in patients with thrombosis and phospholipid-reactive antibodies. *Thromb. Haemost.* **75**, 721.
146. Horbach, D. A., van Oort, E., Donders, R. C., Derksen, R. H., and de Groot, P. G. (1996). Lupus anticoagulant is the strongest risk factor for both venous and arterial thrombosis in patients with systemic lupus erythematosus. Comparison between different assays for the detection of antiphospholipid antibodies. *Thromb. Haemost.* **76**, 916.
147. Forastiero, R. R., Martinuzzo, M. E., Cerrato, G. S., Kordich, L. C., and Carreras, L. O. (1997). Relationship of anti beta2-glycoprotein I and anti prothrombin antibodies to thrombosis and pregnancy loss in patients with antiphospholipid antibodies. *Thromb. Haemost.* **78**, 1008.
148. Swadzba, J., de Clerck, L. S., Stevens, W. J., Bridts, C. H., van Cotthem, K. A., Musial, J., Jankowski, M., and Szczeklik, A. (1997). Anticardiolipin, anti-β2-glycoprotein I, antiprothrombin antibodies, and lupus anticoagulant in patients with systemic lupus erythematosus with a history of thrombosis. *J. Rheumatol.* **24**, 1710.
149. Guerin, J., Smith, O., White, B., Sweetman, G., Feighery, C., and Jackson, J. (1998). Antibodies to prothrombin in antiphospholipid syndrome and inflammatory disorders. *Br. J. Haematol.* **102**, 896.
150. Atsumi, T., Ieko, M., Bertolaccini, M. L., Ichikawa, K., Tsutsumi, A., Matsuura, E., and Koike, T. (2000). Association of autoantibodies against the phosphatidylserine–prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. *Arthritis Rheum*. **43**, 1982.
151. Huber, R., Berendes, R., and Burger, A. (1994). Annexin V: Crystal structure and its implications on function. In "The Annexins" (S. E. Moss, Ed.), Portland Press, London.
152. Andree, H. A. M., Hermens, W. T., and Hemker, H. C. (1992). Displacement of factor Va by annexin V. In "Phospholipid Binding and Anticoagulant Action of Annexin V" (H. A. M. Andree, Ed.), Universitaire Pers Maastricht, Maastricht.
153. Krikun, G., Lockwood, C. J., Wu, X. X., Zhou, X. D., Guller, S., Calandri, C., Guha, A., Nemerson, Y., and Rand, J. H. (1994). The expression of the placental anticoagulant protein, annexin V, by villous trophoblasts: Immunolocalization and *in vitro* regulation. *Placenta* **15**, 601.
154. Rand, J. H., Wu, X. X., Guller, S., Gil, J., Guha, A., Scher, J., and Lockwood, C. J. (1994). Reduction of annexin-V (placental anticoagulant protein-I) on placental villi of women with antiphospholipid antibodies and recurrent spontaneous abortion. *Am. J. Obstet. Gynecol.* **171**, 1566.
155. Gris, J. C., Quere, I., Sanmarco, M., Boutiere, B., Mercier, E., Amiral, J., Hubert, A. M., Ripart-Neveu, S., Hoffet, M., Tailland, M. L., Rousseau, O., Monpeyroux, F., Dauzat, M., Sampol, J., Daures, J. P., Berlan, J., and Mares, P. (2000). Antiphospholipid and antiprotein syndromes in non-thrombotic, non-autoimmune women with unexplained recurrent primary early foetal loss. The Nimes Obstetricians and Haematologists Study-NOHA. *Thromb. Haemost.* **84**, 228.
156. Lakos, G., Kiss, E., Regeczy, N., Tarjan, P., Soltesz, P., Zeher, M., Bodolay, E., Szucs, G., Szakony, S., Sipka, S., and Szegedi, G. (2000). Antiprothrombin and antiannexin V antibodies imply risk of thrombosis in patients with systemic autoimmune diseases. *J. Rheumatol.* **27**, 924.
157. Nakamura, N., Ban, T., Yamaji, K., Yoneda, Y., and Wada, Y. (1998). Localization of the apoptosis-inducing activity of lupus anticoagulant in an annexin V-binding antibody subset. *J. Clin. Invest.* **101**, 1951.
158. Nakamura, N., Kuragaki, C., Shidara, Y., Yamaji, K., and Wada, Y. (1995). Antibody to annexin V has antiphospholipid and lupus anticoagulant properties. *Am. J. Haematol.* **49**, 347.
159. Rand, J. H., Wu, X. X., Andree, H. A., Lockwood, C. J., Guller, S., Scher, J., and Harpel, P. C. (1997). Pregnancy loss in the antiphospholipid-antibody syndrome—a possible thrombogenic mechanism. *N. Engl. J. Med.* **337**, 154.
160. Rand, J. H., Wu, X. X., Andree, H. A., Ross, J. B., Rusinova, E., Gascon-Lema, M. G., Calandri, C., and Harpel, P. C. (1998). Antiphospholipid antibodies accelerate plasma coagulation by inhibiting annexin-V binding to phospholipids: A "lupus procoagulant" phenomenon. *Blood* **92**, 1652.
161. Smirnov, M. D., and Esmon, C. T. (1994). Phosphatidylethanolamine incorporation into vesicles selectively enhances factor Va inactivation by activated protein C. *J. Biol. Chem.* **269**, 816.
162. Weidmann, C. E., Wallace, D. J., Peter, J. B., Knight, P. J., Bear, M. B., and Klinenberg, J. R. (1988). Studies of IgG, IgM and IgA antiphospholipid antibody isotypes in systemic lupus erythematosus. *J. Rheumatol.* **15**, 74.
163. Staub, H. L., Harris, E. N., and Khamashta, M. A. (1989). Antibody to phosphatidylethanolamine in a patient with lupus anticoagulant and thrombosis. *Ann. Rheum. Dis.* **48**, 166.

164. Falcon, C. R., Hoffer, A. M., and Carreras, L. O. (1990). Evaluation of the clinical and laboratory associations of antiphosphatidylethanolamine antibodies. *Thromb. Res.* **59**, 383.
165. Bertolaccini, M. L., Roch, B., Amengual, O., Atsumi, T., Khamashta, M. A., and Hughes, G. R. V. (1998). Multiple antiphospholipid tests do not increase the diagnostic yield in antiphospholipid syndrome. *Br. J. Rheumatol.* **37**, 1229.
166. Sugi, T., and McLntyre, J. A. (1996). Phosphatidylethanolamine induces specific conformational changes in the kininogens recognizable by antiphosphatidylethanolamine antibodies. *Thromb. Haemost.* **76**, 354.
167. McIntyre, J. A., and Wagenknecht, D. R. (2000). Antiphosphatidylethanolamine (aPE) antibodies: A survey. *J. Autoimmun.* **15**, 185.
168. Reverdiau-Moalic, P., Delahousse, B., Body, G., Bardos, P., Leroy, J., and Gruel, Y. (1996). Evolution of blood coagulation activators and inhibitors in the healthy human fetus. *Blood.* **88**, 900.
169. Berard, M., Chantome, R., Marcelli, A., and Boffa, M. C. (1996). Antiphosphatidylethanolamine antibodies as the only antiphospholipid antibodies. I. Association with thrombosis and vascular cutaneous diseases. *J. Rheumatol.* **23**, 1369.
170. Boffa, M. C., Berard, M., Sugi, T., and McIntyre, J. A. (1996). Antiphosphatidylethanolamine antibodies as the only antiphospholipid antibodies detected by ELISA. II. Kininogen reactivity. *J. Rheumatol.* **23**, 1375.
171. Sugi, T., Katsunuma, J., Izumi, S., McIntyre, J. A., and Makino, T. (1999). Prevalence and heterogeneity of antiphosphatidylethanolamine antibodies in patients with recurrent early pregnancy losses. *Fertil. Steril.* **71**, 1060.
172. Jones, D. W., Gallimore, M. J., Harris, S. L., and Winter, M. (1999). Antibodies to factor XII associated with lupus anticoagulant. *Thromb. Haemost.* **81**, 387.
173. Shi, W., Chong, B., Hogg, P., and Chesterman, C. (1993). Anticardiolipin antibodies block the inhibition by β2-glycoprotein I of the factor Xa generating activity of platelets. *Thromb. Haemost.* **70**, 342.
174. Nomura, S., Fukuhara, S., Komiyama, Y., Takahashi, H., Matsuura, E., Nakagaki, T., Funatsu, A., Sugo, T., Matsuda, M., and Koike, T. (1994). β2-Glycoprotein I and anticardiolipin antibody influence factor Xa generation but not factor Xa binding to platelet-derived microparticles. *Thromb. Haemost.* **71**, 526.
175. Esmon, C. T., and Owen, W. G. (1981). Identification of an endothelial cell cofactor for thrombin-catalysed activation of protein C. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2249.
176. Esmon, N. L., Owen, W. G., and Esmon, C. T. (1982). Isolation of a membrane-bound cofactor for thrombin-catalyzed activation of protein C. *J. Biol. Chem.* **257**, 859.
177. Ye, J., Esmon, N. L., Esmon, C. T., and Johnson, A. E. (1991). The active site of thrombin is altered upon binding to thrombomodulin. Two distinct structural changes are detected by fluorescence, but only one correlates with protein C activation. *J. Biol. Chem.* **266**, 23016.
178. Suzuki, K., Stenflo, J., Dahlbäck, B., and Teodorsson, B. (1983). Inactivation of human coagulation Factor V by activated protein C. *J. Biol. Chem.* **258**, 1914.
179. Dahlback, B. (1991). Protein S and C4b-binding protein: Components involved in the regulation of the protein C anticoagulant system. *Thromb. Haemost.* **66**, 49.
180. de Groot, P. G., and Derksen, R. H. W. M. (1994). Protein C pathway, antiphospholipid antibodies and thrombosis. *Lupus.* **3**, 229.
181. Cariou, R., Tobelem, G., Soria, C., and Caen, J. (1986). Inhibition of protein C activation by endothelial cells in the presence of lupus anticoagulant. *N. Engl. J. Med.* **314**, 1193.
182. Cariou, R., Tobelem, G., Bellucci, S., Soria, J., Soria, C., Maclouf, J., and Caen, J. (1988). Effect of lupus anticoagulant on antithrombotic properties of endothelial cells—Inhibition of thrombomodulin-dependent protein C activation. *Thromb. Haemost.* **60**, 54.
183. Taskiris, D. A., Settas, L., Makris, P. E., and Marbet, G. A. (1990). Lupus anticoagulant—antiphospholipid antibodies and thrombophilia. Relation to protein C-protein S-thrombomodulin. *J. Rheumatol.* **17**, 785.
184. Marciniak, E., and Romond, E. H. (1989). Impaired catalytic function of activated protein C: A new *in vitro* manifestation of lupus anticoagulant. *Blood* **71**, 2426.
185. Malia, R. G., Kitchen, S., Greaves, M., and Preston, F. E. (1990). Inhibition of activated protein C and its cofactor protein S by antiphospholipid antibodies. *Br. J. Haematol.* **76**, 101.
186. Ieko, M., Ichikawa, K., Triplett, D. A., Matsuura, E., Atsumi, T., Sawada, K., and Koike, T. (1999). Beta2-glycoprotein I is necessary to inhibit protein C activity by monoclonal anticardiolipin antibodies. *Arthritis Rheum.* **42**, 167.
187. Atsumi, T., Khamashta, M. A., Amengual, O., and Hughes, G. R. V. (1997). Up-regulated tissue factor expression in antiphospholipid syndrome. *Thromb. Haemost.* **77**, 222.
188. Merrill, J. T., Zhang, H. W., Shen, C., Antonov, I. V., Lahita, R. G., and Myones, B. L. (1997). Fluid phase interaction between the anticoagulant protein S and β2-glycoprotein I: Inhibition of protein S capture by immobilized C4b binding protein. *Arthritis Rheum.* **40**, S300.
189. Colucci, M., Balconi, G., Lorenzet, R., and Locati, P. D. (1983). Cultured human endothelial cells generate tissue factor in response to endotoxin. *J. Clin. Invest.* **71**, 1893.
190. Herbert, J. M., Savi, P., Laplace, M. C., and Lale, A. (1992). IL-4 inhibits LPS-, IL-1 beta- and TNF alpha-induced expression of tissue factor in endothelial cells and monocytes. *FEBS Lett.* **310**, 31.
191. Nemerson, Y. (1988). Tissue factor and hemostasis. *Blood* **71**, 1.
192. Peterson, L. C., Valentin, S., and Hedner, U. (1995). Regulation of the extrinsic pathway system in health and disease: The role of factor VIIa and tissue factor pathway inhibitor. *Thrombo. Res.* **79**, 1.

193. Amengual, O., Atsumi, T., Khamashta, M. A., and Hughes, G. R. V. (1998). The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. *Thromb. Haemost.* **79**, 276.
194. Branch, D. W., and Rodgers, G. M. (1993). Induction of endothelial cell tissue factor activity by sera from patients with antiphospholipid syndrome: A possible mechanism of thrombosis. *Am. J. Obstet. Gynecol.* **168**, 206.
195. Kornberg, A., Blank, M., Kaufman, S., and Shoenfeld, Y. (1994). Induction of tissue factor-like activity in monocytes by anti-cardiolipin antibodies. *J. Immunol.* **153**, 1328.
196. Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., and Masaki, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* **332**, 411.
197. Levin, E. R. (1995). Endothelins. *N. Engl. J. Med.* **333**, 356.
198. Nakamura, S., Naruse, M., Naruse, K., Demura, H., and Uemura, H. (1990). Immunocytochemical localization of endothelin in cultured bovine endothelial cells. *Histochemistry* **94**, 475.
199. Marsen, T. A., Simonson, M. S., and Dunn, M. J. (1995). Thrombin induces the preproendothelin-1 gene in endothelial cells by a protein tyrosine kinase-linked mechanism. *Cir. Res.* **76**, 987.
200. Inoue, A., Yanagisawa, M., Takuwa, Y., Mitsui, Y., Kobayashi, M., and Masaki, T. (1989). The human preproendothelin-1 gene. *J. Biol. Chem.* **264**, 14954.
201. Atsumi, T., Khamashta, M. A., Haworth, R. S., Brooks, G., Amengual, O., Ichikawa, K., Koike, T., and Hughes, G. R. V. (1998). Arterial disease and thrombosis in the antiphospholipid syndrome: A pathogenic role for endothelin 1. *Arthritis Rheum.* **41**, 800.
202. Pierangeli, S. S., Colden-Stanfield, M., Liu, X., Barker, J. H., Anderson, G. L., and Harris, E. N. (1999). Antiphospholipid antibodies from antiphospholipid syndrome patients activate endothelial cells *in vitro* and in vivo. *Circulation* **99**, 1997.
203. Atsumi, T., Khamashta, M. A., Andujar, C., Leandro, M. J., Amengual, O., Ames, P. R. and Hughes, G. R. V. (1998). Elevated plasma lipoprotein(a) level and its association with impaired fibrinolysis in patients with antiphospholipid syndrome. *J. Rheumatol.* **25**, 69.
204. Yamazaki, M., Asakura, H., Jokaji, H., Saito, M., Uotani, C., Kumabashiri, I., Morishita, E., Aoshima, K., Ikeda, T., and Matsuda, T. (1994). Plasma levels of lipoprotein(a) are elevated in patients with the antiphospholipid antibody syndrome. *Thromb. Haemost.* **71**, 424.
205. Jurado, M., Paramo, J. A., Gutierrez-Pimentel, M., and Rocha, E. (1992). Fibrinolytic potential and antiphospholipid antibodies in systemic lupus erythematosus and other connective tissue disorders. *Thromb. Haemost.* **68**, 516.
206. Vu, T. K., Hung, D. T., Wheaton, V. I., and Coughlin, S. R. (1991). Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64**, 1057.
207. Gharavi, A. E., Pierangeli, S. S., Colden-Stanfield, M., Liu, X. W., Espinola, R. G., and Harris, E. N. (1999). GDKV-induced antiphospholipid antibodies enhance thrombosis and activate endothelial cells in vivo and in vitro. *J. Immunol.* **163**, 2922.
208. Dunoyer-Geindre, S., Kruithof, E. K., Galve-de Rochemonteix, B., Rosnoblet, C., Gruenberg, J., Reber, G., and de Moerloose, P. (2001). Localization of β2-glycoprotein 1 in late endosomes of human endothelial cells. *Thromb. Haemost.* **85**, 903.
209. Hawihorne, J. N., and Ansell, G. B. (1982). "Phospholipids." Elsevier Biomedical Press, Amsterdam.
210. Fadok, V. A., Voelker, D. R., Campbell, P. A., Cohen, J. J., Bratton, D. L., and Henson, P. M. (1992). Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *J. Immunol.* **148**, 2207.
211. Savill, J., Fadok, V., Henson, P., and Haslett, C. (1993). Phagocyte recognition of cells undergoing apoptosis. *Immunol. Today* **14**, 131.
212. Price, B. E., Rauch, J., Shia, M. A., Walsh, M. T., Lieberthal, W., Gilligan, H. M., O'Laughlin, T., Koh, J. S., and Levine, J. S. (1996). Anti-phospholipid autoantibodies bind to apoptotic, but not viable, thymocytes in a β2-glycoprotein I-dependent manner. *J. Immunol.* **157**, 2201.
213. Pittoni, V., Ravirajan, C. T., Donohoe, S., Machin, S. J., Lydyard, P. M., and Isenberg, D. A. (2000). Human monoclonal anti-phospholipid antibodies selectively bind to membrane phospholipid and β2-glycoprotein I (β2-GPI) on apoptotic cells. *Clin. Exp. Immunol.* **119**, 533.
214. Urowitz, M. B., Bookman, A. A., Koehler, B. E., Gordon, D. A., Smythe, H. A., and Ogryzlo, M. A. (1976). The bimodal mortality pattern of systemic lupus erythematosus. *Am. J. Med.* **60**, 221.
215. Homcy, C. J., Liberthson, R. R., Fallon, J. T., Gross, S., and Miller, L. M. (1982). Ischemic heart disease in systemic lupus erythematosus in the young patient: Report of six cases. *Am. J. Cardiol.* **49**, 478.
216. Asherson, R. A., Harris, N., Gharavi, A., and Hughes, G. R. V. (1986). Myocardial infarctio in systemic lupus erythematosus and "lupus-like" disease. *Arthritis Rheum.* **29**, 1292.
217. Asherson, R. A., Chan, J. K., Harris, E. N., Gharavi, A. E., and Hughes, G. R. (1985). Anticardiolipin antibody, recurrent thrombosis, and warfarin withdrawal. *Ann. Rheum. Dis.* **44**, 823.
218. Pauzner, R., Rosner, E., and Many, A. (1986). Circulating anticoagulant in systemic lupus erythematosus: Clinical manifestations. *Acta Haematol.* **76**, 90.
219. Jungers, P., Liote, F., Dautzenberg, M. D., Gazengel, C., Dougados, M., Tron, F., and Bach, J. F. (1984). Lupus anticoagulant and thrombosis in systemic lupus erythematosus. *Lancet.* **1**, 574.
220. Doherty, N. E., and Siegel, R. J. (1985). Cardiovascular manifestations of systemic lupus erythematosus. *Am. Heart J.* **110**, 1257.
221. Brown, M. S., and Goldstein, J. L. (1994). The hyperlipoproteinemias and other disorders of lipid metabo-

lism. In "Harrison's Principles of Internal Medicine" (K. D. Isselbacher, Ed.), 13th ed. McGraw-Hill, New York.

222. Witztum, J. L., and Steinberg, D. (1991). Role of oxidized low density lipoprotein in atherogenesis. *J. Clin. Invest.* **88**, 1785.
223. Goldstein, J. L., Ho, Y. K., Basu, S. K., and Brown, M. S. (1979). Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 333.
224. O'Brien, K. D., Alpers, C. E., Hokanson, J. E., Wang, S., and Chait, A. (1996). Oxidation-specific epitopes in human coronary atherosclerosis are not limited to oxidized low-density lipoprotein. *Circulation* **94**, 1216.
225. Palinski, W., Yla-Herttuala, S., Rosenfeld, M. E., Butler, S. W., Socher, S. A., Parthasarathy, S., Curtiss, L. K., and Witztum, J. L. (1990). Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* **10**, 325.
226. Salonen, J. T., Yla-Herttuala, S., Yamamoto, R., Butler, S., Korpela, H., Salonen, R., Nyyssonen, K., Palinski, W., and Witztum, J. L. (1992). Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* **339**, 883.
227. Steinbrecher, U. P., Parthasarathy, S., Leake, D. S., Witztum, J. L., and Steinberg, D. (1984). Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3883.
228. Vaarala, O., Alfthan, G., Jauhiainen, M., Leirisalo-Repo, M., Aho, K., and Palosuo, T. (1993). Crossreaction between antibodies to oxidised low-density lipoprotein and to cardiolipin in systemic lupus erythematosus. *Lancet* **341**, 923.
229. Amengual, O., Atsumi, T., Khamashta, M. A., Tinahones, F., and Hughes, G. R. V. (1997). Autoantibodies against oxidised low-density lipoprotein in antiphospholipid syndrome. *Br. J. Rheumatol.* **36**, 964.
230. Tinahones, F. J., Guadrado, M. J., Khamashta, M. A., Mujic, F., Gomez-Zumaquero, J. M., Collantes, E., and Hughes, G. R. V. (1998). Lack of cross-reaction between antibodies to beta2-glycoprotein-I and oxidized low-density lipoprotein in patients with antiphospholipid syndrome. *Br. J. Rheumatol.* **37**, 746.
231. Romero, F. I., Amengual, O., Atsumi, T., Khamashta, M. A., Tinahones, F. J., and Hughes, G. R. V. (1998). Arterial disease in lupus and secondary antiphospholipid syndrome: Association with anti-beta2-glycoprotein I antibodies but not with antibodies against oxidized low-density lipoprotein. *Br. J. Rheumatol.* **37**, 883.
232. Hasunuma, Y., Matsuura, E., Makita, Z., Katahira, T., Nishi, S., and Koike, T. (1997). Involvement of β2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. *Clin. Exp. Immunol.* **107**, 569.
233. Hörkkö, S., Miller, E., Dudl, E., Reaven, P., Curtiss, L. K., Zvaifler, N., Terkeltaub, R., Pierangeli, S., Branch, D. W., Palinski, W., and Witztum, J. L. (1996). Antiphospholipid antibodies are directed against epitopes of oxidized phospholipids. *J. Clin. Invest.* **98**, 815.
234. Hörkko, S., Miller, E., Branch, D. W., Palinski, W., and Witztum, J. L. (1997). The epitopes for some antiphospholipid antibodies are adducts of oxidized phospholipid and beta2 glycoprotein 1 (and other proteins). *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10356.
235. Hörkko, S., bird, D. A., Miller, E., Itabe, H., Leitinger, N., Subbanagounder, G., Berliner, J. A., Friedman, P., Dennis, E. A., Curtiss, L. K., Palinski, W., and Witztum, J. L. (1999). Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid–protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. *J. Clin. Invest.* **103**, 117.
236. Watson, A. D., Leitinger, N., Navab, M., Faull, K. F., Horkko, S., Witztum, J. L., Palinski, W., Schwenke, D., Salomon, R. G., Sha, W., Subbanagounder, G., Fogelman, A. M., and Berliner, J. A. (1997). Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo. *J. Biol. Chem.* **272**, 13597.
237. Kobayashi, K., Matsuura, E., Liu, Q., Furukawa, J., Kaihara, K., Inagaki, J., Atsumi, T., Sakairi, N., Yasuda, T., Voelker, D. R., and Koike, T. (2001). A specific ligand for beta(2)-glycoprotein I mediates autoantibody-dependent uptake of oxidized low density lipoprotein by macrophages. *J. Lipid. Res.* **42**, 697.
238. Griffith, R. L., Virella, G. T., Stevenson, H. C., and Lopes-Virella, M. F. (1988). Low density lipoprotein metabolism by human macrophages activated with low density lipoprotein immune complexes. A possible mechanism of foam cell formation. *J. Exp. Med.* **168**, 1041.
239. Lopes-Virella, M. F., Binzafar, N., Rackley, S., Takei, A., La Via, M., and Virella, G. (1997). The uptake of LDL-IC by human macrophages: Predominant involvement of the Fc gamma RI receptor. *Atherosclerosis* **135**, 161.
240. Khoo, J. C., Miller, E., Pio, F., Steinberg, D., and Witztum, J. L. (1992). Monoclonal antibodies against LDL further enhance macrophage uptake of LDL aggregates. *Arterioscler. Thromb.* **12**, 1258.
241. Kiener, P. A., Rankin, B. M., Davis, P. M., Yocum, S. A., Warr, G. A., and Grove, R. I. (1995). Immune complexes of LDL induce atherogenic responses in human monocytic cells. *Arterioscler. Thromb. Vasc. Biol.* **15**, 990.
242. Morganelli, P. M., Rogers, R. A., Kitzmiller, T. J., and Bergeron, A. (1995). Enhanced metabolism of LDL aggregates mediated by specific human monocyte IgG Fc receptors. *J. Lipid Res.* **36**, 714.
243. George, J., Afek, A., Gilburd, B., Blank, M., Levy, Y., Aron-Maor, A., Levkovitz, H., Shaish, A., Goldberg, I., Kopolovic, J., Harats, D., and Shoenfeld, Y. (1998). Induction of early atherosclerosis in LDL-receptor-deficient mice immunized with β2-glycoprotein I. *Circulation* **98**, 1108.
244. George, J., Harats, D., Gilburd, B., Afek, A., Levy, Y., Schneiderman, J., Barshack, I., Kopolovic, J., and Shoenfeld, Y. (1999). Immunolocalization of beta2-

glycoprotein I (apolipoprotein H) to human atherosclerotic plaques: Potential implications for lesion progression. *Circulation* **99**, 2227.
245. Branch, D. W., Scott, J. R., Kochenour, N. K., and Hershgold, E. (1985). Obstetric complications associated with the lupus anticoagulant. *N. Engl. J. Med*. **313**, 1322.
246. Lubbe, W. F., Butler, W. S., Palmer, S. J., and Linggins, G. C. (1984). Lupus anticoagulant in pregnancy. *Br. J. Obstet. Gynaecol*. **91**, 357.
247. Nayar, R., and Lage, J. M. (1994). Placental changes in a first trimester missed abortion in maternal systemic lupus erythematosus with antiphospholipid syndrome; a case report and review of the literature. *Hum. Pathol*. **27**, 201.
248. Out, H. J., Bruinse, H. W., Christiaens, G. C., van Vliet, M., Meilof, J. F., de Groot, P. G., Smeenk, R. J., and Derksen, R. H. (1991). Prevalence of antiphospholipid antibodies in patients with fetal loss. *Ann. Rheum. dis*. **50**, 553.
249. Salafia, C. M., and Parke, A. L. (1997). Placental pathology in systemic lupus erythematosus and phospholipid antibody syndrome. *Rheum. Dis. Clin. North Am*. **23**, 85.
250. di Somone, N., Meroni, P. L., de Papa, N., Raschi, E., Caliandro, D., De Carolis, C. S., Khamashta, M. A. Atsumi, T., Hughes, G. R., Balestrieri, G., Tincani, A., Casali, P., and Caruso, A. (2000). Antiphospholipid antibodies affect trophoblast gonadotropin secretion and invasiveness by binding directly and through adhered beta2-glycoprotein I. *Arthritis Rheum*. **43**, 140.

40

THE CLINICAL ASPECTS OF THE ANTIPHOSPHOLIPID SYNDROME

Munther A. Khamashta
Graham R. V. Hughes

The antiphospholipid syndrome (APS) is a noninflammatory autoimmune disease. The most critical pathologic process is thrombosis, which results in most of the clinical features suffered by these patients. Recurrent thrombosis together with an adverse pregnancy history and the presence of persistently elevated levels of anti-phospholipid antibodies (aPL) defines the syndrome [1].

The last decade saw a rapid increase in worldwide interest related to APS, firstly because the biological peculiarity of the disease in which autoantibodies cause thrombosis, and secondly because APS is recognized as one of the most frequent causes of acquired thrombophilia [2].

DIAGNOSTIC APPROACH TO THE ANTIPHOSPHOLIPID SYNDROME

The diagnosis of APS is first and foremost clinical (the patient must have one or more thrombotic or obstetric features of the condition). Laboratory testing for aPL is used to confirm or refute the diagnosis. The 1999 International Consensus Statement on preliminary classification criteria for definite APS provides simplified criteria for the classification of APS (Table 1) [3]. These criteria have been validated [4]. A patient with APS must manifest at least one of two clinical criteria (vascular thrombosis or pregnancy morbidity) and at least one of two laboratory criteria (positive lupus anticoagulant (LA) or medium-to-high titer β_2-glycoprotein I-dependent IgG or IgM anti-cardiolipin antibody (aCL), confirmed on two separate occasions, at least 6 weeks apart). Many of the patients reported to have the syndrome have SLE and can be regarded as having secondary APS. Some patients do not have any underlying systemic disease. These patients may be regarded as having primary APS [5]. For research and classification purposes, the term primary is useful, although there appear to be few differences in complications related to aPL antibody or in antibody specificity in the presence or absence of SLE [6, 7]. Although some patients with primary APS progress to systemic lupus, most do now show such progression [8].

Whereas LA is reported as being positive or negative, aCL are reported in terms of international units (designated GPL for IgG binding and MPL for IgM binding). In most laboratories there is substantial concordance between LA activity and aCL antibodies, with approximately 70% of patients with definite APS having both LA and aCL antibodies. However, these antibodies may not be identical, either because they detect different epitopes altogether, or because they have different affinities to various epitopes in different test systems. Because there is no definitive association of specific clinical manifestations with particular aPL, both LA and aCL testing should be routinely carried out in seeking the diagnosis of APS.

The aCL is the more sensitive test for APS, while LA is more specific. However, the specificity of aCL antibodies for APS increases with an increasing antibody titer [9]. Low positive aCL results should be viewed with

TABLE 1 International Consensus Statement on Preliminary Criteria for the Classification of the Antiphospholipid Syndrome

Clinical criteria

1. Vascular thrombosis
   One or more clinical episodes of arterial, venous, or small vessel thrombosis, occurring within any tissue or organ. With the exception of superficial venous thrombosis, thrombosis must be confirmed by imaging or Doppler studies or histopathology. For histopathological confirmation, thrombosis should be present without significant evidence of inflammation in the vessel wall.
2. Pregnancy morbidity
   A. One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation, or
   B. One or more premature births of a morphologically normal neonate at or before the 34th week of gestation, or
   C. Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation.

Laboratory criteria

1. Anti-cardiolipin antibodies
   Anti-cardiolipin antibodies of IgG and/or IgM isotype in blood, present in medium or high titer, on two or more occasions, at least 6 weeks apart, measured by a standardized enzyme-linked immunosorbent assay for ß$_2$-Glycoprotein I-dependent anti-cardiolipin antibodies.
2. Lupus anticoagulant
   Lupus anticoagulant present in plasma, on two or more occasions at least 6 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis in the following steps:
   - Prolonged phospholipid-dependent coagulation demonstrated on a screening test, for example, activated partial thromboplastin time, kaolin clotting time, dilute Russell's viper venom time, dilute prothrombin time, textarin time.
   - Failure to correct the prolonged coagulation time on the screening test by mixing with normal, platelet-poor plasma.
   - Shortening or correction of the prolonged coagulation time on the screening test by addition of excess phospholipid.
   - Exclusion of other coagulopathies, for example, factor VIII inhibitor, or heparin, as appropriate.

[a] Antiphospholipid syndrome is considered definite when at least one of the clinical criteria and one of the laboratory criteria are met. Modified from Reference 3.

suspicion; they may be found in up to 5% of normal individuals and should not be used to make the diagnosis of APS.

The demonstration that autoimmune aCL are directed against a β_2-glycoprotein I or an epitope formed by the interaction of phospholipids and β_2-glycoprotein I led to the development of assays for anti-β_2-glycoprotein I antibodies and other phospholipid binding plasma proteins [10]. A number of studies have highlighted the significance of testing for antibodies directed to β_2-glycoprotein I and prothrombin–phosphatidylserine complex as an alternative enzyme-linked immunosorbent assay with higher specificity than the conventional aCL assay [11, 12]. Because of the lack of standardization of these assays, their immediate application remains questionable. However, some authorities recommend testing for anti-β_2-glycoprotein I in patients strongly suspected to have APS, but in whom tests for LA and aCL are negative [13, 14].

Clinicians should recognize that the International Consensus Criteria were developed primarily for research purposes to ensure more uniform characterization, as well as subcategorization, of patients included in studies. We view this objective as crucial for credible investigative efforts and for appreciation of subtleties of treatment. As with other autoimmune conditions, such as SLE, there are individuals who present with one or more clinical or laboratory features suggestive of APS but in whom the diagnosis cannot be made by the relatively strict International Consensus Criteria. In such cases, experienced clinical judgement is required for best care.

CLINICAL FEATURES

Thrombosis

Arterial and venous thrombosis can be present in APS, distinguishing this from other prothrombotic states such as protein C, S, or anti-thrombin III deficiency, where only venous thrombosis occurs. Any organ and any size of vessel (small, medium, or large) can be affected; thus, the range of clinical features is extremely wide.

In the venous circulation, thrombosis of the deep veins of the lower extremities has been reported most frequently (occasionally after the use of oral contraceptive pills containing estrogen). It is often recurrent and may be accompanied by pulmonary embolism. It has been estimated that up to 19% of patients with deep vein thrombosis and/or pulmonary thromboembolism are suffering from aPL coagulopathy and may demonstrate a positive LA test, aCL, or both. Other reported sites of thrombosis include the axillary, ocular, renal, hepatic and sagittal veins, and the inferior vena cava.

The APS is now considered one of the most frequent causes of the Budd-Chiari syndrome [15]. aPL have been implicated in the development of adrenal vein thrombosis leading to Addison's disease [16]. Venous events usually occur at single sites and these can recur at the same or different sites, months or years apart.

Unlike other known clotting disorders, arterial thromboses are a major feature of the APS. Occlusions of the intracranial arteries has been reported most frequently, with the majority of patients presenting with stroke and transient ischemic attacks (TIAs). Other arterial thromboses have involved the retina, coronary, mesenteric, and peripheral arteries. The clinical presentation depends on the anatomic site occluded. Malignant hypertension with renal insufficiency secondary to thrombosis of the renal glomeruli and renal thrombotic microangiopathy (without classic lupus nephritis) has also been associated with the presence of aPL [17]. As with venous thrombosis, arterial events occur at single sites usually and can recur months or years later.

Central Nervous System Manifestations

Central nervous system (CNS) involvement is common in patients with APS. The original description of the syndrome in 1983, stressed the importance of cerebral features in these patients [18] and highlighted the frequency of intractable headache or migraine, epilepsy, chorea, and cerebrovascular accidents (TIAs or visual field defects or progressive cerebral ischemia) [19]. Although the mechanism of neurological involvement in patients with APS is thought to be thrombotic in origin, a number of other neuropsychiatric manifestations cannot be explained solely by hypercoagulability. Table 2 summarises CNS manifestations reported to be associated with the presence of aPL.

TABLE 2 Central Nervous System Manifestations Associated with the Presence of Antiphospholipid Antibodies

Cerebrovascular disease
Transient ischemic attacks
Ischemic strokes
Acute ischemic encephalopathy
Cerebral venous thrombosis
Epilepsy
Headache
Chorea
Multiple sclerosis
Transverse myelitis
Idiopathic intracranial hypertension
Other neurological syndromes
Sensorineural hearing loss
Guillain-Barre syndrome
Transient global amnesia
Ocular syndromes
Dystonia-Parkinsonism
Cognitive dysfunction
Dementia
Other psychiatric disorders
Depression
Psychosis

Stroke and TIAs are the most common neurological complications of APS. These are also the most frequently reported presentation of arterial thrombosis in APS [20]. The association between cerebrovascular disease and aPL was described in the early studies [21, 22] and these antibodies are now internationally recognized as an important etiological factor and may be present in up to 7% of all patients who have suffered a stroke [23]. They should be sought especially in young patients with strokes, where they may account for up to 18% [24]. A growing body of evidence supports an association between aPL and ischemic stroke not only in SLE and/or APS but in unselected populations as well [25].

An ischemic stroke can be isolated or multiple and recurrent. The risk of recurrent stroke appears to be increased in APS and patients occasionally present with multi-infarct dementia [26, 27]. It is unclear whether the presence of aCL at the time of initial stroke increases the risk of recurrence in an unselected population. Levine *et al*. [28] examined in a prospective study 81 consecutive patients with aPL who developed focal cerebral ischemia. The mean age of this cohort was younger than the average atherothromboembolic stroke victim with women being more commonly affected than men. Significantly, the frequency of conventional stroke risk factors was lowest in the group of stroke patients with the highest levels of IgG aCL reactivity. Moreover, patients with highest IgG aCL had the shortest times to subsequent thrombo-occlusive events, mainly represented by cerebral infarction often occurring within the first year of follow-up, supporting that IgG aCL represented a risk factor for recurrent stroke.

Angiograms typically demonstrate thrombotic occlusion of intracranial branch or trunk vessels, but in one-third of the cases, these studies may be normal. Magnetic resonance imaging (MRI) is consistently more sensitive than computed tomography in detecting infarcts in patients with APS [29]. Brain MRI in aPL patients with ischemic stroke shows cortical abnormalities consistent with large vessel occlusion. aPL patients often present small foci of high signal in brain white matter, which are often defined as consistent with the

presence of small vessel disease. In some cases, emboli from cardiac valvular vegetations may account for stroke or TIAs, and echocardiograms are advisable, particularly where there is no evidence of large vessel occlusion [30–33].

A less common form of cerebral thrombotic disease associated with aPL is sagittal venous sinus thrombosis [34]. As with stroke, venous sinus thrombosis in patients with APS occurs at a younger age than in individuals without aPL and shows a higher rate of postthrombosis migraine and more cerebral infarctions in imaging studies. However, other causes such as factor V Leiden mutation resulting in activated protein C resistance should be excluded.

Acute ischemia of the eye is a major feature of APS, with retinal and choroidal vessel involvement being the most common finding [35–37]. Patients usually complain of transient blurred vision or amaurosis fugax, transient diplopia, decreased vision, transient field loss associated with headache, or photopsy. It is important to distinguish this aPL-induced thrombotic retinopathy from that seen in SLE patients as a result of vasculitis or atherosclerosis. Optic neuropathy also occurs in SLE patients. One study found an association with aPL mainly when optic neuropathy was unilateral [38].

Sudden sensorineural hearing loss, often presenting as sudden deafness, can occur in some patients with APS. Toubi *et al*. [39] reported a 27% prevalence of positivity for aCL in 30 unselected patients with sudden or progressive sensorineural hearing loss. Naarendorp and Spiera [40] reported on 6 SLE patients with sudden sensorineural hearing loss; all 6 patients were positive for aCL or LA. They concluded that acute onset of sensorineural hearing loss in the presence of aPL may be a manifestation of APS, and that anticoagulation treatment was to be recommended for these patients.

Clinical syndromes mimicking multiple sclerosis (MS), mainly in its relapsing–remitting pattern, are reported to occur in association with aPL [41–43]. It can be difficult to distinguish MS from APS clinically and on MRI. Certainly the ischemic changes produced by APS in the white matter may be indistinguishable on MRI from those of MS (Fig. 1). In our experience, a careful medical history, a previous history of thrombosis or fetal loss, an abnormal localization of the lesions in brain MRI, and the response to anticoagulant therapy might be helpful in the differential diagnosis. Although controversial, we believe that testing for aPL should become routine in all patients with MS, especially if there are atypical features [41, 44, 45]. For the patient, the importance of a treatable differential diagnosis of APS cannot be understated [46].

Many other neurological abnormalities have been reported in APS, but these are not clearly linked to thrombosis. These include transverse myelopathy, chorea, seizures, Guillain-Barre syndrome, psychosis, and migraine headaches.

One of the most prominent features in patients with APS is headache. This symptom, a common complaint of APS patients in clinical practice, can vary from classic migraine to almost continuous incapacitating headache. Although important in clinical practice, the association between migraine and aPL in the published literature, is still controversial. Tietjen *et al*. [47] studied a large population, and failed to demonstrate an association between the presence of aCL and migraine in subjects younger than 60 years of age. Similar results have been reported previously [48]. Our most recent experience in applying the 1999 American College of Rheumatology criteria for neuropsychiatric syndromes to a large cohort of SLE patients (323 patients), showed a prevalence of headache similar to that of the general population, but aPL were significantly more prevalent in the group of patients with headache compared to those without headache [49]. We did not find a higher prevalence of aPL in migraine than in other types of headache. Interestingly, in some of our patients with severe headache resistant to conventional treatment,

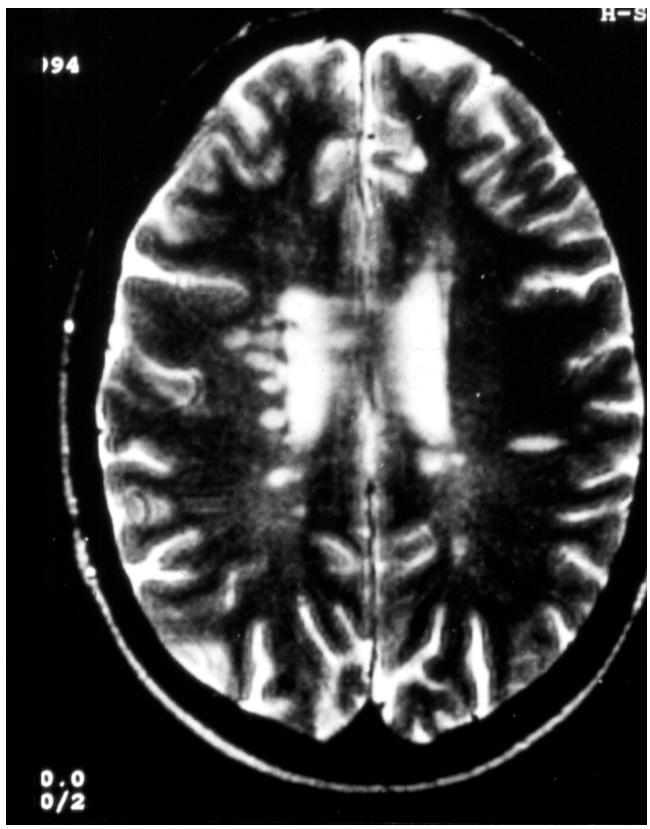


FIGURE 1 Livedo reticularis.

complete or partial remission of the headache was noticed when treatment with aspirin, heparin, or warfarin was started for thrombotic events [50, 51]. Furthermore, the relationship between migraine and stroke has been widely studied [52–55]. The predictive value of variables such as gender, the presence or absence of a family history of migraine, use of oral contraceptives, high blood pressure, diabetes, heart disease, and smoking has been analyzed and related to the development of "migrainous stroke." None of these studies analyzed the presence of aPL as an independent risk factor for stroke in their patients. In our series, 8 patients, all young women, developed stroke after some years of migraine [50]. This highlights the importance of considering the presence of aPL in patients with migrainous stroke or even with severe persistent headache, especially in young women.

Neuropsychiatric manifestations in APS ranges from mild cognitive dysfunction to severe dementia [56–59]. Patients affected by mild cognitive dysfunction often complain of poor concentration or forgetfulness. Verbal memory deficits, decreased psychomotor speed, and decreased overall productivity have been significantly correlated with elevated aPL levels. Some of these patients had never had a history of stroke and gross neurological examinations were normal. Computed tomography scans in these patients are usually normal and MRI studies frequently show scattered areas of nonspecific increased signal intensity. These areas may increase or decrease in both size and number over time and may not represent permanent injury.

The recognition of subtle forms of cognitive dysfunction has been greatly facilitated by the application of formal neuropsychological assessment mainly in patients with SLE. Menon *et al*. [59] and Hanly *et al*. [58] showed that SLE patients persistently positive for aCL (medium high titer) had significantly lower scores on a variety of neuropsychological tests than SLE patients negative for aPL. Denburg *et al*. [57] also found that LA-positive patients performed worse than LA-negative patients in measures of verbal memory, cognitive flexibility, and psychomotor speed. Some of these patients had no previous history of any neuropsychiatric involvement, suggesting a direct relationship between the presence of aPL and cognitive impairment. Whether these cognitive deficits result from recurrent cerebral ischemia or whether there are other underlying mechanisms remains unknown. Anecdotal reports of improvement of cognitive disorders after initiation of anticoagulation therapy for other reasons in APS patients, may provide some support for the theory that arterial thrombosis and/or ischemia represent the primary cause of this type of CNS dysfunction. Although MRI is a highly sensitive method for detecting cerebral lesions, new imaging techniques such as positron emission tomography or single photon emission computed tomography may provide objective information about cerebral damage and the possible underlying mechanisms in APS patients with cognitive dysfunction.

Mosek *et al*. [60] examined the relationship of aPL and dementia in the elderly. They found that 5 of the 87 (6%) demented patients, but none of the 69 controls, had significantly elevated IgG aCL levels. None of the patients had features of an immune-mediated disease. The role of aPL antibodies in these patients, with apparently diffuse brain disease, is currently unknown.

Although depression and psychosis have been associated with aPL, it had been postulated that autoantibodies, and specifically aPL, may represent an adverse response to neuroleptic treatment. Schwartz *et al*. [61] studied 34 unmedicated patients without known autoimmune disorders admitted with acute psychosis. aCL and LA were determined before and after neuroleptic treatment. They found that 32% of the unmedicated psychotic patients had positive aPL (24% IgG aCL and 9% LA). Of the 22 patients followed up after medication, 32% showed moderate titers of IgG aCL and 18% LA positivity. This study showed an increased incidence of aPL in psychotic patients and that the presence of these antibodies cannot be simply assumed to be a result of the treatment.

Seizures have been consistently associated with the presence of aPL in SLE patients and are well described in patients with primary APS [49, 62–65]. The etiology of seizures in APS patients may be associated with cerebral infarction but some authors have suggested that aPL interact directly with neuronal tissue [29]. Intriguingly, aPL can also reduce a γ-aminobutyric acid receptor-mediated chloride current in snail neurones [66]. This effect might lower seizure threshold.

Other syndromes that are rarer but no less important have been associated with the presence of aPL; for example, chorea was reported in early descriptions of the syndrome [18, 67]. In an extensive review of 50 APS patients with chorea, the role of estrogens in this disorder is highlighted (6 patients were receiving the oral contraceptive pill and 4 developed chorea during pregnancy or the puerperium) [68]. Although striatal ischemia can cause this symptom, some studies using positron emission tomography have shown increases in lentiform and caudate nucleus metabolism, which returns to normal when the patient recovers [69].

Skin Manifestations

A wide variety of cutaneous manifestations have been reported in patients with APS (Table 3), and these

very often constitute early signs of the disease. The majority of these lesions can be explained by vascular occlusion, frequently demonstrable by histopathological examination [70].

Livedo reticularis (racemosa) is the most frequently associated cutaneous manifestation in patients with the APS (Fig. 2). Livedo reticularis is a dusky, violaceous, vascular discoloration of the skin with a network pattern, which usually appears over the upper and lower limbs. The association between livedo reticularis and aPL was first reported by Hughes in 1983 [19] and was confirmed in subsequent studies [71–73]. Many cases of Sneddon's syndrome, defined as the clinical triad of stroke, livedo reticularis, and hypertension, may represent undiagnosed APS [74]. The relationship between livedo reticularis and other clinical manifestations and its value as a predictor for the risk of thrombosis need further investigation [75].

Skin ulcers are also common in APS. Histopathological analysis of the skin specimens often demonstrates thrombosis of the small vessels with vascular proliferation and minimal inflammatory changes [76]. Skin ulcers normally appear in the extremities, although they may be more widespread and sometimes leave white atrophic scars. Treatment with steroids or immunosuppression is usually ineffective, and some successfully treated cases can be explained by the coexistence of an underlying connective tissue disease with a vasculitic process [77]. Treatment with antiplatelet agents, oral anticoagulation, or even antifibrinolytic therapy can be effective [78, 79].

The prevalence of subungual splinter hemorrhages is less than 5% in patients with APS [80]. Although the causes are many, including infectious processes and vasculitis, multiple lesions in different fingers in a patient with primary or secondary APS should alert the physician to the occurrence of other thrombotic events [81].

Digital gangrene has been described in primary and secondary APS and widespread superficial skin necrosis appears in around 3% of APS patients. Its onset is

TABLE 3 Skin Manifestations Associated with the Antiphospholipid Syndrome

Livedo reticularis
Cutaneous ulceration
Cutaneous gangrene and necrosis
Livedoid vasculitis
Skin nodules
Erythematous macules
Subungual splinter hemorrhages
Thrombophlebitis
Degos-like lesions
Anetoderma

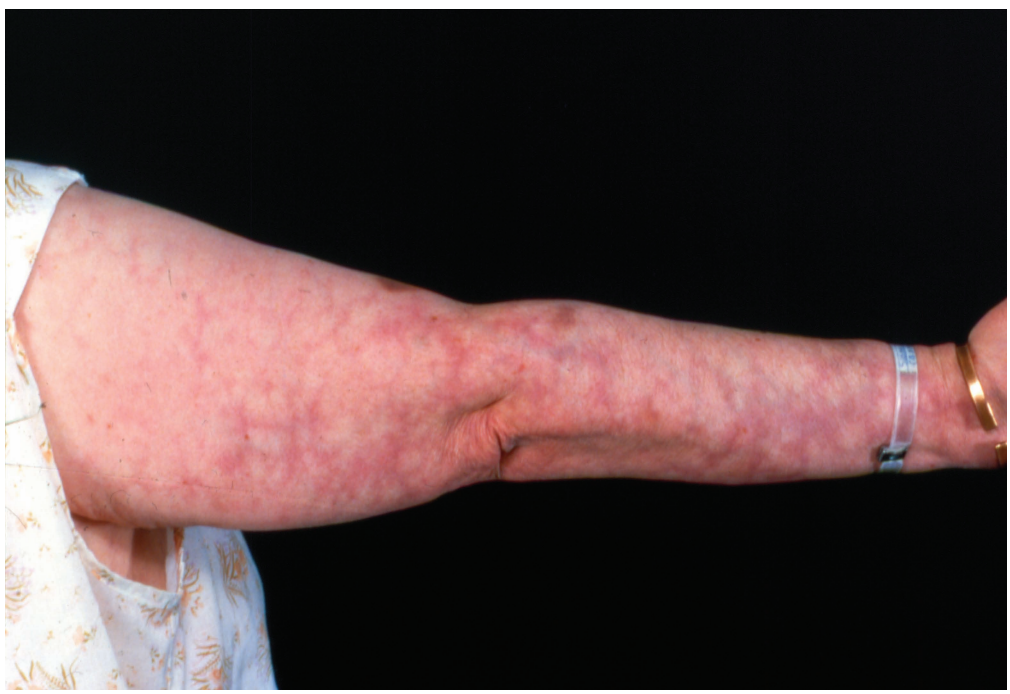


FIGURE 2 Ischemia associated with APS.

often acute; lesions are purpuric and painful and are localized to the limbs, head, and buttocks. There are several case reports of extensive skin necrosis as the first manifestation of APS [82]. Some of them occurred without any obvious trigger factor [83], but in others, skin necrosis followed the onset of infection or surgery [84].

A variety of other cutaneous lesions resembling vasculitis, including purpura, tender nodules, papules, and palmar-plantar erythema, have been described in APS [70]. Histologically, the absence of vasculitis and the finding of noninflammatory thrombosis of small arteries or veins of the dermis and hypodermis are characteristic of skin lesions in APS.

Heart Manifestations

The antiphospholipid syndrome has a variety of cardiac manifestations. The most important are valve disease, coronary artery disease, and, less commonly, cardiomyopathy and intracardiac thrombus. These lesions appear in primary and secondary APS.

A number of studies have documented the prevalence of valve disease in SLE and primary APS patients [30, 85, 86]. Valve disease is present in around 48% of patients with SLE and aPL, and in only 21% of SLE patients negative for aPL. Studies involving only patients with APS show that from 35 to 75% had valvulopathy [31, 32, 87]. In addition, aPL were significantly higher (21%) in a group of unselected patients with heart valve disease when compared with control subjects (9%) [88]. A positive correlation between levels of aPL and the presence of valve damage has been described [89]. In this study, 50% of SLE patients with high levels of aCL had valve disease compared with 37% of patients with lower levels and with only 14% of SLE patients negative for aPL [90]. There is also some data supporting the relationship between valve lesions and the presence of aPL with some evidence suggesting that markers of endothelial cell activation are upregulated in valves from APS patients [91]. Deposition of immunoglobulins in the valves suggested a possible association between this deposition and the activation of the endothelial cells in APS.

Two morphologic echocardiographic patterns can be discerned: valve masses (vegetations) and valvular thickening. The predominant functional abnormality is regurgitation, whereas stenosis is rarely seen. The mitral valve is the most commonly affected site, followed by the aortic valve. Involvement of the tricuspid or pulmonary valve has also been described [92]. Mitral or aortic regurgitation is rarely severe enough to cause symptoms and requirement for valve replacement is distinctly uncommon. Valvular abnormalities of APS is different from that seen in rheumatic heart disease. In APS valvular thickening is diffuse, whereas in rheumatic heart disease thickening is more localized, present at leaflet tips, and often associated with thickening, fusion, and calcification of the chordae tendinae.

A study described the echocardiographic lesions of 29 patients with primary APS [87]. Transesophageal echocardiography was performed in all these patients at the onset of the study and in 13 of 19 patients 1 year later. This second echocardiogram showed no changes in the lesion in 6 patients and new lesions in the remaining 7. All received anticoagulant or antiaggregant treatment during the year of the study. The authors concluded that anticoagulant or antiaggregant treatment does not diminish the size of the valve lesions, although there are anecdotal reports of vegetations resolving with high-intensity anticoagulation [93].

Coronary artery disease has been documented in APS patients. Early descriptions by Hamsten *et al*. in 1986 [94] have been confirmed by prospective studies showing that elevated levels of aCL in a non-SLE population imply an increased risk for the development of myocardial infarction [25, 95, 96]. The prevalence of aCL in patients with myocardial infarction is between 5 and 15% [97, 98]. Although this prevalence may not justify routine screening in patients with myocardial infarction, its measurement may be important in patients under the age of 45 years if they have previous venous or arterial thrombosis or an adverse pregnancy history (e.g., recurrent miscarriages, fetal death). In patients with SLE, particularly women between 35 and 44 years of age, the risk of cardiovascular events is over 10 times higher than in healthy women of similar age [99]. Around 35% of SLE patients are positive for aPL, and the contribution of aPL to the development of myocardial infarction in this population may indeed be substantial.

Different antibodies have been implicated in the development of coronary artery disease in APS patients. aCL is directed against different antigenic structures. Some of them recognize phospholipids, some recognize phospholipid binding proteins such as β_2-glycoprotein I, and some may be directed against cross-reactive epitopes common with oxidized low-density lipoprotein (LDL) [100]. The presence of antioxidized LDL is considered to be a marker of atherosclerosis. Two prospective studies have shown that these antibodies are predictive for myocardial infarction in the general population [98, 101]. aCL levels were associated with antioxidized LDL antibodies, and the joint effect of these two different populations of antibodies was additive for the risk of myocardial infarction. In another study, including a large population of patients with different manifestations of coronary artery disease (109 patients

with coronary artery bypass surgery, 106 patients with balloon angioplasty, 101 patients with acute myocardial infarction, and 99 patients with acute myocardial ischemia), it was shown that anti-oxidized LDL antibody titers were significantly higher in the group with acute myocardial infarction than in other groups in men but not in women. The titers of aCL did not differ among the patient groups. Neither of the autoantibodies was associated with recurrent coronary events. aCL were inversely correlated with dietary intake of vitamin E and polyunsaturated fat in men with coronary heart disease [102].

Kidney Manifestations

Renal involvement is a prominent feature in APS. Initially underestimated, it is currently the subject of numerous studies [103].

Hypertension is the most common clinical manifestation present in more than 40% of patients. It can be present as mild, severe, or even malignant hypertension. Pathogenic mechanisms leading to hypertension include thrombosis in a trunk of the renal artery (renovascular hypertension) and intrarenal vascular lesions [17, 104–106]. These lesions can cause hypertension via stimulation of the renin–angiotensin–aldosterone system. All patients with APS and hypertension should be investigated for renal involvement (especially renal artery stenosis), because hypertension is often the only early clinical manifestation. Although renal involvement, when it occurs, is usually chronic with renal insufficiency slowly progressing, acute renal failure can also occur. In the study by Nochy *et al*. [107], which included 16 patients with primary APS, 90% had hypertension, which was severe in 31% and malignant in 12%. Renal insufficiency was present in 87% and 2 patients had acute renal failure.

Thrombosis of renal arteries and veins has been described in APS patients [105, 108, 109]. This occlusion can be caused by different mechanisms such as *in situ* thrombosis, or in the case of arterial thrombosis, it can also be the consequence of an embolic event resulting from a damaged cardiac valve. Treatment with anticoagulant drugs or transluminal angioplasty has been successful if the problem was detected at an early stage [110].

Anti-phospholipid antibodies have been anecdotally detected in some patients with end-stage renal disease (ESRD), though the data is conflicting [111, 112]. Although Brunet *et al*. [111] found a prevalence of aPL of 31% (aCL = 15.5%, LA = 16.5%) and a clear association between the presence of LA and vascular access thrombosis, Fabrizi *et al*. [112] reported a lower prevalence of aPL (8.8%). The presence of aPL was not associated with factors such as age, cause of ESRD, time on hemodialysis, type of hemodialysis membrane, or thrombotic events. Only one patient positive for LA had recurrent thrombosis of the access graft and native veins.

Although the outcome of renal transplant in patients with SLE is not different from that of other populations, it is possible that the presence of aPL may modify the prognosis. A number of studies have reported a poor outcome, with high rates of graft loss as a result of thrombotic events in patients with SLE positive for aPL [113–115]. Most of the patients included in these studies were not fully anticoagulated. In patients with primary APS, the observed outcome has been even worse, with recurrence of the disease as a thrombotic microangiopathy in the graft despite intensive anticoagulant therapy [116]. Vaidya *et al*. [115] undertook a multicenter study, including 502 ESRD patients waiting for renal transplant. They found that 93 (19%) of these patients were positive for aCL. Only 23 of 93 cases fulfilled the criteria for APS (previous thrombotic episodes, including documented thrombotic microangiopathy or adverse pregnancy history). Eleven of these 23 patients received kidney transplants. Four were treated with oral anticoagulation, and 7 did not receive any anticoagulant drug. All 7 patients without anticoagulant treatment lost their allografts within 1 week as a result of thrombosis. Only 1 of the 4 patients treated lost the graft because of thrombosis. Interestingly, of the remaining 70 aCL-positive patients without any other feature of APS, 37 received a renal transplant, and none of them lost the allografts as a result of thrombosis. These observations suggest that all patients with APS should be fully anticoagulated as part of the posttransplant therapeutic regimen [117].

The histologic findings of the renal lesions in APS have been extensively reviewed [107, 118, 119]. Daugas *et al*. [119] delineated the clinical and histologic manifestations found in patients with APS and renal involvement. APS nephropathy is clinically manifest by a syndrome of vascular nephropathy, associating hypertension, acute and/or chronic renal insufficiency, and low grade proteinuria. Histologically, APS nephropathy is a vaso-occlusive process associating, side-by-side, acute thromboses (thormbotic microangiopathy) and chronic vascular lesions (arterial fibrous intimal hyperplasia, arteriosclerosis, and organized thromboses, with or without recanalization). The authors emphasized the importance of recognizing this characteristic histologic picture for diagnostic and therapeutic purposes. The presence of APS nephropathy in conjunction with lupus nephropathy probably augments the risk of evolution toward ESRD, because it is associated with higher creatinine levels at time of diagnosis, increased interstitial fibrosis, and systemic hypertension.

Lung Manifestations

Patients with APS may develop a broad spectrum of lung disease. Pulmonary thromboembolism and pulmonary hypertension are the most common complications, but microvascular pulmonary thrombosis, pulmonary capillaritis, and alveolar hemorrhage have also been reported [120].

Pulmonary embolism is a frequent complication of deep venous thrombosis and occurs in around 30% of APS patients [121]. It may be the first manifestation of the disease. Recurrent pulmonary embolism may lead to thromboembolic pulmonary hypertension.

The prevalence of pulmonary hypertension in APS associated with SLE and the primary APS has been estimated to 1.8 and 3.5%, respectively [6]. In most cases, the mechanism has been pulmonary emboli, although *in situ* thrombosis remains a possibility [122].

Although quite a rare manifestation, some APS patients may present with diffuse alveolar pulmonary hemorrhage. The common symptoms are fever, cough, dyspnea with or without hemoptysis, hypoxemia, and diffuse pulmonary infiltrates. The diagnosis is usually made by open lung biopsy, which shows microvascular thrombosis and secondary alveolar hemorrhage with or without pulmonary capillaritis. Treatment with corticosteroids usually leads to dramatic improvement [123].

Hematologic Manifestations

The most frequent hematologic manifestation of APS is thrombocytopenia. It is present in 25% of patients though rarely severe enough to cause hemorrhage [124]. The platelet count often remains stable for many years; then, for reasons that are often obscure, the count drops, sometimes catastrophically. Occasionally, patients with APS may present only with severe thrombocytopenia but later develop pregnancy loss or thrombosis. The exact role of aPL in thrombocytopenia is still unclear. More recent data have shown that thrombocytopenia probably results from specific anti-platelet glycoprotein antibodies [125–127]. Galli *et al*. [125] found antibodies to the specific platelet membrane glycoproteins IIb/IIIa and Ib/IX in 40% of patients positive for aPL; there was a significant correlation between these antibodies and thrombocytopenia.

Hemolytic anemia may be present in some patients with APS and is sometimes associated with the presence of thrombocytopenia, the so-called "Evans syndrome." Although a positive direct Coombs' test is not rare in APS patients (10–20%) hemolytic anemia is uncommon. A significant correlation has been found between aPL and hemolytic anemia, although the association with different isotypes of aCL is still controversial. Some authors found an association with IgM isotype [72, 128]; however, this has not been confirmed in other studies [5, 6, 129]. The pathogenic mechanisms for aPL-related hemolytic anemia are not clear. aPL may bind directly to the red blood cell membrane, but factors such as anti-erythrocyte antibodies or immune complexes fixed to red blood cells may also play a role.

Obstetric Manifestations

Recurrent pregnancy loss, typically in the second trimester, is one of the most consistent features of APS [130]. Many cases of APS are diagnosed after investigation for recurrent miscarriage. The prospective fetal loss rate in primary APS is reported to be 50 to 75% [131]. Some studies suggest this rate may be as high as 90% in patients with SLE and secondary APS [132]. The risk of fetal loss is directly related to the antibody titer, particularly the IgG aCL [9, 133], although many women with recurrent miscarriage have IgM aCL antibodies only. It is impossible to predict which women are likely to develop complications in pregnancy, and some women with persistently elevated aPL titers and a history of thromboses with or without thrombocytopenia have no fetal complications at all. Previous poor obstetric outcome remains the most important predictor of future risk [134, 135].

In pregnancies that do not end in miscarriage or fetal loss, there is a high incidence of preeclampsia, intrauterine growth restriction (IUGR), placental abruption, and premature delivery [134, 136]. It is important to note that the most recent classification criteria for APS have been amended to highlight the fact that not only fetal loss but premature birth before 34 weeks as a result of preeclampsia, placental abruption, or IUGR and positive aCL, or LA may allow the patient to be labeled as having APS [3].

Varying high rates of preeclampsia has been reported in women with APS, which contributes to the high rate of preterm delivery in this condition. Raised aCL levels have been reported in association with severe early onset preeclampsia [136]. The weight of evidence supports aPL testing in women with early onset (<34 weeks of gestation) severe preeclampsia.

Units that manage patients with severe manifestations of APS such as thrombosis and previous stillbirth [134, 137] have a higher incidence of complications in pregnancy than those that recruit women predominantly from recurrent miscarriage clinics [138, 139]. In our unit, we run a multidisciplinary team service, where most APS cases have either been identified by rheumatology (association with SLE) or hematology (previous thrombosis) colleagues. Many patients are referred for

specialist management after previous poor obstetric outcome. Our live birth rate is 70 to 80%, and in a previous study from our unit, the incidence of preeclampsia was 18%, the percentage of babies born with birth weights less than the 10th centile for gestational age was 31% and the percentage of infants delivered prematurely (<37 weeks) with a mean gestation of approximately 34 weeks was about 43% [134]. Approximately 70% of these women were delivered by cesarean section, and 7% of babies died in the neonatal period as a result of problems related to prematurity. Regular ultrasound scanning for fetal growth is recommended in these patients and, in specialist units (including our own), assessment of uterine artery Doppler waveforms is performed in the mid-trimester [140]. The presence of mid-trimester uterine artery notches in high-risk pregnancies is associated with preeclampsia, IUGR, and intrapartum asphyxia with a sensitivity of 90% and a positive predictive value of 60% [141]. In high-risk pregnancies, abnormal uterine artery Doppler velocimetry is also of some value in predicting placental abruption, a common feature of APS pregnancies, but gives no indication as to the timing of this event [142].

Catastrophic Antiphospholipid Syndrome

A minority of patients with APS present with an acute and devastating syndrome characterized by multiple simultaneous vascular occlusions throughout the body, often resulting in death. This syndrome, termed catastrophic APS, is defined by the clinical involvement of at least three different organ systems over a period of days or few weeks with histopathological evidence of multiple occlusions of large or small vessels [143]. Although the same clinical manifestations seen with primary and secondary APS occur as part of catastrophic APS, there are important differences in prevalence and in the caliber of the vessels predominantly affected. Ischemia of the kidneys, bowels, lungs, heart, and/or brain are most frequent, but rarely adrenal, testicular, splenic, pancreatic, or skin involvement have been described. Occlusion of small vessels (thrombotic microangiopathy) is characteristic resulting in symptoms related to dysfunction of the affected organs. Depending on the organs involved, patients may present with hypertension and renal impairment, acute respiratory distress syndrome, alveolar hemorrhage and capillaritis, confusion and disorientation, or abdominal pain and distension secondary to bowel infarction. Precipitating factors of catastrophic APS include infections, surgical procedures, withdrawal of anticoagulant therapy, and the use of drugs such as oral contraceptives.

Other Manifestations

Avascular necrosis of bone is an uncommon complication in lupus patients and clearly associated with high steroid dosage. We have noted an increased risk of avascular necrosis in individuals positive for aPL, possibly as a result of small arterial occlusions, notably of the head of the femur [144].

Rare cases of bone-marrow necrosis have been reported secondary to APS [145, 146].

Many patients with APS seem to develop widespread arteriopathy. The systemic narrowing of major arteries is similar in many respects to the widespread endarterial disease seen in some patients after heart–lung transplantation. Thus, aPL might be associated with accelerated vascular disease, including atherosclerosis [147]. More recent work has suggested that aPL are directed against oxidized phospholipids, following the observation that oxidation of cardiolipin is required, even in the present of β_2-glycoprotein I, for it to be recognized by purified IgG fractions and monoclonal antibodies from APS patients [148]. These findings raise the possibility that oxidized LDL, as oxidized cardiolipin, may act as an immunizing antigen leading to the generation of aPL, providing an additional link between atherogenesis and APS.

Some clinicians are convinced that aPL are associated with infertility. Additionally, some clinicians recommend that infertile women who have aPL and are undergoing *in vitro* fertilization should be treated with heparin to improve the rate of pregnancy. However, experts disagree regarding the relationship between aPL and infertility. There is also substantial evidence that treatment with heparin does not alter the rate of pregnancy following *in vitro* fertilization [149, 150].

EPIDEMIOLOGY

The epidemiology of aPL is still being investigated worldwide. Efforts are being made in clinics throughout the world to assess the importance of this factor in recurrent abortion, stroke, myocardial infarction, epilepsy, and so on. Prospective studies have shown an association between aPL and the first episode of venous thrombosis [151] the first myocardial infarction [95], and the first ischemic stroke [25]. A critical issue, therefore, is the identification of patients with aPL who are at increased risk for a thrombotic event.

Anti-phospholipid antibodies, using standardized techniques, are detected in less than 1% of apparently normal individuals and in up to 3% of the elderly population without clinical manifestations of the APS. Among patients with SLE, the prevalence of aPL is

much higher, ranging from 30 to 40% [152]. For otherwise healthy control subjects there are insufficient data to determine what percentage of those with aPL antibodies will eventually have a thrombotic event or a complication of pregnancy consistent with the APS. In contrast, APS may develop in 50–70% of patients with both SLE and aPL after 10–20 years of follow-up [153, 154].

The specificities of aPL antibodies probably differ in various disorders. Large retrospective studies of patients with thrombotic complications suggest that those with high concentrations of IgG aCL appear to be at greatest risk for thrombosis, whereas the risk of clotting appears to be much lower in patients with infection-related aPL [155].

The effect of race only has been adequately studied in African-American and White populations [154, 156, 157]. The frequency of LA and high-titer aCL is significantly less common in African-Americans than in Whites and the APS is more frequent in Arabs compared to the Indian population.

References

1. Hughes, G. R. V. (1993). The antiphospholipid syndrome: Ten years on. *Lancet* **342**, 341–344.
2. Khamashta, M. A. (2000). "Hughes Syndrome—Antiphospholipid Syndrome." Springer-Verlag London.
3. Wilson, W. A., Gharavi, A. E., Koike, T., Lockshin, M. D., Branch, D. W., Piette, J. C., Brey, R., Derksen, R., Harris, E. N., Hughes, G. R., Triplett, D. A., and Khamashta, M. A. (1999). International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: Report of an international workshop. *Arthritis Rheum.* **42**, 1309–1311.
4. Lockshin, M. D., Sammaritano, L. R., and Schwartzman, S. (2000). Validation of the Sapporo criteria for antiphospholipid syndrome. *Arthritis Rheum.* **43**, 440–443.
5. Asherson, R. A., Khamashta, M. A., Ordi-Ros, J., Derksen, R. H., Machin, S. J., Barquinero, J., Outt, H. H., Harris, E. N., Vilardell-Torres, M., and Hughes, G. R. (1989). The "primary" antiphospholipid syndrome: Major clinical and serological features. *Medicine* **68**, 366–374.
6. Vianna, J. L., Khamashta, M. A., Ordi-Ros, J., Font, J., Cervera, R., Lopez-Soto, A., Tolosa, C., Franz, J., Selva, A., Ingelmo, M., *et al*. (1994). Comparison of the primary and secondary antiphospholipid syndrome: A European Multicenter Study of 114 patients. *Am. J. Med.* **96**, 3–9.
7. Cervera, R., Piette, J. C., Font, J., Khamashta, M. A., Shoenfeld, Y., Camps, M. T., Jacobsen, S., Lakos, G., Tincani, A., Kontopoulou-Griva, I., Galeazzi, M., Meroni, P. L., Derksen, R. H., de Groot, P. G., Gromnica-Ihle, E., Baleva, M., Mosca, M., Bombardieri, S., Houssiau, F., Gris, J. C., Quere, I. I., Hachulla, E., Vasconcelos, C., Roch, B., Fernandez-Nebro, A., Boffa, M. C., Hughes, G. R., and Ingelmo, M. (2002). Antiphospholipid syndrome: Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1,000 patients. *Arthritis Rheum.* **46**, 1019–1027.
8. Mujic, F., Cuadrado, M. J., Lloyd, M., Khamashta, M. A., Page, G., and Hughes, G. R. V. (1995). Primary antiphospholipid syndrome evolving into systemic lupus erythematosus. *J. Rheumatol.* **22**, 1589–1592.
9. Harris, N. E., Chan, J. K. H., Asherson, R. A., Aber, V. R., Gharavi, A. E., and Hughes, G. R. V. (1986). Thrombosis, recurrent fetal loss and thrombocytopenia. *Arch. Intern. Med.* **146**, 2153–2156.
10. Roubey, R. A. (1994). Autoantibodies to phospholipid-binding plasma proteins: A new view of lupus anticoagulants and other antiphospholipid autoantibodies. *Blood* **84**, 2854–2867.
11. Amengual, O., Atsumi, T., Khamashta, M. A., Koike, T., and Hughes, G. R. (1996). Specificity of ELISA for antibody to beta 2-glycoprotein I in patients with antiphospholipid syndrome. *Br. J. Rheumatol.* **35**, 1239–1243.
12. Atsumi, T., Ieko, M., Bertolaccini, M. L., Ichikawa, K., Tsutsumi, A., Matsuura, E., and Koike, T. (2000). Association of autoantibodies against the phosphatidylserine–prothrombin complex with manifestations of the antiphospholipid syndrome and with the presence of lupus anticoagulant. *Arthritis Rheum.* **43**, 1982–1993.
13. Cabral, A. R., Amigo, M. C., Cabiedes, J., and Alarcon-Segovia, D. (1996). The antiphospholipid/cofactor syndrome: A primary variant with antibodies to β2 glycoprotein I but no antibodies detectable in standard antiphospholipid assay. *Am. J. Med.* **101**, 472–481.
14. Levine, J. S., Branch, D. W., and Rauch, J. (2002). The antiphospholipid syndrome. *N Engl. J. Med.* **346**, 752–763.
15. Espinosa, G., Font, J., Garcia-Pagan, J. C., Tassies, D., Reverter, J. C., Gaig, C., Cervantes, F., Cervera, R., Bosch, J., and Ingelmo, M. (2001). Budd-Chiari syndrome secondary to antiphospholipid syndrome. Clinical and immunologic characeristics of 43 patients. *Medicine* **80**, 345–354.
16. Asherson, R. A., and Hughes, G. R. (1991). Hypoadrenalism, Addison's disease and antiphospholipid antibodies. *J. Rheumatol.* **18**, 1–3.
17. Amigo, M. C., Garcia-Torres, R., Robles, M., Bochicchio, T., and Reyes, P. A. (1992). Renal involvement in the primary antiphospholipid syndrome. *J. Rheumatol.* **18**, 1181–1185.
18. Hughes, G. R. V. (1983). Thrombosis, abortion, cerebral disease, and the lupus anticoagulant. *Br. Med. J.* **287**, 1088–1089.
19. Hughes, G. R. V. (1984). The Prosser-White oration 1983. Connective tissue disease and the skin. *Clin. Exp. Dermatol.* **9**, 535–544.
20. Toubi, E., Khamashta, M. A., Panarra, A., and Hughes, G. R. (1995). Association of antiphospholipid antibodies with central nervous system disease in systemic lupus erythematosus. *Am. J. Med.* **99**, 397–401.

21. Harris, E. N., Gharavi, A. E., Asherson, R. A., Boey, M. L., and Hughes, G. R. V. (1984). Cerebral infarction in systemic lupus erythematosus: Association with anticardiolipin antibodies. *Clin. Exp. Rheumatol.* **2**, 47.
22. Asherson, R. A., Khamashta, M. A., Gil, A., Vazquez, J. J., Chan, O., Baguley, E., and Hughes, G. R. (1989). Cerebrovascular disease and antiphospholipid antibodies in systemic lupus erythematosus, lupus-like disease, and the primary antiphospholipid syndrome. *Am. J. Med.* **86**, 391–399.
23. Montalban, J., Codina, A., Ordi, J., Vilardell, M., Khamashta, M. A., and Hughes, G. R. V. (1991). Antiphospholipid antibodies in cerebral ischemia. *Stroke* **22**, 750–753.
24. Nencini, P., Baruffi, M. C., Abbate, R., Massai, G., Amaducci, L., and Inzitari, D. (1992). Lupus anticoagulant and anticardiolipin antibodies in young adults with cerebral ischemia. *Stroke* **23**, 189–193.
25. Brey, R. L., Abbott, R. D., Curb, J. D., Sharp, D. S., Ross, G. W., Stallworth, C. L., and Kittner, S. J. (2001). Beta(2)-Glycoprotein 1-dependent anticardiolipin antibodies and risk of ischemic stroke and myocardial infarction: The Honolulu heart program. *Stroke* **32**, 1701–1706.
26. Levine, S. R., Salowich-Palm, L., Sawaya, K. L., Perry, M., Spencer, H. J., Winkler, H. J., Alam, Z., and Carey, J. L. (1997). IgG anticardiolipin antibody titer >40 GPL and the risk of subsequent thrombo-occlusive events and death. A prospective cohort study. *Stroke* **28**, 1660–1665.
27. Asherson, R. A., Mercey, D., Phillips, G., Sheehan, N., Gharavi, A. E., Harris, E. N., and Hughes, G. R. (1987). Recurrent stroke and multi-infarct dementia in systemic lupus erythematosus: Association with antiphospholipid antibodies. *Ann. Rheum. Dis.* **46**, 605–611.
28. Levine, S. R., Brey, R. L., Sawaya, K. L., Salowich-Palm, L., Kokkinos, J., Kostrzema, B., Perry, M., Havstad, S., and Carey, J. (1995). Recurrent stroke and thrombo-occlusive events in the antiphospholipid syndrome. *Ann. Neurol.* **38**, 119–124.
29. Levine, S. R., Deegan, M. J., Futrell, N., and Welch, K. M. (1990). Cerebrovascular and neurologic disease associated with antiphospholipid antibodies: 48 cases. *Neurology* **40**, 1181–1189.
30. Khamashta, M. A., Cervera, R., Asherson, R. A., Font, J., Gil, A., Coltart, D. J., Vazquez, J. J., Pare, C., Ingelmo, M., Oliver, J., *et al*. (1990). Association of antibodies against phospholipids with heart valve disease in systemic lupus erythematosus. *Lancet* **335**, 1541–1544.
31. Cervera, R., Khamashta, M. A., Font, J., Reyes, P. A., Vianna, J. L., Lopez-Soto, A., Amigo, M. C., Asherson, R. A., Azqueta, M., Pare, C., *et al*. (1991). High prevalence of significant heart valve lesions in patients with the 'primary' antiphospholipid syndrome. *Lupus* **1**, 43–47.
32. Galve, E., Ordi, J., Barquinero, J., Evangelista, A., Vilardell, M., and Soler-Soler, J. (1992). Valvular heart disease in the primary antiphospholipid syndrome. *Ann. Intern. Med.* **116**, 293–298.
33. Garcia-Torres, R., Amigo, M. C., de la Rosa, A., Moron, A., and Reyes, P. A. (1996). Valvular heart disease in primary antiphospholipid syndrome (PAPS): Clinical and morphological findings. *Lupus* **5**, 56–61.
34. Deschiens, M. A., Conard, J., Horellou, M. H., Ameri, A., Preter, M., Chedru, F., Samama, M. M., and Bousser, M. G. (1996). Coagulation studies, factor V Leiden, and anticardiolipin antibodies in 40 cases of cerebral venous thrombosis. *Stroke* **27**, 1724–1730.
35. Castanon, C., Amigo, M. C., Banales, J. L., Nava, A., and Reyes, P. A. (1995). Ocular vaso-occlusive disease in primary antiphospholipid syndrome. *Ophthalmology* **102**, 256–262.
36. Dunn, J. P., Noorily, S. W., Petri, M., Finkelstein, D., Rosenbaum, J. T., and Jabs, D. A. (1996). Antiphospholipid antibodies and retinal vascular disease. *Lupus* **5**, 313–322.
37. Wiechens, B., Schroder, J. O., Potzsch, B., and Rochels, R. (1997). Primary antiphospholipid antibody syndrome and retinal occlusive vasculopathy. *Am. J. Ophthalmol.* **123**, 848–850.
38. Giorgi, D., and Balacco Gabrieli, C. (1999). Optic neuropathy in systemic lupus erythematosus and antiphospholipid syndrome (APS): Clinical features, pathogenesis, review of the literature and proposed ophthalmological criteria for APS diagnosis. *Clin. Rheumatol.* **18**, 124–131.
39. Toubi, E., Ben-David, J., Kessel, A., Podoshin, L., and Golan, T. D. (1997). Autoimmune aberration in sudden sensorineural hearing loss: Association with anticardiolipin antibodies. *Lupus* **6**, 540–542.
40. Naarendorp, M., and Spiera, H. (1998). Sudden sensorineural hearing loss in patients with systemic lupus erythematosus or lupus-like syndromes and antiphospholipid antibodies. *J. Rheumatol.* **25**, 589–592.
41. Cuadrado, M. J., Khamashta, M. A., Ballesteros, A., Godfrey, T., Simon, M. J., and Hughes, G. R. (2000). Can neurologic manifestations of Hughes (antiphospholipid) syndrome be distinguished from multiple sclerosis? Analysis of 27 patients and review of the literature. *Medicine* **79**, 57–68.
42. Ijdo, J. W., Conti-Kelly, A. M., Greco, P., Abedi, M., Amos, M., Provenzale, J. M., and Greco, T. P. (1999). Antiphospholipid antibodies in patients with multiple sclerosis and MS-like illnesses: MS or APS? *Lupus* **8**, 109–115.
43. Scott, T. F., Hess, D., and Brillman, J. (1994). Antiphospholipid antibody syndrome mimicking multiple sclerosis clinically and by magnetic resonance imaging. *Arch. Intern. Med.* **154**, 917–920.
44. Karussis, D., Leker, R. R., Ashkenazi, A., and Abramsky, O. (1998). A subgroup of multiple sclerosis patients with anticardiolipin antibodies and unusual clinical manifestations: Do they represent a new nosological entity? *Ann. Neurol.* **44**, 629–634.
45. Sastre-Garriga, J., Reverter, J. C., Font, J., Tintore, M., Espinosa, G., and Montalban, X. (2001). Anticardiolipin antibodies are not a useful screening tool in a nonselected large group of patients with multiple sclerosis. *Ann. Neurol.* **49**, 408–411.

46. Ruiz-Irastorza, G., and Khamashta, M. A. (2000). Warfarin for multiple sclerosis? *Q. J. Med.* **93**, 497–499.
47. Tietjen, G. E., Day, M., Norris, L., Aurora, S., Halvorsen, A., Schultz, L. R., and Levine, S. R. (1998). Role of anticardiolipin antibodies in young persons with migraine and transient focal neurologic events: A prospective study. *Neurology* **50**, 1433–1440.
48. Montalban, J., Cervera, R., Font, J., Ordi, J., Vianna, J., Haga, H. J., Tintore, M., Khamashta, M. A., and Hughes, G. R. (1992). Lack of association between anticardiolipin antibodies and migraine in systemic lupus erythematosus. *Neurology* **42**, 681–682.
49. Sanna, G., Bertolaccini, M. L., Cuadrado, M. J., Laing, H., Khamashta, M. A., Mathieu, A., and Hughes, G. R. V. (2003). Neuropsychiatric manifestations in systemic lupus erythematosus: Prevalence and association with antiphospholipid antibodies. *J. Rheumatol.* **30**, 985–992.
50. Cuadrado, M. J., Khamashta, M. A., and Hughes, G. R. (2000). Migraine and stroke in young women. *Q. J. Med.* **93**, 317–318.
51. Cuadrado, M. J., Khamashta, M. A., D'Cruz, D., and Hughes, G. R. (2001). Migraine in Hughes syndrome—heparin as a therapeutic trial? *Q. J. Med.* **94**, 114–115.
52. Tzourio, C., Tehindrazanarivelo, A., Iglesias, S., Alperovitch, A., Chedru, F., d'Anglejan-Chatillon, J., and Bousser, M. G. (1995). Case-control study of migraine and risk of ischaemic stroke in young women. *Br. Med. J.* **310**, 830–833.
53. Carolei, A., Marini, C., and De Matteis, G. (1996). History of migraine and risk of cerebral ischaemia in young adults. The Italian National Research Council Study Group on Stroke in the Young. *Lancet* **347**, 1503–1506.
54. Merikangas, K. R., Fenton, B. T., Cheng, S. H., Stolar, M. J., and Risch, N. (1997). Association between migraine and stroke in a large-scale epidemiological study of the United States. *Arch. Neurol.* **54**, 362–368.
55. Chang, C. L., Donaghy, M., and Poulter, N. (1999). Migraine and stroke in young women: Case-control study. World Health Organization Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception. *Br. Med. J.* **318**, 13–18.
56. Coull, B. M., and Goodnight, S. H. (1990). Antiphospholipid antibodies, prethrombotic states, and stroke. *Stroke* **21**, 1370–1374.
57. Denburg, S. D., Carbotte, R. M., Ginsberg, J. S., and Denburg, J. A. (1997). The relationship of antiphospholipid antibodies to cognitive function in patients with systemic lupus erythematosus. *J. Int. Neuropsychol. Soc.* **3**, 377–386.
58. Hanly, J. G., Hong, C., Smith, S., and Fisk, J. D. (1999). A prospective analysis of cognitive function and anticardiolipin antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 728–734.
59. Menon, S., Jameson-Shortall, E., Newman, S. P., Hall-Craggs, M. R., Chinn, R., and Isenberg, D. A. (1999). A longitudinal study of anticardiolipin antibody levels and cognitive functioning in systemic lupus erythematosus. *Arthritis Rheum.* **42**, 735–741.
60. Mosek, A., Yust, I., Treves, T. A., Vardinon, N., Korczyn, A. D., and Chapman, J. (2000). Dementia and antiphospholipid antibodies. *Dement. Geriatr. Cogn. Disord.* **11**, 36–38.
61. Schwartz, M., Rochas, M., Weller, B., Sheinkman, A., Tal, I., Golan, D., Toubi, N., Eldar, I., Sharf, B., and Attias, D. (1998). High association of anticardiolipin antibodies with psychosis. *J. Clin. Psychiatry* **59**, 20–23.
62. Herranz, M. T., Rivier, G., Khamashta, M. A., Blaser, K. U., and Hughes, G. R. (1994). Association between antiphospholipid antibodies and epilepsy in patients with systemic lupus erythematosus. *Arthritis Rheum.* **37**, 568–571.
63. Liou, H. H., Wang, C. R., Chen, C. J., Chen, R. C., Chuang, C. Y., Chiang, I. P., and Tsai, M. C. (1996). Elevated levels of anticardiolipin antibodies and epilepsy in lupus patients. *Lupus* **5**, 307–312.
64. Inzelberg, R., and Korczyn, A. D. (1989). Lupus anticoagulant and late onset seizures. *Acta Neurol. Scand.* **79**, 114–118.
65. Peltola, J. T., Haapala, A., Isojarvi, J. I., Auvinen, A., Palmio, J., Latvala, K., Kulmala, P., Laine, S., Vaarala, O., and Keranen, T. (2000). Antiphospholipid and antinuclear antibodies in patients with epilepsy or new-onset seizure disorders. *Am. J. Med.* **109**, 712–717.
66. Liou, H. H., Wang, C. R., Chou, H. C., Arvanov, V. L., Chen, R. C., Chang, Y. C., Chuang, C. Y., Chen, C. Y., and Tsai, M. C. (1994). Anticardiolipin antisera from lupus patients with seizures reduce a GABA receptor-mediated chloride current in snail neurons. *Life Sci.* **54**, 1119–1125.
67. Asherson, R. A., and Hughes, G. R. V. (1988). Antiphospholipid antibodies and chorea. *J. Rheumatol.* **15**, 377–379.
68. Cervera, R., Asherson, R. A., Font, J., Tikly, M., Pallares, L., Chamorro, A., and Ingelmo, M. (1997). Chorea in the antiphospholipid syndrome: Clinical, radiologic, and immunologic characteristics of 50 patients from our clinics and recent literature. *Medicine* **76**, 203–212.
69. Sunden-Cullberg, J., Tedroff, J., and Aquilonius, S. M. (1998). Reversible chorea in primary antiphospholipid syndrome. *Mov. Disord.* **13**, 147–149.
70. Gibson, G. E., Su, W. P., and Pittelkow, M. R. (1997). Antiphospholipid syndrome and the skin. *J. Am. Acad. Dermatol.* **36**, 970–982.
71. Asherson, R. A., Mayou, S. C., Merry, P., Black, M. M., and Hughes, G. R. (1989). The spectrum of livedo reticularis and anticardiolipin antibodies. *Br. J. Dermatol.* **120**, 215–221.
72. Alarcon-Segovia, D., Delezé, M., Oria, C. V., Sanchez-Guerrero, J., Gomez-Pacheco, L., Cabiedes, J., Fernandez, L., and Ponce de Leon, S. (1989). Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus: A prospective analysis of 500 consecutive patients. *Medicine* **68**, 353.
73. Englert, H. J., Loizou, S., Derue, G. G., Walport, M. J., and Hughes, G. R. (1989). Clinical and immunologic features of livedo reticularis in lupus: A case-control study. *Am. J. Med.* **87**, 408–410.

74. Frances, C., and Piette, J. C. (2000). The mystery of Sneddon syndrome: Relationship with antiphospholipid syndrome and systemic lupus erythematosus. *J. Autoimmun.* **15**, 139–143.
75. Hughes, G. R. V. (2000). Speculations on antiphospholipid syndrome in the coming millennium. *J. Autoimmun.* **15**, 269–271.
76. Goldberg, D. P., Lewis, V. L., Jr., and Koenig, W. J. (1995). Antiphospholipid antibody syndrome: A new cause of nonhealing skin ulcers. *Plast. Reconstr. Surg.* **95**, 837–841.
77. Rocca, P. V., Siegel, L. B., and Cupps, T. R. (1994). The concomitant expression of vasculitis and coagulopathy: Synergy for marked tissue ischemia. *J. Rheumatol.* **21**, 556–560.
78. Aguirre, M. A., Jurado, A., Mujic, F., and Cuadrado, M. J. (1998). Oral anticoagulation therapy of chronic skin ulcers in a patient with primary antiphospholipid syndrome. *Clin. Exp. Rheumatol.* **16**, 628–629.
79. Gertner, E., and Lie, J. T. (1994). Systemic therapy with fibrinolytic agents and heparin for recalcitrant nonhealing cutaneous ulcer in the antiphospholipid syndrome. *J. Rheumatol.* **21**, 2159–2161.
80. Mujic, F., Lloyd, M., Cuadrado, M. J., Khamashta, M. A., and Hughes, G. R. (1995). Prevalence and clinical significance of subungual splinter haemorrhages in patients with the antiphospholipid syndrome. *Clin. Exp. Rheumatol.* **13**, 327–331.
81. Frances, C., Piette, J. C., Saada, V., Papo, T., Wechsler, B., Chosidow, O., and Godeau, P. (1994). Multiple subungual splinter hemorrhages in the antiphospholipid syndrome: A report of five cases and review of the literature. *Lupus* **3**, 123–128.
82. Creamer, D., Hunt, B. J., and Black, M. M. (2000). Widespread cutaneous necrosis occurring in association with the antiphospholipid syndrome: A report of two cases. *Br. J. Dermatol.* **142**, 1199–1203.
83. Paira, S., Roverano, S., Zunino, A., Oliva, M. E., and Bertolaccini, M. L. (1999). Extensive cutaneous necrosis associated with anticardiolipin antibodies. *J. Rheumatol.* **26**, 1197–1200.
84. Del Castillo, L. F., Soria, C., Schoendorff, C., Garcia Garcia, C., Diez-Caballero, N., Rodriguez Alen, A., Saez, A. I., Urrutia, S., and Garcia Almagro, D. (1997). Widespread cutaneous necrosis and antiphospholipid antibodies: Two episodes related to surgical manipulation and urinary tract infection. *J. Am. Acad. Dermatol.* **36**, 872–875.
85. Roldan, C. A., Shively, B. K., Lau, C. C., Gurule, F. T., Smith, E. A., and Crawford, M. H. (1992). Systemic lupus erythematosus valve disease by transesophageal echocardiography and the role of antiphospholipid antibodies. *J. Am. Coll. Cardiol.* **20**, 1127–1134.
86. Cervera, R. (2000). Recent advances in antiphospholipid antibody-related valvulopathies. *J. Autoimmun.* **15**, 123–125.
87. Espinola-Zavaleta, N., Vargas-Barron, J., Colmenares-Galvis, T., Cruz-Cruz, F., Romero-Cardenas, A., Keirns, C., and Amigo, M. C. (1999). Echocardiographic evaluation of patients with primary antiphospholipid syndrome. *Am. Heart J.* **137**, 973–978.
88. Bouillanne, O., Millaire, A., de Groote, P., Puisieux, F., Cesbron, J. Y., Jude, B., Hatron, P. Y., and Ducloux, G. (1996). Prevalence and clinical significance of antiphospholipid antibodies in heart valve disease: A case-control study. *Am. Heart J.* **132**, 790–795.
89. Brenner, B., Blumenfeld, Z., Markiewicz, W., and Reisner, S. A. (1991). Cardiac involvement in patients with primary antiphospholipid syndrome. *J. Am. Coll. Cardiol.* **18**, 931–936.
90. Meyer, O., Golstein, M., Nicaise, P., Labarre, C., and Kahn, M. F. (1995). Heart valve disease in systemic lupus erythematosus. Role of antiphospholipid antibodies. *Clin. Rev. Allergy Immunol.* **13**, 49–56.
91. Afek, A., Shoenfeld, Y., Manor, R., Goldberg, I., Ziporen, L., George, J., Polak-Charcon, S., Amigo, M. C., Garcia-Torres, R., Segal, R., and Kopolovic, J. (1999). Increased endothelial cell expression of alpha3beta1 integrin in cardiac valvulopathy in the primary (Hughes) and secondary antiphospholipid syndrome. *Lupus* **8**, 502–507.
92. Hojnik, M., George, J., Ziporen, L., and Shoenfeld, Y. (1996). Heart valve involvement (Libman-Sacks endocarditis) in the antiphospholipid syndrome. *Circulation* **93**, 1579–1587.
93. Agirbasli, M. A., Hansen, D. E., and Byrd III, B. F. (1997). Resolution of vegetations with anticoagulation after myocardial infarction in primary antiphospholipid syndrome. *J. Am. Soc. Echocardiogr.* **10**, 877–880.
94. Hamsten, A., Norberg, R., Bjorkholm, M., de Faire, U., and Holm, G. (1986). Antibodies to cardiolipin in young survivors of myocardial infarction: An association with recurrent cardiovascular events. *Lancet* **1**, 113–116.
95. Vaarala, O., Manttari, M., Manninen, V., Tenkanen, L., Puurunen, M., Aho, K., and Palosuo, T. (1995). Anticardiolipin antibodies and risk of myocardial infarction in a prospective cohort of middle-aged men. *Circulation* **91**, 23–27.
96. Zuckerman, E., Toubi, E., Shiran, A., Sabo, E., Shmuel, Z., Golan, T. D., Abinader, E., and Yeshurun, D. (1996). Anticardiolipin antibodies and acute myocardial infarction in non-systemic lupus erythmatosus patients: A controlled prospective study. *Am. J. Med.* **101**, 381–386.
97. Adler, Y., Finkelstein, Y., Zandeman-Goddard, G., Blank, M., Lorber, M., Lorber, A., Faden, D., and Shoenfeld, Y. (1995). The presence of antiphospholipid antibodies in acute myocardial infarction. *Lupus* **4**, 309–313.
98. Wu, R., Nityanand, S., Berglund, L., Lithell, H., Holm, G., and Lefvert, A. K. (1997). Antibodies against cardiolipin and oxidatively modified LDL in 50-year-old men predict myocardial infarction. *Arterioscler. Thromb. Vasc. Biol.* **17**, 3159–3163.
99. Manzi, S., Meilahn, E. N., Rairie, J. E., Conte, C. G., Medsger, T. A., Jr., Jansen-McWilliams, L., RB, D. A., and Kuller, L. H. (1997). Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: Comparison with the Framingham Study. *Am. J. Epidemiol.* **145**, 408–415.

100. Vaarala, O., Puurunen, M., Lukka, M., Alfthan, G., Leirisalo-Repo, M., Aho, K., and Palosuo, T. (1996). Affinity-purified cardiolipin-binding antibodies show heterogeneity in their binding to oxidized low-density lipoprotein. *Clin. Exp. Immunol.* **104**, 269–274.
101. Puurunen, M., Manttari, M., Manninen, V., Tenkanen, L., Alfthan, G., Ehnholm, C., Vaarala, O., Aho, K., and Palosuo, T. (1994). Antibody against oxidized low-density lipoprotein predicting myocardial infarction. *Arch. Intern. Med.* **154**, 2605–2609.
102. Erkkila, A. T., Narvanen, O., Lehto, S., Uusitupa, M. I., and Yla-Herttuala, S. (2000). Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease. *Arterioscler. Thromb. Vasc. Biol.* **20**, 204–209.
103. Nzerue, C. M., Hewan-Lowe, K., Pierangeli, S., and Harris, E. N. (2002). "Black swan in the kidney": Renal involvement in the antiphospholipid antibody syndrome. *Kidney Int.* **62**, 733–744.
104. Asherson, R. A., Noble, G. E., and Hughes, G. R. (1991). Hypertension, renal artery stenosis and the "primary" antiphospholipid syndrome. *J. Rheumatol.* **18**, 1413–1415.
105. Godfrey, T., Khamashta, M. A., and Hughes, G. R. (2000). Antiphospholipid syndrome and renal artery stenosis. *Q. J. Med.* **93**, 127–129.
106. Rossi, E., Sani, C., Zini, M., Casoli, M. C., and Restori, G. (1992). Anticardiolipin antibodies and renovascular hypertension. *Ann. Rheum. Dis.* **51**, 1180–1181.
107. Nochy, D., Daugas, E., Droz, D., Beaufils, H., Grunfeld, J. P., Piette, J. C., Bariety, J., and Hill, G. (1999). The intrarenal vascular lesions associated with primary antiphospholipid syndrome. *J. Am. Soc. Nephrol.* **10**, 507–518.
108. Asherson, R. A., Buchanan, N., Baguley, E., and Hughes, G. R. (1993). Postpartum bilateral renal vein thrombosis in the primary antiphospholipid syndrome. *J. Rheumatol.* **20**, 874–876.
109. Morgan, R. J., and Feneley, R. C. (1994). Renal vein thrombosis caused by primary antiphospholipid syndrome. *Br. J. Urol.* **74**, 807–808.
110. Remondino, G. I., Mysler, E., Pissano, M. N., Furattini, M. C., Basta, M. C., Presas, J. L., and Allievi, A. (2000). A reversible bilateral renal artery stenosis in association with antiphospholipid syndrome. *Lupus* **9**, 65–67.
111. Brunet, P., Aillaud, M. F., San Marco, M., Philip-Joet, C., Dussol, B., Bernard, D., Juhan-Vague, I., and Berland, Y. (1995). Antiphospholipids in hemodialysis patients: Relationship between lupus anticoagulant and thrombosis. *Kidney Int.* **48**, 794–800.
112. Fabrizi, F., Sangiorgio, R., Pontoriero, G., Corti, M., Tentori, F., Troina, E., and Locatelli, F. (1999). Antiphospholipid (aPL) antibodies in end-stage renal disease. *J. Nephrol.* **12**, 89–94.
113. Radhakrishnan, J., Williams, G. S., Appel, G. B., and Cohen, D. J. (1994). Renal transplantation in anticardiolipin antibody-positive lupus erythematosus patients. *Am. J. Kidney Dis.* **23**, 286–289.
114. Stone, J. H., Amend, W. J., and Criswell, L. A. (1999). Antiphospholipid antibody syndrome in renal transplantation: Occurrence of clinical events in 96 consecutive patients with systemic lupus erythematosus. *Am. J. Kidney Dis.* **34**, 1040–1047.
115. Vaidya, S., Sellers, R., Kimball, P., Shanahan, T., Gitomer, J., Gugliuzza, K., and Fish, J. C. (2000). Frequency, potential risk and therapeutic intervention in end-stage renal disease patients with antiphospholipid antibody syndrome: A multicenter study. *Transplantation* **69**, 1348–1352.
116. Mondragon-Ramirez, G., Bochicchio, T., Garcia-Torres, R., Amigo, M. C., Martinez-Lavin, M., Reyes, P., and Herrera-Acosta, J. (1994). Recurrent renal thrombotic angiopathy after kidney transplantation in two patients with primary antiphospholipid syndrome (PAPS). *Clin. Transplant.* **8**, 93–96.
117. McIntyre, J. A., and Wagenknecht, D. R. (2001). Antiphospholipid antibodies. Risk assessments for solid organ, bone marrow, and tissue transplantation. *Rheum. Dis. Clin. North Am.* **27**, 611–631.
118. Amigo, M. C., and Garcia-Torres, R. (2000). Kidney disease in antiphospholipid syndrome. In "Hughes Syndrome. Antiphospholipid Syndrome" (M. A. Khamashta, Ed.), pp. 70–81. Springer-Verlag, London.
119. Daugas, E., Nochy, D., Huong du, L. T., Duhaut, P., Beaufils, H., Caudwell, V., Bariety, J., Piette, J. C., and Hill, G. (2002). Antiphospholipid syndrome nephropathy in systemic lupus erythematosus. *J. Am. Soc. Nephrol.* **13**, 42–52.
120. Espinosa, G., Cervera, R., Font, J., and Asherson, R. A. (2002). The lung in the antiphospholipid syndrome. *Ann. Rheum. Dis.* **61**, 195–198.
121. Asherson, R. A., and Cervera, R. (1995). Review: Antiphospholipid antibodies and the lung. *J. Rheumatol.* **22**, 62–66.
122. Asherson, R. A., Higenbottam, T. W., Dinh Xuan, A. T., Khamashta, M. A., and Hughes, G. R. (1990). Pulmonary hypertension in a lupus clinic: Experience with twenty-four patients. *J. Rheumatol.* **17**, 1292–1298.
123. Gertner, E. (1999). Diffuse alveolar hemorrhage in the antiphospholipid syndrome: Spectrum of disease and treatment. *J. Rheumatol.* **26**, 805–807.
124. Cuadrado, M. J., Mujic, F., Munoz, E., Khamashta, M. A., and Hughes, G. R. (1997). Thrombocytopenia in the antiphospholipid syndrome. *Ann. Rheum. Dis.* **56**, 194–196.
125. Galli, M., Daldossi, M., and Barbui, T. (1994). Anti-glycoprotein Ib/IX and IIb/IIIa antibodies in patients with antiphospholipid antibodies. *Thromb. Haemost.* **71**, 571–575.
126. Godeau, B., Piette, J. C., Fromont, P., Intrator, L., Schaeffer, A., and Bierling, P. (1997). Specific antiplatelet glycoprotein autoantibodies are associated with the thrombocytopenia of primary antiphospholipid syndrome. *Br. J. Haematol.* **98**, 873–879.
127. Panzer, S., Gschwandtner, M. E., Hutter, D., Spitzauer, S., and Pabinger, I. (1997). Specificities of platelet autoantibodies in patients with lupus anticoagulants in primary antiphospholipid syndrome. *Ann. Hematol.* **74**, 239–242.

128. Fong, K. Y., Loizou, S., Boey, M. L., and Walport, M. J. (1992). Anticardiolipin antibodies, haemolytic anaemia and thrombocytopenia in systemic lupus erythematosus. *Br. J. Rheumatol.* **31**, 453–455.
129. Kokori, S. I., Ioannidis, J. P., Voulgarelis, M., Tzioufas, A. G., and Moutsopoulos, H. M. (2000). Autoimmune hemolytic anemia in patients with systemic lupus erythematosus. *Am. J. Med.* **108**, 198–204.
130. Shehata, H. A., Nelson-Piercy, C., and Khamashta, M. A. (2001). Management of pregnancy in antiphospholipid syndrome. *Rheum. Dis. Clin. North Am.* **27**, 643–659.
131. Perez, M. C., Wilson, W. A., Brown, H. L., and Scopelitis, E. (1991). Anticardiolipin antibodies in unselected pregnant women. Relationship to fetal outcome. *J. Perinatol.* **11**, 33–36.
132. Branch, D. W., Scott, J. R., Kochenour, N. K., and Hershgold, E. (1985). Obstetric complications associated with the lupus anticoagulant. *N. Engl. J. Med.* **313**, 1322–1326.
133. Lynch, A., Marlar, R., Murphy, J., Davila, G., Santos, M., Rutledge, J., and Emlen, W. (1994). Antiphospholipid antibodies in predicting adverse pregnancy outcome. A prospective study. *Ann. Intern. Med.* **120**, 470–475.
134. Lima, F., Khamashta, M. A., Buchanan, N. M., Kerslake, S., Hunt, B. J., and Hughes, G. R. (1996). A study of sixty pregnancies in patients with the antiphospholipid syndrome. *Clin. Exp. Rheumatol.* **14**, 131–136.
135. Ramsey-Goldman, R., Kutzer, J. E., Kuller, L. H., Guzick, D., Carpenter, A. B., and Medsger, T. A., Jr. (1993). Pregnancy outcome and anti-cardiolipin antibody in women with systemic lupus erythematosus. *Am. J. Epidemiol.* **138**, 1057–1069.
136. Dekker, G. A., de Vries, J. I., Doelitzsch, P. M., Huijgens, P. C., von Blomberg, B. M., Jakobs, C., and van Geijn, H. P. (1995). Underlying disorders associated with severe early-onset preeclampsia. *Am. J. Obstet. Gynecol.* **173**, 1042–1048.
137. Branch, D. W., Silver, R. M., Blackwell, J. L., Reading, J. C., and Scott, J. R. (1992). Outcome of treated pregnancies in women with antiphospholipid syndrome: An update of the Utah experience. *Obstet. Gynecol.* **80**, 614–620.
138. Backos, M., Rai, R., Baxter, N., Chilcott, I. T., Cohen, H., and Regan, L. (1999). Pregnancy complications in women with recurrent miscarriage associated with antiphospholipid antibodies treated with low dose aspirin and heparin. *Br. J. Obstet. Gynaecol.* **106**, 102–107.
139. Farquharson, R. G., Quenby, S., and Greaves, M. (2002). Antiphospholipid syndrome in pregnancy: A randomized, controlled trial of treatment. *Obstet. Gynecol.* **100**, 408–413.
140. Caruso, A., De Carolis, S., Ferrazzani, S., Valesini, G., Caforio, L., and Mancuso, S. (1993). Pregnancy outcome in relation to uterine artery flow velocity waveforms and clinical characteristics in women with antiphospholipid syndrome. *Obstet. Gynecol.* **82**, 970–977.
141. Coleman, M. A., McCowan, L. M., and North, R. A. (2000). Mid-trimester uterine artery Doppler screening as a predictor of adverse pregnancy outcome in high-risk women. *Ultrasound Obstet. Gynecol.* **15**, 7–12.
142. Harrington, K., Cooper, D., Lees, C., Hecher, K., and Campbell, S. (1996). Doppler ultrasound of the uterine arteries: The importance of bilateral notching in the prediction of pre-eclampsia, placental abruption or delivery of a small-for-gestational-age baby. *Ultrasound Obstet. Gynecol.* **7**, 182–188.
143. Asherson, R. A., Cervera, R., Piette, J. C., Shoenfeld, Y., Espinosa, G., Petri, M. A., Lim, E., Lau, T. C., Gurjal, A., Jedryka-Goral, A., Chwalinska-Sadowska, H., Dibner, R. J., Rojas-Rodriguez, J., Garcia-Carrasco, M., Grandone, J. T., Parke, A. L., Barbosa, P., Vasconcelos, C., Ramos-Casals, M., Font, J., and Ingelmo, M. (2001). Catastrophic antiphospholipid syndrome: Clues to the pathogenesis from a series of 80 patients. *Medicine* **80**, 355–377.
144. Asherson, R. A., Liote, F., Page, B., Meyer, O., Buchanan, N., Khamashta, M. A., Jungers, P., and Hughes, G. R. (1993). Avascular necrosis of bone and antiphospholipid antibodies in systemic lupus erythematosus. *J. Rheumatol.* **20**, 284–288.
145. Moore, J., Ma, D. D., and Concannon, A. (1998). Nonmalignant bone marrow necrosis: A report of two cases. *Pathology* **30**, 318–320.
146. Paydas, S., Kocak, R., Zorludemir, S., and Baslamisli, F. (1997). Bone marrow necrosis in antiphospholipid syndrome. *J. Clin. Pathol.* **50**, 261–262.
147. George, J., Haratz, D., and Shoenfeld, Y. (2001). Accelerated atheroma, antiphospholipid antibodies, and the antiphospholipid syndrome. *Rheum. Dis. Clin. North Am.* **27**, 603–610, vii.
148. Horkko, S., Olee, T., Mo, L., Branch, D. W., Woods, V. L. J., Palinski, W., Chen, P. P., and Witztum, J. L. (2001). Anticardiolipin antibodies from patients with the antiphospholipid antibody syndrome recognize epitopes in both beta(2)-glycoprotein 1 and oxidized low-density lipoprotein. *Circulation* **103**, 941–946.
149. Branch, D. W. (1998). Antiphospholipid antibodies and infertility: Factor or fallacy. *Lupus* **7**(Suppl 2), S90–S94.
150. Balasch, J., and Cervera, R. (2002). Reflections on the management of reproductive failure in the antiphospholipid syndrome—the clinician's perspective. *Lupus* **11**, 467–477.
151. Ginsburg, K. S., Liang, M. H., Newcomer, L., Goldhaber, S. Z., Schur, P. H., Hennekens, C. H., and Stampfer, M. J. (1992). Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. *Ann. Intern. Med.* **117**, 997–1002.
152. Cervera, R., Khamashta, M. A., Font, J., Sebastiani, G. D., Gil, A., Lavilla, P., Domenech, I., Aydintug, A. O., Jedryka-Goral, A., de Ramon, E., *et al*. (1993). Systemic lupus erythematosus: Clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. The European Working Party on Systemic Lupus Erythematosus. *Medicine* **72**, 113–124.
153. Shah, N. M., Khamashta, M. A., Atsumi, T., and Hughes, G. R. (1998). Outcome of patients with anticardiolipin antibodies: A 10 year follow-up of 52 patients. *Lupus* **7**, 3–6.

154. Petri, M. (2000). Epidemiology of the antiphospholipid antibody syndrome. *J. Autoimmun.* **15**, 145–151.
155. Hunt, J. E., McNeil, H. P., Morgan, G. J., Crameri, R. M., and Krilis, S. A. (1992). A phospholipid-beta 2-glycoprotein I complex is an antigen for anticardiolipin antibodies occurring in autoimmune disease but not with infection. *Lupus* **1**, 75–81.
156. Malaviya, A. N., Marouf, R., Al-Jarallah, K., Al-Awadi, A., Al-Saied, K., Al-Gaurer, S., Khamis, A., Al-Salem, I., Raghupathy, R., Al-Mazidi, M., Serebour, F., Umamaheswaran, I., Mourou, M., Mokhtar, M., Qurtom, M., Raoof Al-Shayeb, A., Tarasevicius, T., and Hussain, M. A. (1996). Hughes syndrome: A common problem in Kuwait hospitals. *Br. J. Rheumatol.* **35**, 1132–1136.
157. Molina, J. F., Gutierrez-Urena, S., Molina, J., Uribe, O., Richards, S., De Ceulaer, C., Garcia, C., Wilson, W. A., Gharavi, A. E., and Espinoza, L. R. (1997). Variability of anticardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. *J. Rheumatol.* **24**, 291–296.

41

THE CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME

Ronald A. Asherson
Ricard Cervera

INTRODUCTION

A widespread coagulopathy, affecting predominantly small vessels supplying organs, rather than the usual large vessel occlusions of larger veins and arteries seen in the classic antiphospholipid syndrome (APS), in association with elevations of anti-phospholipid antibodies (aPL) (often high levels), was defined in 1992 as the catastrophic APS [1] and in 2002 was called Asherson's syndrome. Isolated cases of this syndrome had previously been reported by others and referred to as "devastating noninflammatory vasculopathy" [2], or "occlusive vasculopathy" [3]. Neither of these terms are used today and the adjective "catastrophic" is universally accepted to describe this syndrome, bearing in mind that many of the complications of systemic lupus erythematosus (SLE) may indeed be "catastrophic" too. Since the original attempt to define the condition [1], which was based on 10 reported cases, many more cases have been reported. A series of 50 patients was analyzed and documented in 1998 [4] and a further 80 patients reviewed in 2001 [5]. Additionally, several reviews on the topic have been published [6, 7]. Therefore, although they represent less than 1% of all patients with APS [8], they are usually in a life-threatening medical situation that requires high clinical awareness in order to institute adequate and effective therapy to achieve amelioration of this condition.

DEFINITION

The main characteristics of this syndrome are: (a) a widespread coagulopathy (noninflammatory), affecting predominantly small vessels involving organs; (b) a minority of patients demonstrate features of the classic APS with involvement of large veins (of the lower limbs usually) or cerebral vasculature; (c) the vascular occlusions occur within days to weeks of a "triggering" event or may occur seemingly spontaneously; (d) the patient may be suffering from SLE often accompanied by an APS, there may have been a history of a previous thrombotic event (venous/arterial), or the event may arise *de novo* in a patient with no previous history of thrombotic events (primary APS); (e) serological testing reveals very high levels of aPL in more than 50% of patients; (f) there is often a moderate to severe thrombocytopenia; and (g) laboratory evidence of disseminated intravascular coagulation (DIC) or microangiopatic anemia may be present (Fig. 1).

The majority of patients suffering from catastrophic APS have been found to be suffering from defined SLE or the primary APS [4, 5], while a minority have had primary Sjögren's syndrome [2], rheumatoid arthritis [9], systemic sclerosis [10], ulcerative colitis [5], or relapsing polychondritis [11]. Additionally, several patients have been documented who have developed catastrophic APS in association with carcinoma or lymphoma [4, 5]. Essentially, the same precipitating factors,

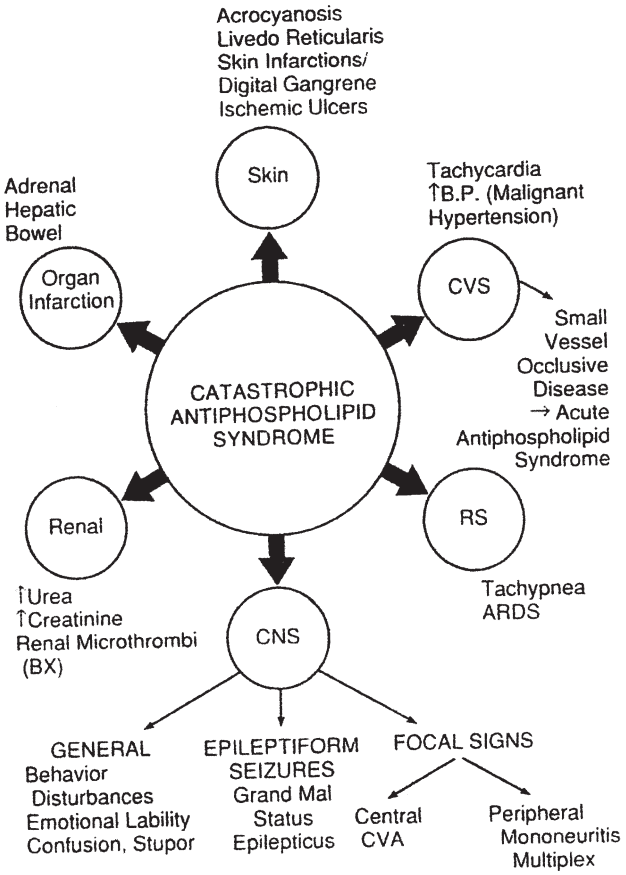


FIGURE 1. Main features of the catastrophic APS.

clinical and laboratory features, and outcome have been documented in the reported cases, irrespective of the etiology of the condition. The patients with SLE have often had a history of a classic APS preceding the development of catastrophic APS and may have been on prophylactic therapy in order to prevent recurrences of thrombotic vascular occlusions (e.g., coumadin, salicylates), as well as on drugs to treat the underlying lupus (immunosupressants, antimalarials, etc.). Control of both the SLE and the APS may have been satisfactory.

PATHOGENESIS

General Characteristics

It appears that, following a sudden "trigger" or insult, the patient is catapulted into multiorgan failure (Table 1) and very often has to be admitted to an intensive care unit for diagnosis and treatment. The trigger may be quite minor or several triggers may be operating simultaneously (the so-called "double" or "treble" hit hypothesis, which applies to any patient presenting with multiorgan failure arising from surgical trauma, sepsis, burns, pancreatitis, etc.) (Fig. 2). The usual factors, which may predispose to thrombosis in patients with a classic APS do not seem to play a role in the pathogenesis of catastrophic APS. These include conditions which are chronic, such as prolonged bed rest, sedentary situations (e.g., long-haul flying), obesity, dyslipidemias, diabetes mellitus, smoking, nephrotic syndrome, and the other hereditary thrombophilias, some recently described (e.g., factor V Leiden mutation, prothrombin mutation). The trigger factors which precipitate catastrophic APS are usually acute and superimposed on patients with the underlying coagulopathy induced by aPL, and what seems to be unique about the patients with catastrophic APS is that, to date, the condition has not been reported and is not seen with other underlying coagulopathies due to the hereditary thrombophilias, implying a basic autoimmune mechanism which is magnified by the "trigger" to cause this often fatal condition. It must be emphasized, however, that in some patients, particular triggers (e.g., warfarin withdrawal) may not result in the catastrophic syndrome, but simply in recurrent thrombosis affecting large vessels. The factors determining which APS patients will develop catastrophic APS rather than recurrent large vessel occlusions are unknown at this time.

TABLE 1 Trigger Factors for the Catastrophic APS

Infections
Trauma (including even minor trauma, such as biopsies, dental extractions, fractures)
Invasive procedures (needle stick injury)
Warfarin withdrawal/low International normalized ratio (INR)
Drug administration (e.g., oral contraceptives)
SLE "flares"
Large clots themselves ("thrombotic storm")

The catastrophic APS may occur in two situations where an underlying hypercoagulable state already exists or develops, that is, carcinoma and the puerperium. Patients with carcinoma may have an underlying hypercoagulable state and this was pointed out by Trousseau in the nineteenth century ("Trousseau's syndrome"). The aPL have been demonstrated in many types of carcinoma patients as well as in those suffering from hematological/lymphoproliferative malignancies [12] and it has been hypothesized that some patients who develop clotting in these conditions may in fact be examples of the APS. Further insults in cancer patients, such as biopsies, have in fact resulted in catastrophic APS when aPL have been present. The sites of the carcinoma are varied and lung, uterus, stomach, and biliary tree have all been reported in association with this con-

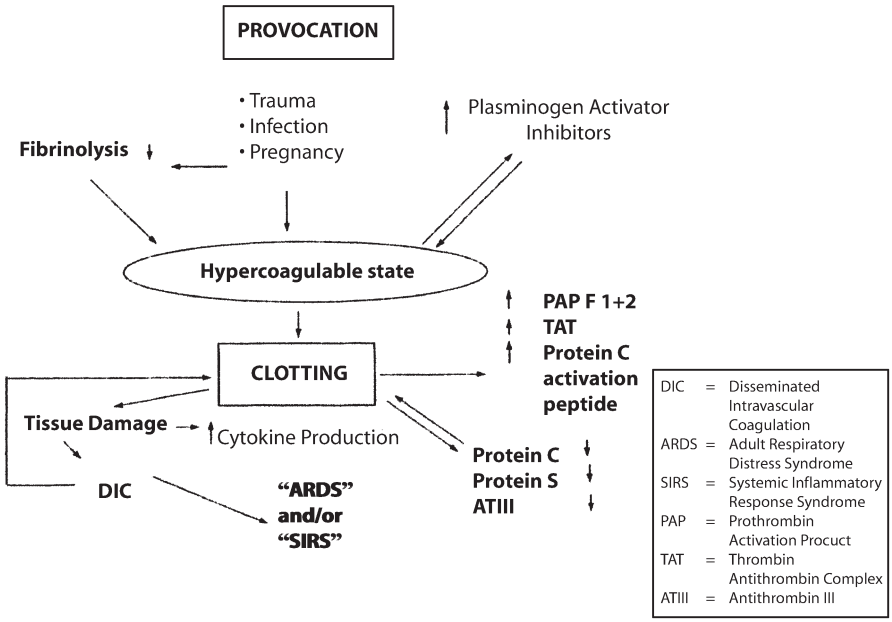


FIGURE 2. Pathogenesis of catastrophic APS.

dition. On the other hand, in the puerperium, there is an alteration in the hormonal milieu as well as in hemostatic equilibrium, both designed and intended to promote healing of the vascular bed consequent on placental implantation. It is well known that classic APS patients may thrombose at this time and, indeed, in patients who have a previous history of thrombosis, it is now recommended that low molecular weight heparin (LMWH) be administered for 6 weeks postpartum in order to prevent vascular occlusions at this time. Several patients with catastrophic APS have been reported who have developed the condition during the puerperium. Catastrophic APS in association with the HELLP (hemolysis, elevated liver enzymes, and low platelet) syndrome, seen during pregnancy, has also been reported [4, 5].

Specific Triggering Factors

Infections

Bacterial infections occurring prior to the onset of catastrophic APS were encountered in 29% of the first series of patients reported [4] and in 36% of the second series [5]. Infections were present in two of the six patients reported by Kitchens [13], and Hayem *et al*. [14] documented a single patient who developed catastrophic APS following a well-diagnosed *Salmonella typhi* infection. In a series of patients reported from Mexico [15], infections were documented in 60% of the eight patients. Other cases also confirmed infections preceding the onset of the condition. Undas *et al*. [16] described a patient whose two episodes of catastrophic APS were clearly precipitated by upper respiratory infections.

This topic has been extensively reviewed [17–19]. By the mechanism of "molecular mimicry" some infectious agents may induce not only aPL but also pathogenetic anti-β_2-glycoprotein I (GPI) autoantibodies. Gharavi *et al*. [17, 18] have identified seven proteins with sequence homology to GDKU and GDKU2 proteins which are the same sequence as found in the β_2GPI binding site for aPL as well as being part of many viruses to which humans are exposed. Immunization of mice with these peptides conjugated to bovine serum albumin in Freund's adjuvant have been shown to produce high levels of aPL and anti-β_2GPI. Other mechanisms may also be responsible for initiating the sudden onset of catastrophic APS following infections. Enterotoxins produced by enterococci/staphylococci as well as other microorganisms ("superantigens") may activate T cells which then may stimulate those B cells already primed for the production of aPL in APS/SLE patients.

Cytokine activation (tumor necrosis factor-α, interleukin-1, mainly) as well as γ-interferon mediated activation of the monocytic/phagocytic system occurs simultaneously. This activation of the inflammatory response leads to phospholipid asymmetry of cell membranes, exposure of anionic phospholipids, normally "hidden" under the surface of endothelial cells and platelets. This endothelial cell activation then initiates the coagulation process resulting in the binding of aPL to β_2GPI. There is also another role for monocytes via tissue factor. Activated endothelial cells secrete

increased amounts of interleukin-l which in turn activates monocytes to produce tissue factor procoagulant. Adherence of monocytes to E-selectin, expressed on activated endothelial cells, induces these monocytes to undergo phenotypic transformation and this induces more expression of tissue factor. The aPL may also bind to adherent monocytes and activate them via an Fc-receptor mediated mechanism and monoclonal aPL have also been shown to induce procoagulant activity [19]. The entire process is clearly extremely complicated with several reactions occurring simultaneously, the end result being thrombosis. The role of cytokine activation and endothelial cells in the pathogenesis of the systemic inflammatory response syndrome (SIRS) and thrombosis has been extensively reviewed by Belmont *et al*. [20].

Generation of Ongoing Clotting by Clot Itself ("Thrombotic Storm")

The paper by Kitchens [13] may be of great importance in the pathogenesis of the condition. Of the six patients he reported as suffering from "thrombotic storm," three were clear examples of catastrophic APS and although aPL were not estimated in the remainder, it is more than probable that they were also examples of the condition. Kitchens hypothsized that newly formed clots in some patients with a preexisting autoimmune hypercoagulable state continue to provide thrombin. Fibrinolysis is depressed by increases in plasminogen activator inhibitors and a state of fibrinolytic "shutdown" occurs. Coagulation activation products including prothrombin activation peptides F 1 and 2, thrombin–antithrombin complexes (TAT), and protein C activation peptide are increased in plasma. At the same time there is a depletion of the body's natural anticoagulant proteins (proteins C and S, antithrombin III). A combination of all these mechanisms leads to continued clotting such as is seen in catastrophic APS. A paper by Amital *et al*. [21] documenting two patients who had developed catastrophic APS which remitted following amputation of the limbs where large vessel clotting had occurred lends credence to this hypothesis, although removal of infected ulcers could also have been partially responsible.

Warfarin Withdrawal/Low International Normalized Ratio

Warfarin withdrawal (e.g., prior to surgical procedure, biopsies, or for hemorrhagic complications attributable to anticoagulation therapy) may be followed by recurrent thrombosis with days to weeks in classic APS patients, but may also lead to catastrophic APS when other trigger factors are also present at the time, for example, infection [4, 5]. In addition, low levels of the international normalized ratio (INR), which are inadequate to protect against thrombosis may also serve to alter the hemostatic milieu when other prothrombotic factors are superimposed. It is well documented that INR levels may fluctuate widely in classic APS patients and this is all the more reason why their frequent estimation is necessary in APS patients and dosages of anticoagulants adjusted accordingly in order to prevent recurrent thrombosis. LMWH is best administered immediately prior to, and following invasive and investigative procedures as well as surgical interventions/biopsies in these patients.

CLINICAL MANIFESTATIONS

General Characteristics

Although patients may present with single organ involvement, for example, dyspnea, deterioration of consciousness, or abdominal pain, the picture very soon changes to that of multiorgan failure and the patient is then usually admitted to an intensive care unit for further investigations and treatment. Unless the clinicians consider a catastrophic APS situation and perform the necessary tests, the diagnosis may very often be missed. A previous history of SLE, thrombosis, etc. should provide clues as to the disease. It should be emphasized that although large vessel occlusions, such as deep vein thrombosis, often complicated by pulmonary embolism and arterial lesions (cerebral, peripheral) do occur (15–20% of patients), the frequency of these lesions is far less than the dominant small vessel occlusive disease affecting organs mainly (intraabdominal, cerebral, cardiac, renal, etc.) which is responsible for most of the clinical picture. Additionally, unusual vessels tend to be involved, for example, scrotal, testicular, ovarian, esophageal, colonic, gastric) which is unlike the distribution seen in patients with the simple or classic APS.

The symptomatology related to the extensive thrombotic disease is of course predicated by the specific organs involved. However, as a consequence of the extensive microvasculopathy and tissue damage, it is accompanied by symptoms related to the SIRS which is essentially not dissimilar to its occurrence with other types of multiorgan failure such as that accompanying septic/endotoxin shock/septicemia, burns, multiple trauma, pancreatitis, etc. Excessive production of cytokines such as tumor necrosis factor-α, interleukin-l, and other cytokines results in a "cytokine storm" which is responsible for such manifestations as cerebral edema

(causing initial confusion and drowsiness), myocardial dysfunction, and the adult respiratory distress syndrome (ARDS) which seems to be the hallmark of the pulmonary involvement in this condition. In some cases, the ARDS may be superimposed on an underlying infective process which itself may have triggered the catastrophic APS by any of the mechanisms described earlier. In addition, there are some of the features of DIC seen in approximately 20% of cases. If is true DIC then this may contribute to the microangiopathic vascular occlusions which take place and may be perpetuating the process itself, as well as leading to diagnostic difficulties in some cases. It is possible that these laboratory features of DIC may simply be a reflection of extensive small vessel endothelial cell damage and ongoing clotting only. The catastrophic APS may be relapsing in a minority of cases but the frequency of this is extremely low as opposed to relapsing thrombotic thrombocytopenic purpura (TTP) .

Specific Organ Involvement

Kidneys

The kidneys are involved in up to 70% of all APS patients. Hypertension (occasionally of the malignant/accelerated variety) may accompany the renal lesions which consist overwhelmingly of renal thrombotic microangiopathy (Fig. 3) which may be demonstrated either by renal biopsy or at postmortem. Death from anuric renal failure is rare, most patients only demonstrating elevated urea and creatinine levels. Hematuria/proteinuria or casts may be seen on urine microscopy.

Lungs

The ARDS dominates pulmonary involvement in most patients. They are dyspneic on initial presentation or rapidly become so. Assisted ventilation usually becomes necessary. Other types of pulmonary pathologies, however, may also be evident and these include embolic disease accompanied by pulmonary infarctions, pulmonary hemorrhage, interstitial infiltration, capillaritis, pulmonary edema, etc. [22]. Major pulmonary arterial occlusion is extremely rare. As mentioned earlier, every effort should be made to identify an infection and the appropriate parenteral antibiotic therapy instituted when indicated.

Nervous System

Gradual progression from initial confusion and disorientation, to drowsiness, semicoma, and coma is seen. In a minority, focal signs, with frank hemiplegias or hemipareses may be evident and seizures (even *status epilepticus*) may occur, although rarely. The focal signs may be consequent on large vessel occlusions similar to those seen with a classic APS. Diffuse microvascular damage dominates the picture though pathologically. Mononeuritis multiplex has been recorded in a few cases and is due to focal vascular involvement of peripheral nerves by small vessel vasculopathy. Severe hypertension may cause hypertensive encephalopathy in

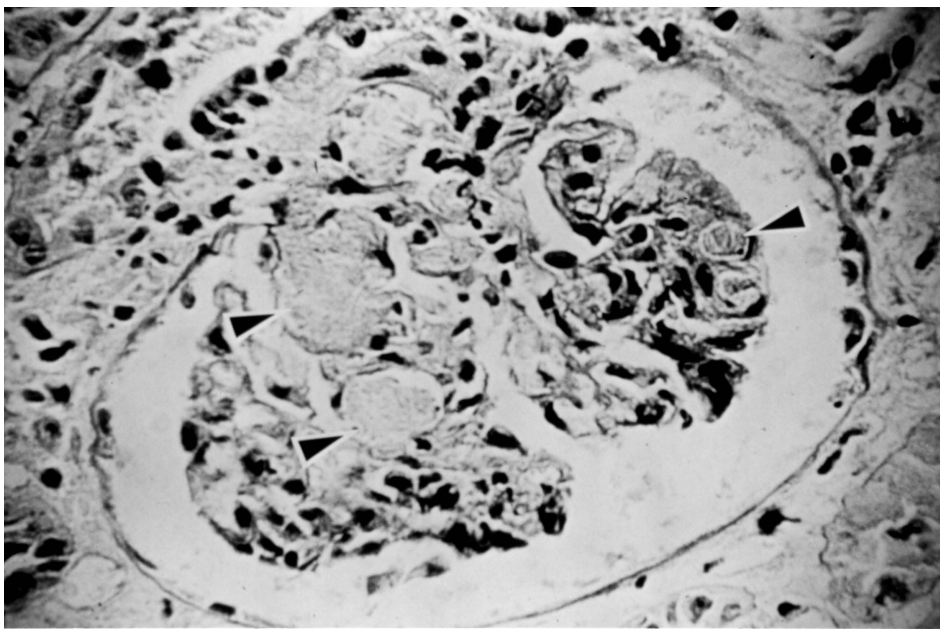


FIGURE 3. Renal microangiopathy.

some. Retinal vascular involvement affecting venules and arterioles may also be seen.

Skin

The dermatological complications seen in the catastrophic APS are essentially similar to those encountered in the simple or classic APS and include *livedo reticularis*, digital ischemia or frank gangrene, superficial skin necrosis, or ischemic (often infected) ulceration of the lower limbs. Frequently, several differing skin lesions may be encountered in the same patient. They are all caused by microvascular occlusive disease of skin vessels except for major ischemic problems which are related to larger vascular occlusions.

Heart

Cardiac microangiopathy leading to a reduced ejection fraction because of myocardial ischemia may be associated with hypotension. Generalized cardiac dysfunction and reduced contractility because of the cytokine storm may also contribute to this state. Cardiac failure may ensue. Myocardial infarctions may be seen when larger vessels are involved and may accompany the microangiopathy. Valve lesions (mitral and aortic) or intracardiac thrombus are often detected in patients with long-standing and preexisting APS for many years.

Gastrointestinal System

Abdominal pain is frequent in patients with catastrophic APS as a presenting feature and may be complained of in approximately one-third of patients. It is due to bowel ischemia. Infarction and peritonitis may occur. Other thrombotic lesions may account for hepatic infarctions (uncommon), thrombotic pancreatitis (usually detected by enzyme elevations only), or ischemic ulceration of the esophagus, stomach, duodenum, small bowel, or colon. A Budd-Chiari syndrome may rarely be encountered. Death may supervene from septic peritonitis or mediastinitis (in the case of esophageal perforation).

Adrenal Glands

The frequency of adrenal involvement in patients with catastrophic APS is extraordinarily high compared to that seen in simple/classic APS patients. It is much more frequent than occlusion of the renal venous trunks which are close by and may be explained by flow or shear dynamics. It is usually bilateral, silent, and forms part of the multiorgan failure. When it occurs acutely, from bilateral adrenal hemorrhage and ensuing hemorrhagic infarction, it may be accompanied by abdominal pain and the differential diagnosis then is from other intraabdominal causes of pain. It has been reported postpartum. A few patients with catastrophic APS have given a history of Addison's disease in the past, often many years previously. Bilateral adrenal hemorrhage has been described as a complication of anticoagulation therapy and with other underlying coagulation abnormalities which predispose to clotting, for example, protein C deficiency, when it may be associated with skin necrosis.

Bone Marrow

Bone marrow necrosis has more recently been described in patients with catastrophic APS and the frequency of this complication is surprisingly high [5] (Fig. 4). It is a relatively uncommon complication of neoplastic diseases, lymphomas, acute leukemia, following radiotherapy, with sickle-cell disease, and severe infections (particularly septic shock states). This complication has been seen in very few patients with simple/classic APS. It seems to be more common in pregnancy or the puerperium.

Other Organs

As in the classic APS, any other organ may be affected by the catastrophic APS, and thrombosis in retina, testes, ovaries, and tendons (Fig. 5), among others, have all been reported.

LABORATORY

More than 50% of patients demonstrate very high levels of anti-cardiolipin antibodies (aCL) and/or positive lupus anticoagulant (LA) tests. The isotype is IgG in 99% of patients. Rarely, an IgM isotype alone may be encountered. Similarly, elevations of antibodies to β_2GPI have been high when measured and are also of the IgG isotype predominantly.

The ANA may be low positive in patients with primary APS and much higher in those suffering from defined SLE. SLE patients usually demonstrate elevated levels of antibodies to double-stranded (ds)DNA. Antibodies to RNP, SS-A/Ro and SS-B/La, may be positive in a minority.

Laboratory features of DIC may be present in up to 20% of cases. These include elevated D-dimers and fibrin degradation products (FDP). Fibrinogen levels may be elevated or reduced. It is unclear whether these features actually indicated the presence of DIC or are

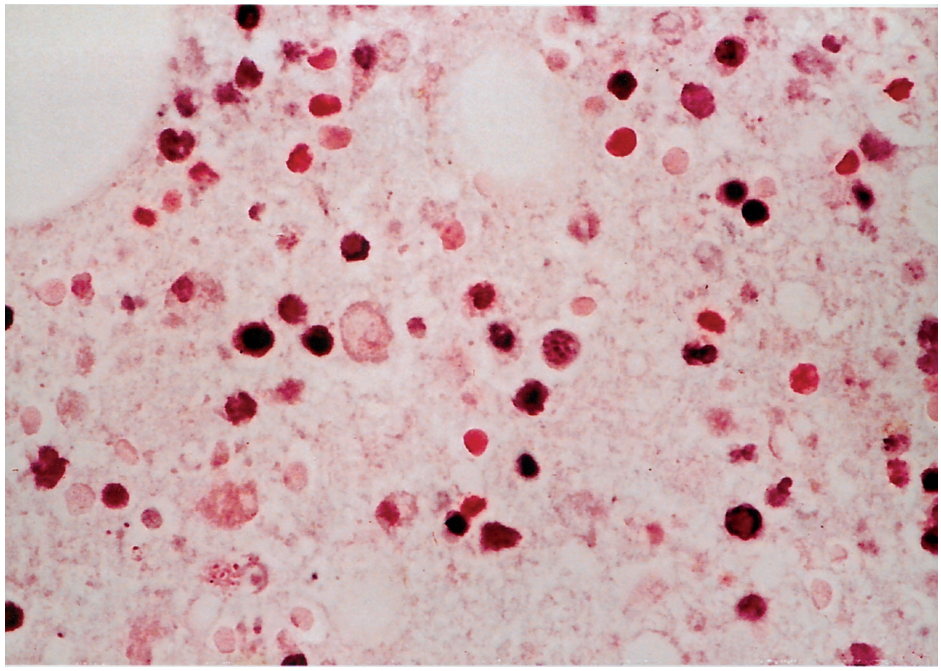


FIGURE 4. Bone marrow necrosis.

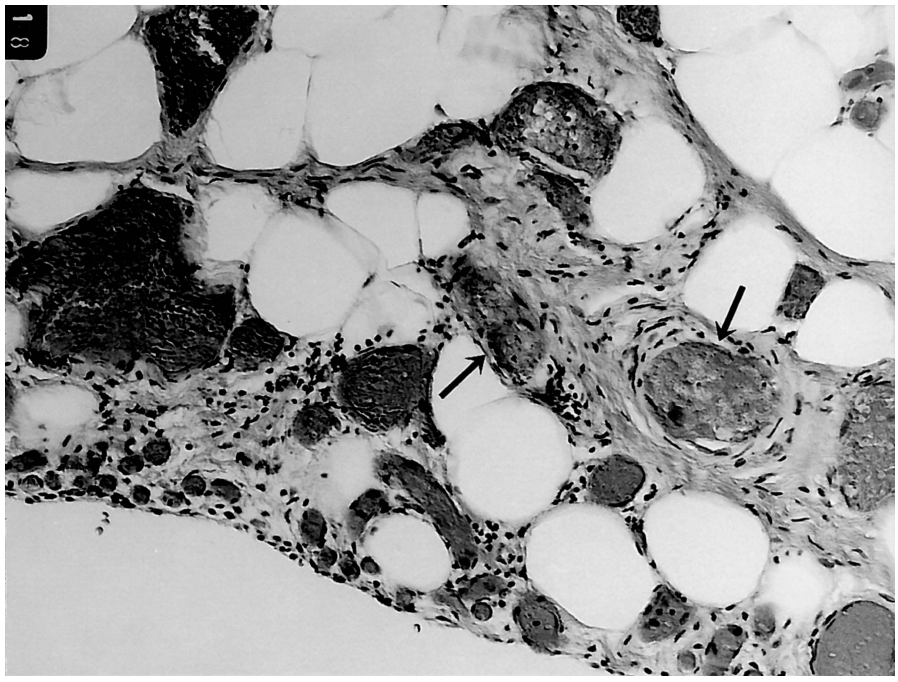


FIGURE 5. Necrosis in tendons.

merely consequent on extensive endothelial damage and ongoing clotting.

The platelet count is decreased in more than 50% of patients and severe thrombocytopenia (below 50×10^9/liter) is seen in a minority. A microangiopathic hemolytic anemia may be present (scanty schistocytes) and may accompany the thrombocytopenia.

DIAGNOSIS

A high index of clinical suspicion is necessary for the diagnosis of catastrophic APS in patients with evidence of both large and small vessel occlusion, affecting multiple organs and systems. The diagnosis may be unduly delayed because, although some of the manifestations

may be clearly evident clinically, for example, deep venous thrombosis (DVT) or stroke, others may present less clearly, for example, renal involvement or nonspecific abdominal pain in cases of mesenteric ischemia or pancreatic thrombosis. In addition, absence of aPL may confuse the clinician. These antibodies may return during the recovery phase or later [23]. Although catastrophic APS may often be the first manifestation of the illness, a previous history of SLE, the primary APS or major vascular occlusive disease might assist the physician in diagnosis. A preliminary set of criteria for the diagnosis of catastrophic APS is presented in Table 2, based on the cumulated published experience of 130 cases with this syndrome [4, 5].

Clinical Diagnosis

Multiorgan presentation and failure is the rule. Involvement of the kidneys is present in 78% of the patients with catastrophic APS, followed by pulmonary (66%), central nervous system (56%), skin (50%), cardiac (50%), gastrointestinal (38%), hepatic (34%), adrenal (13%), and urogenital involvement (6%) [4, 5].

TABLE 2 Preliminary Criteria for the Classification of Catastrophic APS [7]

1. Evidence of involvement of three or more organs, systems, and/or tissues[a]
2. Development of manifestations simultaneously or in less than a week
3. Confirmation by histopathology of small vessel occlusion in at least one organ or tissue[b]
4. Laboratory confirmation of the presence of anti-phospholipid antibodies (lupus anticoagulant and/or anti-cardiolipin antibodies)[c]

Definite catastrophic APS
All four criteria

Probable catastrophic APS
All four criteria, except for only two organs, systems, and/or tissues involvement
All four criteria, except for the absence of laboratory confirmation at least 6 weeks apart due to the early death of a patient never tested for aPL before the catastrophic APS
1, 2, and 4
1, 3, and 4 and the development of a third event in more than a week but less than a month, despite anticoagulation.

[a] Usually, clinical evidence of vessel occlusions, confirmed by imaging techniques when appropriate. Renal involvement is defined by a 50% rise in serum creatinine, severe systemic hypertension (>180/100 mm Hg), and/or proteinuria (>500 mg/24 h).

[b] For histopathological confirmation, significant evidence of thrombosis must be present, although vasculitis may coexist occasionally.

[c] If the patient had not been previously diagnosed as having an APS, the laboratory confirmation requires that presence of anti-phospholipid antibodies must be detected on two or more occasions at least 6 weeks apart (not necessarily at the time of the event), according to the proposed preliminary criteria for the classification of definite APS [24].

Renal Involvement

In these patients, a renal biopsy is indicated in order to diagnose intrarenal arteriolar or glomerular thromboses (renal thrombotic microangiopathy). If renal infarction or renal artery trunk thrombosis is to be excluded, renal angiography (arterial or venous) should be performed.

Pulmonary Involvement

If the radiograph of the chest shows pulmonary infiltrates, a lung biopsy may be indicated in order to identify the presence of hyaline membrane formation, consistent with ARDS or the presence of erythrocytes and iron-staining macrophages, characteristic of the intraalveolar pulmonary hemorrhage. Unusually pulmonary "capillaritis" may be diagnosed on biopsy. If there is a suspicion of pulmonary embolism, a scintigraph study of lungs or an angiogram of the pulmonary circulation must be performed. Additionally, routine sputum cultures and serologies are obligatory in order to rule out any underlying precipitating or complicating pulmonary infection.

Nervous System Involvement

Neuroimaging studies, such as computed tomography or magnetic resonance imaging (MRI) scans of the brain, may reveal major cerebral infarctions or small foci of high signal intensity in subcortical white matter representing areas of subcortical ischemia. In cases of suspected cerebral venous sinus thrombosis, a cerebral angio-MRI is indicated. In order to confirm the presence of status epilepticus, electroencephalography is essential. Electromyography is necessary if mononeuritis multiplex is suspected, and a biopsy of the affected nerves is indicated if the diagnosis is unclear.

Skin Involvement

Diagnosis of these lesions is mainly clinical in patients with multisystemic thrombotic phenomena. Routine ulcer cultures are obligatory in order to rule out infection. Noninflammatory thrombosis in small arteries and/or veins throughout the dermis and the subcutaneous fatty tissue are the main histological findings.

Cardiovascular Involvement

Echocardiographic studies, preferably transesophageal echocardiography, is the technique which allows diagnosis of valvular disease or the presence of intracardiac thrombus in these patients. Routine blood cultures are obligatory in order to rule out true infective endocarditis.

Gastrointestinal, Hepatic, and Pancreatic Involvement

Diagnosis of intestinal ischemia may be confirmed by angiography. Abdominal echography may be useful in case of acute pancreatitis as this will reveal an edematous pancreas or acalculous ischemic gallbladder necrosis. Routine blood cultures are obligatory in order to rule out hepatic abscess. A computed tomography scan of the abdomen may be useful in differentiating this condition from true hepatic infarction and in demonstrating splenic infarctions.

Adrenal Involvement

Diagnosis of the condition is mainly clinical, with a high index of suspicion. Confirmation may be obtained by laboratory findings of hypoglycemia, hyponatremia, hyperkalemia, and mild azotemia combined with a positive test of acute cortisol response to injection of 250 μg of aqueous synthetic corticotrophin. A computed tomography scan will show typical changes of adrenal infarction or hemorrhage.

Urogenital Involvement

Just as with skin involvement, diagnosis of urogenital involvement is mainly clinical in a patient with multiple thrombotic phenomena and scrotal pain. Echography of the testes may be useful in differentiating a testicular torsion.

Ocular Involvement

Fundoscopic examination is compulsory for diagnosis. In some patients, the retinal vessels may appear normal and then, a diagnosis of retrobulbar optic neuritis should be suspected. In these patients, an evoked visual potential may show alteration in conduction velocity of the optic nerve and an MRI scan may reveal small foci of high signal intensity in the optic nerve.

Laboratory Diagnosis

Although the diagnosis of catastrophic APS is based on the clinical picture (multiorgan involvement and failure), the laboratory findings are very important to confirm the clinical suspicion. The following laboratory evaluations are recommended.

Blood Cell Count

Blood cell count may show hemolytic anemia and thrombocytopenia occasionally severe (below 50×10^9/liter). In cases of thrombocytopenia, we suggest that a second platelet count be performed on a sample obtained in citrate as anticoagulant in order to rule out a "pseudo-thrombocytopenia" secondary to EDTA aggregation.

Biochemical Tests

The alterations of these biochemical tests will depend on the organs involved. Routine blood and urine tests, renal function (urea, creatinine), arterial-blood gas level, and cardiac, hepatic, and pancreatic enzymes should be determined.

Coagulation Tests

The activated partial thromboplastin time (aPTT) may be increased if the patient has a lupus anticoagulant. The dilute Russell viper venom time (dRVVT) should also be performed, particularly if the aPTT is negative. In some cases, there are laboratory features of DIC (increased prothrombin time, decreased fibrinogen, and increased fibrinogen/fibrin degradation products). At the time of the acute event, fibrinogen levels may be normal or even increased. Finally, the thrombin time is usually slightly increased but the effect of heparin needs to be taken into account if thrombin time elevations are encountered.

Peripheral Blood Smear

This may reveal scanty fragmented red cells (schistocytes), which are characteristic of microangiopathic hemolysis. In patients with TTP, a related condition, schistocytes are usually plentiful.

Coombs Test

The positivity of Coombs test suggests the existence of autoimmune hemolysis. The Coombs test may, however, be negative.

Anti-phospholipid Antibody Profile

This should include both LA and aCL (IgG and IgM isotypes). Negative testing of aPL may be seen during

the course of thrombotic events, probably due to their consumption, making the diagnosis difficult at the time of the acute event [23]. In these cases, a new sample obtained during the recovery phase should be analyzed. Many laboratories also are able to perform tests to detect antibodies to the cofactor of aCL (β_2-GPI).

Immunology Tests

The ANA may be positive in these patients, although usually at low titers (below 1/320). Higher titers of antinuclear antibodies (ANA) and anti-dsDNA antibodies may be found in patients with defined SLE or "lupus-like disease." The presence of anti-extractable nuclear antigens (ENA) antibodies (anti-Ro, anti-La, anti-RNP, and anti-Sm) is uncommon.

Other Tests

Increased thrombin and plasmin generation markers, such as thrombin–antithrombin and plasmin–antiplasmin complexes, respectively, are usually found. Moreover, other markers of endothelial cell activation elevated in catastrophic APS are cytokines (particularly, interleukin-1 and tumor necrosis factor-α) and the adhesion molecules (intercellular adhesion molecule-1 and E-selectin). However, at the present time, these determinations have only a research value and awaiting these results should not delay other diagnostic and therapeutic procedures. Von Willebrand factor antigen, as an index of endothelial cell damage, however, can now be performed by most laboratories and its estimation is most useful.

DIFFERENTIAL DIAGNOSIS

A careful differential diagnosis is necessary in the presence of multiorgan thrombotic events in a particular patient and this should include catastrophic APS and other microangiopathic syndromes which have in common thrombotic microangiopathy, hemolytic anemia, thrombocytopenia, and involvement of central nervous and renal systems. These include TTP, hemolytic–uremic syndrome (HUS), heparin-induced thrombocytopenia, and the HELLP (hemolysis, elevated liver enzymes and low platelet) syndrome. Additionally, almost one-third of patients with catastrophic APS develop serological or hematological evidence of DIC during the course of their multiorgan illness [4, 5]. All these laboratory features of DIC found in these patients may be explicable on the basis of extensive small vessel endothelial damage. Therefore, DIC should also be included in the differential diagnosis.

Finally, the differential diagnosis should include marantic endocarditis complicated by multiple embolic events, cryoglobulinemia, vasculitis, multiple cholesterol emboli, and heparin-induced thrombocytopenia–thrombosis syndrome. In the latter condition, patients develop thrombosis at any site, especially the skin, within 10–14 days after starting heparin and the first sign is a falling platelet count. This is due to the presence of an antibody which binds to platelet factor 4 and heparin [25, 26]. Platelet aggregation studies and enzyme-linked immunoabsorbent (ELISA)-based assays to identify this platelet factor 4–heparin complex are necessary to establish the diagnosis of this condition. TTP, heparin-induced thrombocytopenia, and DIC will be discussed in some detail.

Thrombotic Thrombocytopenic Purpura

In this condition, studies have identified antibodies in plasma which recognize a proteolytic enzyme (metalloprotease) which is present in normal plasma [27]. This enzyme cleaves large multimers of von Willebrand factor. When it is reduced or rendered inefficient by antibodies directed toward it, these large multimers accumulate and become entangled in subendothelial fibrous components, maximizing von Willebrand factor mediated adhesion of platelets to the subendothelium following vascular damage. Patients with acute TTP have been reported with IgG autoantibodies to this enzyme. In chronic relapsing TTP, the enzyme is reduced or absent. The existence of these large multimers of von Willebrand factor and antibodies to metalloproteases have not been investigated in patients with catastrophic APS and indeed levels of the enzyme have also not been measured. The characteristic pentad of TTP consisting of the early onset of neurological symptoms, renal disease, hemolytic anemia, thrombocytopenia, and fever is seen in 50% of patients with this condition. Several of these features may be present in patients with catastrophic APS. Fever, however, is uncommon in patients with catastrophic APS unless caused by infection. The aPL are not usually present in TTP patients and when they are demonstrated are usually at a low level. There have been several case reports of patients with TTP and aPL. The distinguishing features between the two conditions is the fact that renal disease is usually more serious in patients with catastrophic APS with hypertension, renal infarcts, and renal failure, whereas in TTP it is more subtle and milder. Often there are only abnormalities on urinalysis. Schistocytes are usually abundant in TTP on peripheral smear whereas in catastrophic APS they are scanty if present at all. Microangiopathic hemolytic anemia may be present in both conditions. The clinical presen-

tations may, however, be similar and differentiation difficult in a minority of patients. Catastrophic APS, unlike TTP, has been associated with relapses only infrequently [27–31].

Heparin-Induced Thrombocytopenia

In cases where patients have been placed on heparin prior to the onset of multiorgan failure, this condition has to be seriously considered. Veins, arteries, and the microcirculation may all be involved. Appropriate platelet studies and ELISA-based assays in order to identify platelet factor 4–heparin complex must be undertaken in order to definitively establish a diagnosis of the condition [25, 26].

Disseminated Intravascular Coagulation

Disseminated intravascular coagulation (DIC) is the expression of several pathological processes and occurs as a consequence of the inappropriate activation of the coagulation system induced by tissue factor and other factors released from hypoxic or infarcted tissues [32]. Tissue damage results in thrombin generation at the surface of damaged microvessels. This produces widespread microvascular thrombosis and, in an attempt to maintain the patency of blood vessels, excessive plasmin is generated, resulting in local and systemic fibrinolysis [33].

DIC commonly results from obstetric problems (such as amniotic fluid embolism or placental abruption), intravascular hemolysis by transfusion reactions, sepsis or viremia, burns, disseminated malignancy, or acute leukemia [33]. In the largest series of patients with catastrophic APS, DIC features were present in 28% [5].

Thrombosis and hemorrhage are the major clinical manifestations of this condition, but clinically, microvascular thrombosis in the context of DIC is more often a laboratory phenomenon than a clinical event. Therefore, the commonest presenting clinical manifestation is cutaneous bleeding (petechiae and hemorrhage). Renal involvement may lead to acute renal failure with oliguria and acute tubular necrosis. Generalized central nervous system dysfunction leads to coma and hypoxia and a picture identical to ARDS may be seen in case of lung involvement. Hypotension, shock, and cardiac arrest may occur if the cardiovascular system is involved.

The laboratory diagnosis is based on the presence of thrombocytopenia (usually severe) and prolonged prothrombin, partial activated thromboplastin, and thrombin times, accompanied by the presence of products of fibrinogen/fibrin degradation and low fibrinogen concentrations as well as low levels of antithrombin and protein C concentrations. Schistocytes may be present, but in low number, in contrast to TTP [32].

TREATMENT

The optimal management of catastrophic APS is not known [34] but must have three clear aims: to treat any precipitating factors (prompt use of antibiotics if infection is suspected, amputation for any necrotic organ, high awareness in patients with APS who undergo an operation or an invasive procedure . . .), to prevent and to treat the ongoing thrombotic events and to suppress the excessive cytokine "storm" [13].

Regarding the prevention of catastrophic APS in a patient with classic APS who must undergo an operation or an invasive procedure, high-dose thromboprophylaxis with LMWH appear to be most effective in reducing the risk of postoperative deep venous thrombosis as well as possible catastrophic APS [35–38].

The literature describing treatment of catastrophic APS is confusing since there have been no prospective studies because of the rarity of the condition. Therefore, the treatment of catastrophic APS is not currently standardized. Analysis of the largest series of patients with this condition shows that the combination of anticoagulation plus steroids plus plasma exchange and/or intravenous gammaglobulins has the highest survival rate (70%) [4, 5]. Therefore, when there is a high clinical suspicion of catastrophic APS, the following treatment is suggested (Fig. 6).

First Line Therapies

Anticoagulation

Intravenous heparin administration is necessary to inhibit ongoing clotting and to lyse existing clots which may contribute to the ongoing thrombosis. It is usually necessary to administer more than the usual doses of heparin, monitoring treatment by serial aPTT in order to achieve the correct range. It is recommended that an initial intravenous bolus of 5000 units be followed by continuous infusion of 1500 units/h of heparin, with accurate control of the aPTT ratio. If the clinical course is satisfactory, intravenous heparin must be maintained for 7 to 10 days and then should be replaced by oral anticoagulant therapy (warfarin or coumadin). Although it has been recommended that the INR should be kept over 3.0 [39], a lower range (between 2.5 and 3) could, however, be as effective, with less hemorrhagic problems. Heparin should not be withdrawn before achieving a correct INR with oral anticoagulants.

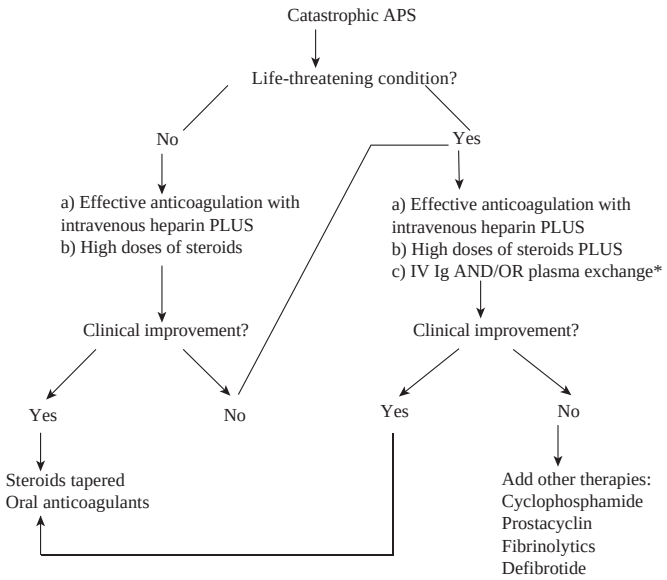


FIGURE 6. Treatment algorithm of catastrophic APS. IVIg, intravenous gammaglobulins. * specially indicated if schistocytes are present.

Steroids

Intravenous steroids are necessary to overcome the excessive cytokine response in these patients [13]. In case of a life-threatening condition, we suggest commencing with intravenous pulses of methylprednisolone (1000 mg daily for 3 to 5 days), followed by high doses of intravenous methylprednisolone (1–2 mg/kg daily). This dose should be tapered according to the therapeutic response.

Second Line Therapies

These are indicated in case of clinical worsening, or in presence of a life-threatening situation.

Gammaglobulins

Intravenous gammaglobulins have been effective in achieving a prompt reduction of the aPL titers in some patients. They are also useful in patients with severe thrombocytopenia (unresponsive to high dose of steroids). The recommended dose is 400 mg/kg daily (approximately 25 g/day) for 5 days.

Plasma exchange

The rationale of this treatment is based on the fact that this is the treatment of choice in patients with TTP all of whom develop microangiopathy. Plasma exchange also removes aPL and will thus benefit the patient. In addition, fresh frozen plasma contains the natural anticoagulants and should be beneficial as these compounds are used up in the clotting process. Therefore, it would be specially indicated in those patients with catastrophic APS who show laboratory features of microangiopathy (schistocytes). plasma exchange should be continued for a minimum of 3 to 5 days but there are no guidelines with regard to the management and duration of this treatment. Neuwelt *et al.* [40], however, used plasma exchange for up to 3 years in a patient with catastrophic APS. It should be taken into account that plasma exchange may interfere with anticoagulant treatment.

Other Therapies

The following therapies should be considered in patients presenting with resistant multiorgan thromboses apparently not responding to the treatment of choice just described.

Fibrinolytics

They may be justified in cases of patients who do not respond to heparin [13]. The main fibrinolytics employed are streptokinase, urokinase, and tissue plasminogen activator and these may be followed by heparin as has been described. Hemorrhage induced by these fibrinolytics can be treated with blood transfusions.

Cyclophosphamide

The largest series [4, 5] did not demonstrate any benefit from the additional use of cyclophosphamide. On the contrary, the addition of cyclophosphamide was associated with a lower survival rate, although these patients may have had more severe disease. However, this drug might be used in cases of severe catastrophic APS, SLE flares, or in those patients with very high levels of aCL to prevent any aPL rebound following plasma exchange or intravenous gammaglobulins infusion [6]. The usual dose that it has been employed is an intravenous pulse of 0.5–1 g/m^2.

Unusual Therapies

The following therapies have not been administered to any significant number of patients with catastrophic APS.

Prostacyclin

Prostacyclin is a naturally occurring substance produced by vascular endothelium. It causes vasodilation

of all vascular beds and it is an extremely potent endogenous inhibitor of platelet aggregation. Prostacyclin has only been evaluated in one patient with systemic sclerosis and catastrophic APS [10]. Continuous intravenous prostacyclin infusion (5 ng/kg/min) was administerd for 7 days but the patient died when therapy with this compound was discontinued.

Ancrod

This substance is a purified fraction of Malayan pit viper venom that has been shown to correct prostacyclin stimulating factor and vascular plasminogen activator deficiencies. However, its use has been described in only one patient who responded to this compound [41].

Defibrotide

This compound is a single-stranded alkali metal salt of DNA fraction that has been shown to act as a potent inhibitor of endothelin I, thrombin-induced platelet aggregation, thromboxane synthesis, as well as a potent inhibitor of a clot formation. Just like ancrod, defibrotide has also been occasionally employed succesfully in patients with catastrophic APS. It is not generally available at the present time and has to be obtained from the manufacturer (CRINAS, Italy).

Intensive Care Therapies

In case of a life-threatening condition or clinical worsening, it must be necessary to admit the patients to an intensive care unit. Rapid and progressive renal failure may require dialysis, the clinical picture of ARDS usually needs mechanical ventilation and close monitoring of inotropic drugs may be required in cases of cardiogenic shock. All these measures play an essential role in the survival rate of patients with catastrophic APS.

In conclusion, the catastrophic APS is a potentially life-threatening condition with a high mortality which requires high clinical awareness. Therefore, it is essential that it be diagnosed early and treated aggresively. The combination of high doses of heparin plus steroids plus intravenous gammaglobulins and/or plasma exchange is the treatment of choice in patients with this severe condition. Additionally, preventive measures in patients with APS may be effective if the development of the catastrophic APS is to be avoided.

References

1. Asherson, R. A. (1992). The catastrophic antiphospholipid antibody syndrome. *J. Rheumatol.* **19**, 508–512.
2. Ingram, S. B., Goodnight, S. H., and Bennet, R. N. (1987). An unusual syndrome of a devastating non-inflamatory vasculopathy associated to anticardiolipin antibodies. Report of two cases. *Arthritis Rheum.* **30**, 1167–1171.
3. Greisman, S. G., Thayaparan, R.-S., Godwin, T. A., and Lockshin, M. D. (1991). Occlusive vasculopathy in systemic lupus erythematosus—Association with anticardiolipin antibody. *Arch. Intern. Med.* **151**, 389–392.
4. Asherson, R. A., Cervera, R., Piette, J. C., *et al.* (1998). Catastrophic antibody syndrome. Clinical and laboratory features of 50 patients. *Medicine* **77**, 195–207.
5. Asherson, R. A., Cervera, R., Piette, J. C., *et al.* (2001). Catastrophic antiphospholipid syndrome: Clues to the pathogenesis from a series of 80 patients. *Medicine* **80**, 355–376.
6. Asherson, R. A., and Cervera, R. (2000). The catastrophic antiphospholipid antibody syndrome: A review of pathogenesis, clinical features and treatment. *IMAJ* **2**, 268–273.
7. Asherson R. A., Cervera R., deGroot P. G., *et al.* Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. *Lupus 2003*, in press.
8. Cervera, R., Piette, J. C., Font, J., *et al.* (2002). Antiphospholipid syndrome: Clinical and immunologic manifestations and patterns of disease expression in a cohort of 1000 patients. *Arthritis Rheum.* **46**, 1019–1027.
9. Voisin, L., Derumeaux, G., Borg, J. Y., Mejjad, O., Vittecoq, O., Tayod, J., Thomine, E., Tron, F., and Le Loët, X. (1995). Catastrophic antiphospholipid syndrome with fatal course in rheumatoid arthritis. *J. Rheumatol.* **22**, 1586–1588.
10. Kane, D., McSweeney, F., Swan, N., and Bresnihan, B. (1998). Catastrophic antiphospholipid antibody syndrome in primary systemic sclerosis. *J. Rheumatol.* **25**, 810–812.
11. Saji, M., Nakajima, A., Sendo, W., Tanaka, M., Koseki, Y., Ichikawa, N., Taniguchi, A., Akama, H., Harigai, M., Terai, C., Hara, M., and Kamatani, N. (2003). Catastrophic antiphospholipid syndrome with complete abdominal aorta occlusion associated with polychondritis. *Clin. Exp. Rheumatol.* in press.
12. Asherson, R. A., and Cervera, R. (2000). Antiphospholipid antibodies and malignancies. In "Cancer and Autoimmunity" (Y. Shoenfeld and M. E. Gershwin, Eds.), pp. 93–103. Elsevier, Amsterdam.
13. Kitchens, C. S. (1998). Thrombotic storm: When thrombosis begets thrombosis. *Am. J. Med.* **104**, 381–385.
14. Hayem, G., Kassis, N., Nicaise, P., Bouvet, P., Andremont, A., Labarre, C., Kahn, M.-F., and Meyer, O. (1999). Systemic lupus erythematosus-associated catastrophic antiphospholipid syndrome occurring after typhoid fever. A possible role of Salmonella lipopolysaccharide in the occurrence of diffuse vasculopathy–coagulopathy. *Arthritis Rheum.* **42**, 1056–1061.
15. Rojas-Rodríguez, J., García-Carrasco, M., Ramos-Casals, M., Enríquez-Coronel, G., Colchero, C., Cervera, R., and Font, J. (2000). Catastrophic antiphospholipid syndrome: Clinical description and triggering factors in 8 patients. *J. Rheumatol.* **27**, 238–240.

16. Undas, A., Swadzba, J., Undas, R., and Musial, J. (1998). Three episodes of acute multiorgan failure in a woman with secondary antiphospholipid syndrome. *Pol. Arch. Med. Wewn.* **100**, 556–560.
17. Gharavi, E., Cucurull, E., Tang, H., Celli, C. M., Wilson, W. A., and Gharavi, A. E. (1999). Induction of antiphospholipid antibodies by immunization with viral peptides. *Lupus* **8**, 449–455.
18. Gharavi, A. E., Pierangeli, S. S., and Harris, N. (2000). New developments in viral peptides and antiphospholipid antibody induction. *J. Autoimmun.* **15**, 227–230.
19. Asherson, R. A., and Shoenfeld, Y. (2000). The role of infection in the pathogenesis of catastrophic antiphospholipid syndrome—Molecular mimicry? *J. Rheumatol.* **27**, 12–14.
20. Belmont, H. M., Abramson, S. B., and Lie, J. T. (1996). Pathology and pathogenesis of vascular injury in systemic lupus erythematosus. *Arthritis Rheum.* **39**, 9–22.
21. Amital, H., Levy, Y., Davidson, C., Lundberg, I., Harju, A., Asborson, R. A., and Shoenfeld, Y. (2001). Catastrophic antiphospholipid syndrome: remission following leg amputation in 2 cases. *Semin. Arthritis Rheum.* **31**, 127–132.
22. Espinosa, G., Cervera, R., Font, J., and Asherson, R. A. (2002). The lung in the antiphospholipid syndrome. *Ann. Rheum. Dis.* in press.
23. Drenkard, C., Sanchez-Guerrero, J., and Alarcón-Segovia, D. (1989). Fall in antiphospholipid antibody at time of thromboocclusive episodes in systemic lupus erythematosus. *J. Rheumatol.* **16**, 614–617.
24. Wilson W. A., Gharavi, A. E., Koike, T., *et al.* (1999). International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Report of an international workshop. *Arthritis. Rheum.* **42**, 1309–1311.
25. Lasne, D., Saffroy, R., Bachelot, C., *et al.* (1997). Tests for heparin-induced thrombocytopenia in primary antiphospholipid syndrome [letter]. *Br. J. Haematol.* **97**, 939.
26. Kelton, J. G., and Warkentin, T. E. (1995). Diagnosis of heparin-induced thrombocytopenia. Still a journey, not yet a destination. *Am. J. Clin. Pathol.* **104**, 611–613.
27. Moake, J. L. (1998). Moschcowitz, multimers, and metalloprotease. *N. Engl. J. Med.* **339**, 1629–1631.
28. Rock, G., Porta, C., and Bobbio-Pallavicini, E. (2000). Thrombotic thrombocytopenic purpura treatment in year 2000. *Heamtologica* **85**, 410–419.
29. Musio, F., Bohen, E. M., Yuan, C. M., and Welch, P. G. (1998). Review of thrombotic thrombocytopenic purpura in the setting of systemic lupus erythematosus. *Semin. Arthritis Rheum.* **28**, 1–19.
30. Ridolfi, R. L., and Bell, W. R. (1981). Thrombotic thrombocytopenic purpura. Report of 25 cases and review of the literature. *Medicine* **60**, 413–428.
31. Murphy, W. G., Moore, J. C., Warkentin, T. E., Hayward, E. P., and Kelton, J. G. (1992). Thrombotic thrombocytopenic purpura. *Blood Coagul. Fibrinol.* **3**, 655–659.
32. Cate, H., Timmerman, J., and Levi, M. (1999). The pathophysiolgy of disseminated intravascular coagulation. *Thromb. Haemost.* **82**, 713–717.
33. Muller-Berghaus, G., ten Cate, H., and Levi, M. (1998). Disseminated intravascular coagulation. In "Cardiovascular Thrombosis: Thrombocardiology and Thromboneurology." (M. Verstraete, V. Fuster, E. J. Topol, Eds.), pp. 781–799. Lippincott-Raven, New York. 781–799.
34. Maddison, P. J., Thorpe, C., Seale, J. R. C., Ahmed, W., and Whiteley, G. S. (2000). Grand rounds from International Lupus Centres. "Catastrophic" antiphospholipid syndrome. *Lupus* **9**, 484–488.
35. Erkan, D., Leibowitz, E., Berman, J., and Lockshin, M. D. (2003). Perioperative medical management of antiphospholipid syndrome: Hospital for Special Surgery experience, review of literature, and recommendations. *J. Rheumatol.* **29**, 843–849.
36. Menon, G., and Allt-Graham, J. (1993). Anaesthesic implications of the anti-cardiolipin syndrome. *Br. J. Anaesth.* **70**, 587–590.
37. Madan, R., Khoursheed, M., Kukla, R., al-Mazidi, M., and Behbehani, A. (1997). The anaesthetist and the antiphospholipid syndrome. *Anaesthesia* **52**, 72–76.
38. Geerts, W. H., Heit, J. A., Clagett, G. P., Pineo, G. F., Colwell, C. W., Anderson, F. A., Jr., and Wheeler, H. B. (2001). Prevention of venous thromboembolism. *Chest* **119**(Suppl. 1), 132S–175S.
39. Khamashta, M. A., Cuadrado, M. J., Mujic, F., Taub, N. A., Hunt, B J., and Hughes, G. R. V. (1995). The management of thrombosis in the antiphospholipid antibody syndrome. *N. Engl. J. Med.* **332**, 993–997.
40. Neuwelt, C. M., Daikh, M., Linfoot, D. I., *et al.* (1997). Catastrophic antiphospholipid syndrome: Response to repeated plasmapheresis over three years. *Arthritis Rheum.* **40**, 1534–1539.
41. Dosekun, A. K., Pollak, V. E., Glas-Greenwalt, P., *et al.* (1984). Ancrod in systemic lupus erythematosus with thrombosis. Clinical and fibrinolysis effects. *Arch. Intern. Med.* **144**, 37–42.

42

HEMATOLOGY: COAGULATION PROBLEMS

Sandor S. Shapiro
Stephen E. F. Spurgeon

LUPUS ANTICOAGULANTS

Lupus anticoagulants (LACs) are immunoglobulins that interfere with *in vitro* phospholipid-dependent coagulation tests [1]. The term was introduced by Feinstein and Rapaport [2] in 1972, although the earliest observations of the phenomenon were reported some 20 years before [3, 4]. Initially recognized in the context of systemic lupus erythematosus (SLE), LACs are now known to occur frequently in patients with other disorders, as well as in individuals with no apparent underlying disease. It is now recognized that LACs and anti-cardiolipin antibodies (ACAs) are separate entities, but both are members of a diverse group of immunoglobulins that bind to phospholipid-bound proteins including, but not limited to, β2-glycoprotein-I (β2GPI) and prothrombin. LACs and ACAs are both associated with a high risk of arterial and venous thromboembolic events [5–8], although the mechanism of this thrombotic risk is still unknown.

In 1980, Thiagarajan *et al*. [9] proposed for the first time a mechanism for LAC action in coagulation tests. They isolated an IgMλ Waldenstrom paraprotein with LAC activity and demonstrated that it had antibody activity against negatively charged phospholipids—phosphatidylserine, phosphatidylinositol, and phosphatidic acid—but not against phosphatidylcholine or phosphatidylethanolamine. They subsequently showed activity of a number of LAC plasmas and sera against cardiolipin, as well [10]. In 1983, Harris *et al*. developed a radioimmunoassay, and subsequently an ELISA, for ACAs and demonstrated a close relationship between the ACA and LAC tests [11].

In this chapter we use the term anti-phospholipid antibody (APL) to denote the presence of a LAC and/or ACA, despite the fact that, as indicated, this is, strictly speaking, a misnomer. We discuss the diagnosis of LACs, the possible thrombotic mechanisms associated with APLs, and the treatment of thrombosis in patients with APLs. We begin with a discussion of tests for LACs, and the relative value of LAC and ACA tests in assessing the risk of thrombosis. For specifics of the ACA ELISA and a more extensive analysis of patient populations using that test, see Chapter 41.

LABORATORY DIAGNOSIS OF LUPUS ANTICOAGULANTS

The minimum laboratory criteria for diagnosis of a LAC have been defined by international agreement [12], and in essence require (1) prolongation of one or more phospholipid-dependent coagulation tests; (2) demonstration that this prolongation is due to the presence of an inhibitor (i.e., that it is not corrected by the addition of an equal volume of normal plasma); and (3) demonstration that the inhibition is not due to interference with the thrombin-fibrinogen reaction and is not directed against the functional activity of a specific coagulation factor.

Several tests are in common use for the detection of LACs, including the activated partial thromboplastin

time (APTT), the tissue thromboplastin inhibition test (TTI) [13, 14], the kaolin clotting time (KCT) [15, 16], the dilute Russell viper venom time (DRVVT) [17], and the dilute APTT [18, 19]. Except for the standard APTT, the other tests depend on the use of reduced concentrations of phospholipid reagents for enhanced sensitivity. The prothrombin time is an insensitive test for LACs, but may be prolonged because of concomitant hypoprothrombinemia [20]. In general, prolongation of any of these tests can be caused by deficiencies of specific clotting factors, by specific inhibitors to clotting factors, or by general inhibitors such as heparin, as well as by LACs. Deficiencies can be ruled out by correction of the prolonged test on addition of an equal volume of normal plasma. In the absence of correction, a specific coagulation inhibitor or the presence of heparin must be ruled out before a LAC can be diagnosed. Heparin can be excluded by the history, as well as by performance of a thrombin time—a test that is prolonged by heparin but is normal in the presence of a LAC. The most common specific coagulation factor inhibitor is an antibody against factor VIII, and the presence of such an inhibitor can prolong the KCT, APTT, dilute APTT, and TTI. The TTI is simply a prothrombin time done in the presence of a high dilution of the usual tissue thromboplastin reagent and, at such dilutions, is sensitive to abnormalities in the intrinsic pathway [21]. The DRVVT should be insensitive to the presence of factor VIII inhibitors, since it is initiated by directly activating factor X to factor Xa. In one study, however, two factor VIII inhibitors were reported to cause minimal prolongation of the DRVVT [22]. In our hands even high-titer antibodies to factor VIII did not prolong the DRVVT [17]. Thus it appears that the DRVVT may be the most specific of the tests for LACs.

The relative sensitivity of LAC tests is more difficult to ascertain. Part of the difficulty stems from the fact that the sensitivity of many of the tests depends on the method of plasma preparation (since the presence of residual platelets, particularly if the plasma is frozen before testing, decreases sensitivity [23]), on the source of phospholipid [22, 24, 25], and on the type of activating agent [26, 27], and has been reported to be decreased in the presence of high levels of factor VIII [28] and fibrinogen [29]. In addition, valid comparisons of tests are difficult to make because of variations in test reagents between laboratories, because tests have frequently been modified from their original descriptions, and because of variability in apparent test sensitivities from patient to patient.

With these caveats, a review of the literature suggests that, overall, the KCT is the most sensitive test, followed by the dilute APTT, the DRVVT, and the TTI [12, 15–19, 30–33]. It is important to recognize that, even using the most sensitive phospholipid reagent, the APTT is the least sensitive of all the tests, and that the DRVVT, the dilute APTT, and the KCT may be positive in some patients with a normal standard APTT [14, 16]. For example, Lo *et al*. [30] reported that of 18 patients positive by the KCT, 17 were positive by the DRVVT, TTI, and dilute APTT, but only 16 were positive by the APTT. Several other of the studies mentioned have also compared the KCT with other LAC tests and have found it to be somewhat more sensitive. On the other hand, the KCT, the dilute APTT, and the TTI are more or less sensitive to abnormalities of the entire intrinsic pathway, and are, therefore, prolonged in the presence of the most common coagulation factor inhibitors.

With the exception of the KCT, all other tests require addition of phospholipid, and results of those tests vary with the source of phospholipid. While the phospholipid content of most coagulation reagents is poorly characterized, there is a suggestion that the lower the PS content, the more sensitive the reagent is to the presence of LACs [25, 34].

Several confirmatory tests have been proposed in the event a prolonged LAC test is discovered. In the platelet neutralization procedure [21] the APTT is repeated after addition of a platelet concentrate; prolongations due to the presence of a LAC are generally normalized by the platelet concentrate. Triplett *et al*., in their original publication [21], found that 13 of 13 APTTs that were prolonged because of the presence of a LAC were normalized by the addition of platelets; in contrast, 10 of 11 patients with specific inhibitors had no shortening of the APTT under these conditions. However, occasional LACs are not corrected by the platelet neutralization procedure. A somewhat similar test was introduced by Rosove *et al*. [35], in which high concentrations of phospholipid are used to normalize the prolonged APTT (high PL-APTT). However, the high PL-APTT may not always be normal in patients with LACs. In fact, one study [29] found that the high PL-APTT was positive in only 10 of 18 patients with LACs, tending to be negative in LAC patients who did not have SLE. Furthermore, this test does not distinguish well between LACs and factor VIII inhibitors [31]. In summary, a positive confirmatory platelet neutralization procedure or high PL-APTT is very useful, but a negative test does not absolutely rule out a LAC. A third test was developed by Rauch *et al*. [36, 37], who demonstrated that LACs react with hexagonal-phase phosphatidylethanolamine and that addition of this phospholipid to the PTT would neutralize the prolongation induced by presence of a LAC [38].

Since no single test has been shown to identify all patients with LACs, while several tests identify 90% of this population, our recommendations are as follows.

1. Sensitive screening tests for LACs should be used. In addition to an APTT, done using one of the 'LAC-sensitive' reagents, to obtain maximum yields, at least one more sensitive test should be employed, such as the DRVVT, the KCT, the TTI, or the dilute APTT.

2. If the LAC screening test is prolonged, a mixing study must be done (equal volumes of normal and patient plasma). If the mixing study is prolonged, a thrombin time must be done to rule out the presence of heparin or other interference with the thrombin-fibrinogen reaction. A prolonged thrombin time casts doubt on the meaning of the LAC screening test and should be investigated further. In many laboratories a reptilase time would then be done, since it is normal in the presence of heparin. Abnormalities of both the APTT and the thrombin time can occur in diffuse intravascular coagulation because of the presence of fibrin split products or, much less commonly, because of the presence of an abnormal fibrinogen or a circulating antithrombin.

3. If the thrombin time is normal, the diagnosis rests between a specific coagulation inhibitor and a LAC. Because of the specificity of the DRVVT, most common specific coagulation factor inhibitors, such as factor VIII and factor IX antibodies, can generally be excluded. A factor V inhibitor would prolong the DRVVT, although these rare inhibitors generally reveal their presence by significant bleeding. Thus, in the presence of clinically significant bleeding, specific tests for a factor V inhibitor should be initiated.

4. A confirmatory test, such as the platelet neutralization procedure, the high phospholipid test, or the hexagonal phase PE test, should be used.

PREVALENCE OF LUPUS ANTICOAGULANTS AND ANTI-CARDIOLIPIN ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

In a comprehensive review of the literature Love and Santoro [5] estimated, using only large, unselected series, that the prevalence of LACs and ACAs in patients with SLE was 28 and 42%, respectively. Similar figures have been reported in more recent studies [7, 39]. Since several different LAC tests were used in the various series, and the upper limit of normal for the ACA tests also varied, the actual figures may be even higher. For example, in three series using an upper limit of only 2 SD above the mean the prevalence of ACAs in SLE was above 50% [40–42]. The differences in incidence of LACs and ACAs in SLE is not merely a matter of test sensitivities. Love and Santoro [5] analyzed series in which patients with SLE had both tests performed: only 45% of ACA-positive patients were also LAC positive, while only 59% of LAC-positive patients were also ACA positive. These findings are consistent with the concept that the two tests identify different populations of antibodies and that these two populations of antibodies can be physically separated.

The prevalence of LACs or ACAs in autoimmune diseases other than SLE has been commented on in several reports. Fort *et al*. [43] measured ACAs in 243 patients with a variety of rheumatic disorders. An elevated frequency of ACAs was found in patients with SLE (38%), rheumatoid arthritis (33%), and psoriatic arthritis (28%). Another study of 90 patients with rheumatoid arthritis (using a cutoff of 4 SD above the normal mean) found 44 (49%) to be ACA positive [44]. Harris *et al*. [45] also studied ACA levels in a variety of collagen–vascular diseases: 39% of SLE patients and 32% of patients with other rheumatic diseases were noted to be positive. LACs and/or ACAs have also been found in lymphoproliferative disorders [46, 47], in acute infections [48], during drug therapy [49, 50], and in individuals with no underlying disease [47].

PREVALENCE OF THROMBOSIS IN PATIENTS WITH LUPUS ANTICOAGULANTS OR ELEVATED ANTI-CARDIOLIPIN ANTIBODIES

The risk of thrombosis in LAC/ACA patients has been examined by many authors, largely through retrospective analysis. By combining the published data, it appears that about one-third of all patients with LACs have had at least one thrombotic event [52]. Among SLE patients, 42% of the LAC-positive and 40% of the ACA-positive individuals had a history of thrombosis; in contrast, the prevalence of thrombosis in LAC-negative or ACA-negative SLE patients was only 10–18% [5]. Generally, the follow-up periods giving rise to these figures have not been specified, but in three studies of LAC-positive patients with SLE or other connective tissue disorders followed for at least 5 years the prevalence of thrombosis was 50–70% [53–55]. When only unselected series were evaluated, the prevalence of thrombosis in LAC/ACA-positive patients with SLE was as high as 62% [7, 39, 56]; the length of follow-up in these studies was not given.

Thrombotic events involve both the arterial and venous circulation; in LAC-positive patients with SLE or related disorders, approximately 60% of thrombotic events were venous and 40% were arterial [46, 47, 53, 54, 57, 58]. The most common site of venous thrombosis was a lower extremity, while the most common

site of arterial thrombosis was the cerebral arterial system.

Thrombosis also occurs in LAC-positive or ACA-positive patients with non-SLE autoimmune disorders [41, 57] and in individuals with no apparent underlying disorders; however, the prevalence of thrombosis in these groups has not been firmly established. In two series of patients with rheumatoid arthritis, the combined prevalence of thrombosis was 10% in ACA-positive patients and 6% in ACA-negative patients [43, 44]. The frequency of thrombosis in patients with LACs secondary to drug therapy is not certain, but is probably low. Gastineau *et al*. [46] reported that none of 14 patients with drug-related LACs had experienced thrombotic events. On the other hand, Triplett and co-workers [12] reported a 24% incidence of thrombosis in 34 patients with drug-induced LACs. Since most of these patients were receiving procainamide or quinidine, and may have been at increased risk because of underlying cardiac disease, it would have been informative to know the prevalence of thrombosis in a matched group of LAC-negative patients on the same drugs. Thrombosis in patients with LACs secondary to 1ong-term chlorpromazine therapy seems to be an infrequent occurrence [49], although it has been reported occasionally [51]. Thrombosis has also been reported in two LAC-positive patients receiving haloperidol and fluphenazine [50].

Patients with AIDS have a high incidence of LACs and elevated ACAs [59–64]. However, the risk of thrombosis in this group appears to be minimal.

MECHANISMS

The causes of the increased thrombotic risk are unknown, although several mechanisms have been posited. For example, Carreras and co-workers [65, 66]. observed inhibition of prostacyclin (PGI_2) synthesis and/or release by aortic rings or cultured endothelial cells exposed to LAC plasma or IgG. However, subsequently there have been several conflicting reports using similar systems [57, 67–71], leaving the role of this mechanism unresolved. Several investigators have reported that plasma or IgG from patients with LACs interferes with thrombin/thrombomodulin-dependent protein C activation or with thrombomodulin function [72]. Protein C in its activated form inactivates coagulation factors Va and VIIIa; protein S is a cofactor in this reaction, and Marciniak and Romond have reported defective phospholipid- and platelet-dependent degradation of factor Va in clotting plasma from patients with LACs [73]. This important observation suggests that LACs may interfere with the Ca^{2+}-dependent interaction of activated protein C with phospholipid in a manner analogous to that previously shown for factor X and prothrombin [1, 2]. It is of interest that several reports have described depressed levels of protein S, although not of protein C, in many individuals with LACs, with or without SLE [1, 74, 75].

Other mechanisms also may be involved. For example, there are conflicting reports that patients with LACs have decreased cell-related fibrinolytic activity [74, 76–79], although no direct evidence for such a mechanism has been adduced. It is possible that LACs might alter platelet function, leading to an increased thrombotic risk, a suggestion consistent with the observation that thrombocytopenia is frequently seen in patients with LACs [1, 13, 80, 81]. It is clear from this discussion that no single mechanism has so far been put forth that can explain the risks of thrombosis in patients with LACs and ACAs.

The most important new mechanistic insight into this problem has come from the realization that patients with LACs/ACAs do not, as a rule, make antibodies to cardiolipin or other phospholipids, but rather to a variety of proteins having in common an ability to form a complex with phospholipids [1, 8, 70, 82]. These proteins probably form neoepitopes on combining with phospholipid and, perhaps by this mechanism, become antigenic. Of these, β_2-glycoprotein I (apolipoprotein H) and prothrombin have had the most attention. β_2-glycoprotein I is required for detection of the vast majority of ACAs in the standard ACA ELISA, and an ELISA for β_2-glycoprotein I has been advocated by several investigators as a more specific predictor of thrombosis than the standard ACA ELISA [83–90]. $Beta_2$-glycoprotein I is also required for the activity of many LACs [91–93], and a number of human and murine monoclonal antibodies to this protein act as LACs when added to normal plasma [94]. However, it has been shown that many other LACs require the presence of prothrombin for their activity [92, 93, 95], and coagulation tests may show differential sensitivity to these two types of LACs [93]. In addition, antibodies to protein C [96], protein S [96], prekallikrein [97], tissue factor pathway inhibitor (TFPI) [98], and annexin V [99, 100] have been described. In each case it is possible to picture a disturbance in function that might give rise to a thrombotic risk. Furthermore, mounting evidence suggests that the endothelium itself may be the target of at least some anti-phospholipid antibodies [101–104] and that plasma thrombomodulin levels may reflect vascular injury induced by such antibodies [105, 106]. Thus no one mechanism may explain the multiple clinical manifestations of what is called the antiphospholipid syn-

drome, and separate causes may predominate that account for the venous thrombosis, arterial thrombosis, fetal loss, and thrombocytopenia seen in this syndrome. Were this to be the case, it is possible that the predictive value of current laboratory tests may depend on the clinical event being evaluated and that new tests may be required to define specific clinical risks.

Several *in vitro* studies have implicated annexin V in the thrombotic diathesis seen in the APS. Annexin V is a protein with a high, calcium-dependent, affinity for anionic phospholipids. It has been suggested that annexin V may have anticoagulant properties because it can displace bound prothrombin or factor Xa from phospholipid surfaces. It has been reported that ACAs can inhibit annexin V binding to a phospholipid surface in a β2-glycoprotein-I-dependent manner [107]. Much of the data on annexin V has been generated by Rand [108]. Rand *et al*. have shown that annexin V expression is decreased on the apical surface of placental villi in APL-positive patients with a history of recurrent spontaneous abortions [109] and on membranes isolated from villi cultured with APL IgG fractions [110]. Furthermore, they have reported that, by displacing annexin V, APLS do, in fact, create a procoagulant environment [111]. However, Willems and co-workers [112, 113] found that the high-affinity binding of annexin V to phospholipid membranes and membranes of activated platelets was unaffected by the presence of ACA–β2-glycoprotein-I complexes, which could not displace annexin V from procoagulant surfaces.

LUPUS ANTICOAGULANTS, ANTICARDIOLIPIN ANTIBODIES AND RELATED ANTIBODIES AS RISK FACTORS FOR THROMBOSIS

The relative value of LACs and ACAs as predictors of thrombosis has been examined by several authors. Harris *et al*. [11] and Boey *et al*. [53] evaluated groups of patients with SLE and other autoimmune diseases, the former by ACA status and the latter by LAC testing: 38% of ACA-positive and 58% of LAC-positive patients had a history of thrombosis. Love and Santoro [5], in their review, noted that 56% of unselected LAC-positive SLE patients had a history of thrombosis as opposed to only 39% of unselected ACA-positive patients. Galli *et al*. [114], based on a review of several studies, suggested that LAC-positivity is a better predictor of thrombosis than is ACA-positivity. More recently, these authors presented intriguing evidence that DRVVT-positivity was a stronger predictor of thrombotic risk than KCT-positivity [93, 115, 116].

Several studies have suggested that thrombotic risk increases with the ACA titer [42]. One series [45] found that 80% of patients with an ACA titer >7 SD above the normal mean had a history of thrombosis. In another report, 11 of 15 patients with ACA >9 SD above the normal mean had a history of thrombosis; two others had pulmonary hypertension [11]. Hamsten *et al*. [117], in a study of 62 non-SLE patients under the age of 45 years who survived acute myocardial infarction, found 13 patients who were ACA-positive. Seven of eight with an ACA titer >5 SD above the normal mean had additional cardiovascular morbidity; in contrast, only one of five patients with lesser elevations had a new cardiovascular event. Deleze *et al*. [118] noted that the odds ratio of recurrent fetal loss in patients with SLE whose ACA titer was >2 SD above normal was 6.6 compared with ACA-negative SLE patients; SLE patients with ACA titers >5 SD above normal had an odds ratio of 10.5. On the other hand, Triplett *et al*. [119] were not able to document a difference in the incidence of thrombosis between high- and low-titer ACA-positive individuals.

Some of the variability in the results quoted may relate to variation in ACA titers in individuals. For example, Ishii *et al*. [120] found that ACA-positive patients could be separated into two groups on the basis of variation in their ACA titers: group A remained positive during stable disease, whereas group B became negative during stable disease. They found that the incidence of thrombosis was 33% in group A and only 3% in group B. Cerebral infarction was found in 23% of group A and in only 3% of group B; spontaneous abortions were found in 41% of married women in group A as opposed to 7% in group B. Another study of 500 SLE patients found that 47% of patients with persistently positive ACA titers had venous thrombotic events, whereas only 6.1% of intermittently positive patients had venous thrombotic events [42], only 3% of the persistently negative group had a history of venous thrombosis.

Currently a great deal of effort is being directed to an analysis of the relative merits of the measurement of antibodies to a number of proteins, particularly β_2-glycoprotein-I and prothrombin, as indices of thrombotic risk [121–126]. Nojima *et al*. [127] studied a group of 168 SLE patients, of whom 49 had thrombotic complications, 14 had experienced fetal loss, and 38 were thrombocytopenic. They found that elevated IgG anti-β2-glycoprotein-I and IgG anti-prothrombin were significant risk factors for arterial, but not venous, thrombosis. They also found that the presence of antibodies to protein S was a significant risk factor for venous thrombosis and that annexin V antibody elevations directly correlated with a history of fetal loss.

However, these interesting correlations have not been observed by all investigators [128, 129], and large prospective studies are still needed to settle this important issue.

Antibodies to prothrombin are present in 60–75% of individuals with LACs, and mild hypoprothrombinemia—in many cases insufficient to prolong the PT significantly—is present in perhaps 25% of these. A small percentage of patients have moderate or severe depression of prothrombin levels. It is of some interest that such individuals may have a lower than expected risk of thrombosis. Lechner and Pabinger-Fasching [47] reported that none of the three patients in their study with significant prothrombin deficiency had a history of thrombosis. When they reviewed the literature they found that none of the 17 reported LAC patients with prothrombin deficiency had thrombotic complications recorded. Another study of lupus patients reported three additional patients with prothrombin deficiency who had no history of thrombosis [130].

On balance, it appears that coagulation-based tests, particularly the DRVVT, may be better predictors of thrombotic risk than the ACA. However, the issue remains to be decided and studies are currently ongoing to that end.

THE ROLE OF GENETIC RISK FACTORS IN THE THROMBOTIC RISK IN PATIENTS WITH LUPUS ANTICOAGULANTS AND ANTICARDIOLIPIN ANTIBODIES

As mentioned previously, not all patients with persistent LACs or ACAs develop thrombosis. This has led to efforts to identify contributing risk factors in APL patients. In this section, we review the data relating to four of the most studied risk factors [131–133]: (1) factor V Leiden (FVL), (2) the prothrombin G20210A mutation, (3) plasminogen activator inhibitor-1 (PAI-1) polymorphisms, and (4) plasma homocysteine levels and methylenetetrahydrofolate reductase (MTHFR) mutations.

1. Factor V Leiden (FVL) is a point mutation in the factor V gene that results in the substitution of glycine for arginine at codon 506. This mutated factor V is activated normally by thrombin—and, therefore, functions normally as a coagulation cofactor. However, the activated factor V is resistant to cleavage by activated protein C (APC), thus removing an important feedback inhibition of the clotting system, and conferring a risk of thrombosis, primarily venous thrombosis [134–136]. FVL is present in ~5% of the Caucasian population and up to 20% of unselected patients with venous thrombosis, and is the most common of the known genetic risk factors for venous thrombosis.

2. The G20210A prothrombin gene mutation—substitution of adenine for guanine at position 20,210 in the nucleotide sequence—is present in 2–3% of the population and in ~6% of unselected patients experiencing their first venous thrombosis [137]. The mutation does not affect the structure of prothrombin, but results in higher circulating levels of the protein.

3. PAI-1 is a major regulator of fibrinolysis. Elevated PAI-1 concentrations have been observed in a number of thrombotic disorders, including coronary artery disease and myocardial infarction [138]. A genetic polymorphism in the promoter region of the PAI-1 gene, in which either four or five guanines are present, has been described [139]. The 5 guanine (5G), but not the 4G, allele binds a repressor and, as a result, individuals homozygous for the 4G allele have 25% higher PAI-1 plasma levels than 5G/5G individuals [139]. Furthermore, the 4G allele is more easily stimulated by cytokines [140] and by triglycerides in the presence of insulin [132, 138]. Several studies have raised the possibility that the 4G/4G genotype may be a thrombotic risk factor [132, 138].

4. The plasma homocysteine level is an independent risk factor for atherosclerosis, arterial thrombosis [141] and, possibly, venous thrombosis, as well [142]. Elevated plasma homocysteine levels can occur as the result of deficiencies of vitamins B_{12} or B_6 or folic acid, chronic renal failure, hypothyroidism, certain malignancies, drugs, and inherited enzyme abnormalities [133, 141]. One enzyme involved in homocysteine metabolism is MTHFR, which catalyzes the conversion of homocysteine to methionine. Two common genetic variants in the MTHFR gene have been studied, 677C→T (C677T) and 1298A→C (A1298C). Each of these mutant alleles is present in 30–40% of individuals [143, 144]. C677T homozygotes (C677T +/+), but not heterozygotes (C677T +/–), have elevated plasma homocysteine levels. No relationship has been identified between plasma homocysteine and the A1298C mutation [143, 144]. Fragmentary data raise the possibility that compound heterozygosity for the two mutations may be associated with elevated homocysteine levels [143–145]. However, in view of the complexity of plasma homocysteine regulation, the thrombotic risk associated with these mutations is not clear [146]. It is important to emphasize that, even within the normal range, atherosclerotic risk is directly related to the homocysteine level. In view of the ease of reducing homocysteine levels by ~40% with simple dietary supplementation [147], the advisability of intervening in this manner in LAC/ACA-positive patients should be borne in mind (see later).

We will now review the data concerning the possible contribution of these genetic factors to the thrombotic risk in patients with LACs and/or ACAs. Fijnheer *et al*. [56] studied 173 SLE patients, 43 of whom had a history of thrombosis, and found that the presence of either a LAC or a moderate-to-high-titer ACA was a risk factor for both venous and arterial thrombosis. Nine of the 173 patients (5%) had FVL; 4 had a history of venous thrombosis. Only 2 of the 173 SLE patients—both with a history of thrombosis—were positive both for a LAC and FVL. Although suggestive, these numbers are too small to draw statistical conclusions.

Hansen *et al*. reviewed the histories of 99 LAC/ACA-positive patients, 78 of whom had thrombosis—41 venous, 25 arterial, 12 both venous and arterial [148]. They found that hypertension, hyperlipidemia, tobacco use, and diabetes were significant risk factors for arterial thrombosis. FVL and prothrombin G20210A appeared to be predictors of venous thrombosis. Specifically, 8 of the 99 patients were FVL-positive and all experienced thrombosis—4 venous, 1 arterial, and 3 both venous and arterial. Three of the 99 patients had the G20210A mutation, and all had thrombotic events —2 venous and 1 both arterial and venous. These numbers, though highly suggestive, did not attain statistical significance when each mutation was analyzed separately—only when the FVL and the G20210A data were grouped together did the differences become significant.

Galli *et al*. [149] evaluated 152 consecutive unselected LAC-positive patients—48 with SLE, other autoimmune diseases, or cancer—and found an increased thrombotic risk in patients with FVL: 5 individuals had FVL and all had a history of venous thrombosis. Of the remaining 147 patients, 68 (46%) had a history of thrombosis. This difference just achieved statistical significance ($p = 0.047$). The presence of prothrombin G20210A or MTHFR C677T did not confer additional thrombotic risk. The authors concluded that (1) the prevalence of FVL in the LAC-positive patients is similar to that in the general population, and (2) the likelihood of venous thrombosis is significantly greater when both abnormalities are present. However, conclusions from this excellent study, like the others, must be tempered because of the small numbers of patients with inherited thrombophilic risk factors.

Ames *et al*. [150] examined the influence of FVL and MTHFR C677T in 49 individuals with idiopathic (non-SLE) anti-phospholipid antibodies, in 70 patients with a history of spontaneous venous thrombosis (APL status not stated, but 17 of whom were heterozygous for protein C, protein S, or antithrombin), and in 193 healthy subjects. FVL prevalence was 14, 18, and 4%, respectively, in the three groups. When these data were reanalyzed, the prevalence of thrombosis in APL-positive patients was 55 and 67% for the FVL-positive and FVL-negative patients, respectively. Thus these data do not indicate an increased risk in APL patients due to FVL. However, among the 27 APL-positive patients with thrombosis, 7 patients were C677T +/+. Two of the latter also had FVL. These 7 patients experienced their first thrombotic event, on average, at a significantly earlier age than patients who were C677T heterozygous or negative. It should be noted that this study did not measure plasma homocysteine levels, so the direct contribution of elevated homocysteine could not be evaluated. Cappucci *et al*. [151] did measure homocysteine levels in C677T +/+ APL-positive patients and found that these patients had significantly higher homocysteine levels and also experienced their first thrombotic event at a significantly earlier age than patients with APL or C677T +/+ alone.

The role of the PAI-1 polymorphism in APL patients has been investigated in several studies. Forastiero *et al*. [152] studied a group of 105 consecutive unselected APL-positive patients, 69 with and 36 without APS, as well as 200 healthy subjects. The prevalence of FVL, MTHFR C677T, and PAI-1 4G/4G mutations was similar in all three groups, while the prevalence of the prothrombin G20210A mutation was three times higher in the APS-positive than in the APS-negative or control groups (8.7, 2.8, and 2.0%, respectively). However, combinations of G20210A or FVL with PAI-1 4G/4G were significantly more frequent in the APS patients than in the other two groups (5.8, 0, and 0.5%, respectively).

On the other hand, Tassies *et al*. [153]. found that APL-positive patients with a history of arterial thrombosis had a significantly higher frequency of at least one 4G allele (4G/4G or 4G/5G) than patients without arterial thrombosis. Although not statistically significant, PAI-1 activity was somewhat higher in these patients. These differences were not seen for venous thrombosis. Interestingly, although all patients and controls had normal plasma levels of TNF-α and IL-1, four patients had increased levels of IL-6 and all four experienced arterial thrombosis. As previously mentioned, PAI-1 activity is upregulated by cytokines [140], and it is possible that plasma PAI-1 levels may increase in response to inflammation to a greater extent in individuals with the 4G allele.

However, several studies have found no additive effect of these genetic risk factors in APS. Thus, Yasuda *et al*. [154] found no correlation between the PAI-1 4G/5G polymorphism and risk of venous or arterial thrombosis in patients with APLs, and Bentolila *et al*. [155] and Bertolaccini *et al*. [156] found no difference in prevalence of the prothrombin G20210A mutation

between APL patients with and without thrombosis and control groups. Thus, it is not yet clear what role genetic mutations play in the thrombotic risk of the antiphospholipid syndrome. It is unlikely that this state of affairs will change until large prospective studies are designed to examine the issue. However, despite the limitations of our present knowledge, it may be prudent to assume that genetic thrombotic risk factors play some additive role in the clinical events in APS and, thus, it seems logical to screen APL patients for these genetic mutations. Because patients who are homozygous for the MTHFR mutation and APLs may have an earlier age of onset of thrombotic events, presumably due to increased homocysteine levels, we recommend measurement of plasma homocysteine levels in all LAC/ACA-positive individuals. Patients with elevated levels, and even patients in the upper two quartiles of the normal range, should be treated with folate/B_{12}/B_6.

TREATMENT OF THROMBOSIS IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS WITH LUPUS ANTICOAGULANTS OR ANTI-CARDIOLIPIN ANTIBODIES

In this section we review the clinical literature relating to thrombosis in LAC/ACA-positive individuals and attempt to provide guidelines for treatment. However, it must be appreciated that adequate prospective studies of this problem are lacking, and therefore our approach, like that of others, is based on small series and anecdotal reports, intuition, and generalizations from other thromboembolic conditions.

Anticoagulation

In any individual with a risk factor for thrombosis, the question of long-term anticoagulation inevitably arises. Should long-term anticoagulation be instituted on discovery that an individual is persistently positive for a LAC or ACA, on eliciting a history of past thrombosis in such a person, or only after diagnosing an acute thrombotic episode, where it is clear that the risk of recurrence is very high [5, 157]? If only in the last setting, should the type of thrombosis play any role in the decision? In each case, it is important to reiterate that only persistent LAC/ACA positivity is strongly associated with thrombotic risk. Thus it is necessary to perform tests at least twice, at least 1 month apart.

While several retrospective studies make it highly likely that anticoagulation decreases the rate of recurrent thrombosis after a first event [157–159], current clinical opinion would argue against long-term anticoagulation solely due to the discovery of a persistently positive LAC or ACA test. An exception may be the use of anticoagulants temporarily during periods of high risk, such as major surgery or prolonged confinement to bed. One retrospective study of 23 patients undergoing 18 vascular and 33 nonvascular surgical procedures concluded (albeit with no control group) that antithrombotic treatment of LAC-positive patients (steroids, antiplatelet agents, and anticoagulants were used in various combinations) appeared to protect against postoperative thrombotic complications [160]. Another study of 9 patients came to a similar conclusion [161]. In our opinion, it would be reasonable to treat LAC/ACA-positive patients with low-dose heparin (5000 units twice daily subcutaneously) in the event of surgery or during prolonged bed rest.

In the event of acute venous or peripheral arterial thrombosis, treatment should be instituted in the standard way with heparin and oral anticoagulants. Oral anticoagulants should be begun on the first day, as soon as effective heparinization has been established. If the pretreatment APTT is only mildly prolonged by the presence of the LAC, the adequacy of heparinization can be followed with the APTT. When the preheparinization APTT is prolonged more than 10 s above normal, other approaches can be used. The substitution of a phospholipid reagent relatively insensitive to the presence of LACs may allow the APTT to be used for monitoring. Otherwise, the thrombin time may be followed. This test is sensitive to heparin, but is not prolonged by LACs. A reasonable approach would be to heparinize to a thrombin time of about 100–120 s (with a normal of about 15 s). Error on the side of a greater prolongation may not be a serious problem, since at least one study used as an end point a prolongation of the thrombin time to more than 100 s without encountering bleeding problems [162]. Heparinization should be continued for at least 5 days, to include at least 4 days of therapeutic range oral anticoagulation.

Oral anticoagulation should be continued for at least 3 months, as is recommended for any patient with an acute thrombotic episode. Should anticoagulation be continued for life? Collected data suggest that in the absence of continued anticoagulation recurrences are frequent [5, 157, 163]. In two series [47, 57], following cessation of anticoagulation a second deep venous thrombosis occurred in 22 of 35 patients, although the intervening time interval was not always stated. Another study noted 32 separate thromboembolic

episodes in 15 LAC patients, implying a high recurrence rate [164]. Recurrent thrombosis was reported in 6 patients (5 of whom carried a diagnosis of SLE) with high-titer ACAs (>7 SD above normal) 6–12 weeks after coumadin withdrawal: 5 had deep vein thrombosis, while the sixth suffered a myocardial infarction [165]. All had been asymptomatic during prior coumadin therapy of 3 months to 3 years duration. As previously mentioned, in a study of younger men (under age 45) with myocardial infarction, not on long-term anticoagulant therapy, 8 of 13 ACA-positive individuals had reinfarcted within 36 months [117]. In another study, 4 of 6 LAC-positive patients under age 40 with cerebral infarction suffered recurrent arterial thrombosis during a follow-up of 1–7 years [166].

The intensity of anticoagulation with coumadin has been the subject of a number of studies. Although "low" or "intermediate" intensity anticoagulation (INR 2–3) has been reported to be effective in biochemical [167] and clinical studies [168, 169], it appears likely that achieving an INR of 2.5–3.0 may only be sufficient to prevent venous recurrences, and that more intense anticoagulation, to an INR > 3.0, may be required to prevent arterial recurrences [157, 158] In view of the likelihood that recurrences will involve the same side of the vascular tree—arterial vs venous—as the initial episode [158], levels of anticoagulation need to be monitored closely.

In summary, available information suggests that LAC-positive and strongly ACA-positive patients who have suffered a thrombotic episode are at high risk of recurrence. Long-term, probably lifelong, oral anticoagulation seems indicated. Anticoagulation after a venous thrombosis should aim for an INR of 2.5–3.0 and after an arterial episode for an INR of 3.0–3.5, recognizing that, particularly in patients with LACs and a prolonged prothrombin time, the INR may not reflect the degree of anticoagulation and more specialized tests may be required.

The utility of aspirin for the prevention of thrombosis in LAC/ACA-positive individuals has been the subject of conflicting studies. In one large study with a 5-year follow-up, aspirin did not offer protection to LAC/ACA-positive male physicians [170]. On the other hand, aspirin seemed protective in women with LACs/ACAs and a history of pregnancy loss [171], at least when administered together with heparin [172]. Therefore, it would seem reasonable to use aspirin in patients who develop recurrent thrombosis while on adequate oral anticoagulation, especially in cases of arterial thrombosis, including CNS events (see later). However, the combination of aspirin with oral anticoagulants should be employed cautiously, since many LAC/ACA-positive patients with SLE are thrombocytopenic.

Ancrod, an enzyme extracted from Malayan pit viper venom, has been used to treat thrombosis in several LAC-positive patients with SLE [162]. This substance has both anticoagulant and thrombolytic effects. Further studies of its effectiveness may be indicated.

Steroid and Other Immunosuppressive Therapy

Steroids and other immunosuppressive agents have been used frequently in an effort to decrease or eradicate ACAs or LACs. In no case should treatment be undertaken before ascertaining that patients are persistently positive. Even then it is important to be aware of the variability of ACA titers in evaluating results of therapy. For example, in one study, IgG and IgM ACA titers were determined in 53 SLE patients who were followed for over 2 years [173]. Only 21 patients with IgG ACAs (40%) and 25 with IgM ACAs (47%) remained in their original category (negative, low-positive, high-positive). Marked changes, such as from negative to high-positive titers, were sometimes seen. Furthermore, an occasional high-titer IgG ACA has been noted to disappear over time in the absence of therapy or a change in disease activity [174].

The relation between disease activity and LAC/ACA titers is not clear. In one study of 47 patients, elevated IgG and IgM ACA titers were associated with disease activity in only 23% and 9% of patients, respectively [173]. In another study [42], 15 of 19 patients had normal IgG ACA titers during inactive disease; 6 of the 15 became positive when the disease was reactivated. Of the 4 patients with elevated IgG ACA titers during inactive disease, on disease reactivation 2 had no change in antibody titers while in the other 2 titers became normal. Several other studies also give evidence that ACA titers frequently vary quite independently of disease activity [87, 175, 176].

With these caveats in mind, we review the data on steroid treatment of LACs and ACAs. Alarcon-Segovia *et al*. [42] reported that in 8 of 10 patients with active SLE and elevated IgG ACAs, titers became normal within 6 months of instituting steroid treatment; an additional 2 patients became normal after 6–10 months. Titers of IgM ACA fell with treatment in all but 1 of 10 patients. The mean starting prednisone dose in this group was 53 mg/day. Others have reported less successful responses to steroids [120, 173, 175, 177].

The literature examining steroid effects on LACs is somewhat sparser. Without therapy LACs usually

persist, although spontaneous disappearance has been observed both with and without remission of the disease [13]. Treatment with steroids has produced variable results. In some cases LACs disappeared if the primary disorder responded to steroids [13, 178]. Lubbe *et al*. [179] found that the prolonged APTT decreased to normal in five of six LAC-positive women (four had SLE) after 4–8 weeks treatment with prednisone (40–50 mg/day) for recurrent abortion. In contrast, Elias and Eldor [57] found no change in the APTT in eight LAC-positive patients receiving 40–50 mg per day of prednisone. Furthermore, occasional patients have responded to steroid therapy with disappearance of LAC activity, but with no change in ACA titer [177].

The therapeutic efficacy of reducing LAC/ACA levels with steroids is, in any case, unclear. Thrombosis [174] and spontaneous abortion [180] have been reported in patients whose elevated ACA titers responded to steroid therapy. Thus, although steroid therapy has produced a decrease in ACA titer or LAC activity in some patients, the efficacy of steroids in forestalling thrombotic events has generally not been proven. For patients with recurrent thrombosis, long-term anticoagulation must still be regarded as the treatment of choice.

Immunosuppressive therapy with other agents, particularly cyclophosphamide and azathioprine, has been used in some cases [44, 57, 120, 181], but insufficient data are available to draw firm conclusions.

Plasmapheresis has been used in an effort to reduce LAC activity or ACA titer [182, 183], although removal of IgG, half of which is in the extravascular space, is relatively inefficient even with multiple exchanges. In addition, because of continuing IgG synthesis, the effects of plasmapheresis are transient [182], so that this approach is unlikely to have long-term beneficial effects. The combination of regular plasmapheresis with steroid and/or azathioprine treatment may occasionally be effective [184]. Repeated plasmapheresis accompanied by methylprednisolone administration has been used with apparent success in three women with a serious skin necrosis syndrome associated with LACs [185]. Two of the women had previously been treated unsuccessfully with corticosteroids.

Hydroxychloroquine has been used for the treatment of lupus, and several observations have suggested that this agent may have a role in prevention of thrombosis in the setting of APLs [186, 187]. Hydroxychloroquine has been reported to inhibit thrombos formation in an experimental model of APL-induced thrombosis in mice [188]. However, no controlled clinical evaluation of the efficacy of this drug has yet been carried out.

Treatment of Cerebrovascular Events

Monoclonal antibodies to anionic phospholipids react with brain tissue [189], and neurologic impairment has been induced in mice in whom the "antiphospholipid" syndrome has been induced by immunization with monoclonal human anti-cardiolipin antibody [190]. Studies in humans of the relationship between cerebrovascular symptomatology and presence of LACs/ACAs suffer from the paucity of reports in which LACs were measured. In Love and Santoro's analysis [5] 38% of LAC-positive SLE patients had "neurologic disorders" compared with only 21% of LAC-negative patients; 49% of patients with elevated ACAs, compared with only 12% of ACA-negative patients, had neurologic symptoms. Elevated ACAs appeared to be more strongly associated with neurologic disorders of vascular origin (transient ischemic attack, stroke, retinal artery occlusion, amaurosis fugax) than with psychiatric disorders. Several series have investigated the relationship between cerebrovascular occlusive events and elevated ACAs (independent of SLE) and have demonstrated a significant association [57, 58, 191–195].

When examined from another standpoint, roughly one-fourth of ACA-positive patients with cerebrovascular disorders have SLE [196, 197]. However, more than one-half of the patients in these series had at least one other risk factor for cerebrovascular accidents, such as smoking or hypertension. Over one-half of the patients had abnormal cerebral arteriograms [130, 192, 146]. One-third to one-half of the patients had associated systemic thrombotic disease [196, 197]; some had a cardiac source for emboli [198]. Many patients, perhaps the majority, had recurrent events [166, 197, 199]. However, the relationship between LAC or ACA titer and frequency of recurrence has not been established.

Effectiveness of therapy is difficult to judge, since only small series have been published. An analysis of the treatment of 45 LAC/ACA-positive patients with cerebrovascular or neurologic disease noted that 10 of the 25 patients treated with antiplatelet agents (aspirin, dipyridamole, or both) had new events during a mean follow-up period of 12 months [197]. Another author reported a good therapeutic effect of 80-mg aspirin daily, over a follow-up period of 10 months, in a patient who previously had recurrent cerebral ischemic events [200].

Coumadin therapy has been used in LAC/ACA-positive patients with cerebral ischemia. Fisher and McGehee [201] reported that four patients treated with coumadin for a mean of 7 years remained free of recurrence. Another review notes that of four patients treated with oral anticoagulants alone or in combina-

tion with other medication, two on coumadin therapy became asymptomatic, one became asymptomatic when aspirin was added to coumadin, and one continued to have transient ischemic attacks [196]. Two other patients had recurrences while receiving aspirin but became asymptomatic during follow-up periods of 2–2.5 years when coumadin was added [196, 202].

Corticosteroids have been used in patients with cerebrovascular ischemic events, although their utility has not been proven. Levine *et al.* [197] noted that of 13 patients treated with steroids alone or in combination with other modalities, 5 subsequently had transient or permanent events during a 1-year follow-up. Immunosuppressive agents other than prednisone have been tried occasionally, with fairly poor results [166, 196, 203].

In summary, no therapy consistently prevents recurrent events in LAC/ACA-positive patients with cerebrovascular disorders [192, 196]. Oral anticoagulation will obviously be used in patients with associated peripheral thrombotic events. If the cerebral event is embolic from a cardiac source, oral anticoagulation would also be the treatment of choice [197]. In other situations initial therapy lies between aspirin and anticoagulation, a choice that cannot be made on the basis of available evidence. Coumadin appears to have been effective in some patients; on the other hand, aspirin is the only treatment proven effective in decreasing the frequency of recurrent cerebral ischemic events in patients with transient ischemic attacks, irrespective of LAC/ACA status [204]. Clearly, a therapeutic trial is needed in LAC/ACA-positive patients. Ticlopidine, an antiplatelet agent, has been shown to be effective in decreasing the frequency of recurrent cerebral ischemic events in patients with stroke [205]. This agent may have a place in the treatment of LAC/ACA-positive patients as well. When coumadin or aspirin alone has proven ineffective, the combination may be tried [147], but, as previously mentioned, the frequency of thrombocytopenia in this patient population should be kept in mind. A role for corticosteroids or other immunosuppressive agents has not been established, and these drugs probably should be used only in the event of failure of other agents or perhaps as adjuncts to oral anticoagulants and aspirin.

For a more complete treatment of this area, the reader should refer to the review by Brey and Levine [206].

Treatment of Recurrent Fetal Loss

Fetal loss in the first trimester occurs in up to 80% of untreated pregnancies in women with LACs/ACAs and a history of recurrent miscarriage [207]. Several approaches have been taken to the treatment of this vexing problem. Lubbe *et al.* [179] were the first to advocate the use of prednisone (40–60 mg per day) and aspirin (75 mg per day). The utility of this approach was substantiated in several trials, and resulted in an increase in successful pregnancies from about 6% in untreated patients to 50–80% in treated patients [54, 208–210]. However, this therapy is not without complications: cushingoid features occur in almost 100% of women, bothersome acne in most, and, less commonly, the more serious complications of gestational diabetes and hypertension.

Because of concern regarding steroid side effects, other regimens have been explored. Low-dose aspirin (75–80 mg daily) has been effective in some patients. But examination of placentas has occasionally shown persistence of vascular thrombosis [209], and some pregnant women have experienced thrombotic episodes while on this regimen [211].

Heparin therapy has been used as an alternative to steroids. Rosove *et al.* [212], in a study of 14 women with at least one prior fetal loss, used twice daily subcutaneous heparin beginning at the time of patient referral, which averaged 10-weeks gestational age. The mean daily heparin dose was 24,700 units, with a range of 10,000–36,000 units. Heparin or coumadin was continued for variable periods thereafter. Using this regimen, heparin side effects were minor and 14 of the 15 pregnancies resulted in live births at a mean gestational age of 36 weeks. The placentas showed fewer gross infarcts than were reported in previous pregnancies. Since then several controlled studies have shown that the combination of low-dose subcutaneous heparin, administered twice daily in amounts that prolong the APTT to the upper limit of normal or just beyond, together with 80-mg aspirin daily, probably is as beneficial as full-dose heparin and results in a live birth rate of 70–80% [213–218].

Intravenous immunoglobulin (IVIG) has been used in a number of pregnancies [219], despite the fact that IVIG preparations contain detectable amounts of ACAs [220]. However, more recent data, including one controlled trial, suggest that IVIG has little additional to offer beyond low-dose heparin and aspirin [221, 222].

OTHER POSSIBLE RISK FACTORS FOR THROMBOSIS

Approximately 10–15% of SLE patients have thrombotic events in the absence of LACs or ACAs [5]. In these patients other mechanisms may be involved. For example, deficiency of antithrombin (previously called

antithrombin), protein C, or protein S may be present [1]. Antithrombin is an inhibitor of many activated coagulation proteins, most importantly thrombin and factor Xa. Activated protein C is a specific inactivator of factors Va and VIIIa, and protein S is a necessary cofactor in this reaction. Approximately 60% of protein S in the bloodstream circulates as a complex with C4b-binding protein (a protein involved in the regulation of the complement cascade), and about 40% is found free. Only free protein S can serve as a cofactor for activated protein C. In addition, some evidence suggests that protein S itself may have an inhibitory effect on the coagulation system [223].

Several investigators have measured the concentrations of protein C, protein S, and antithrombin in patients with SLE in relation to APL status [130, 224–227]. In general, levels of antithrombin and protein C were not related to the presence of LACs/ACAs or of thrombosis. However, 35–45% of SLE patients had depressed levels of free protein S, and this finding tended to correlate with the presence of LACs/ACAs, although there were many exceptions. However, little or no correlation was found between the presence of a LAC/ACA, subnormal free protein S, and thrombosis.

The role of factor V Leiden, the prothrombin G20210A and the MTHFR mutations, and the PAI-1 4G/5G polymorphism, has already been discussed.

The nephrotic syndrome is a common complication of SLE. Several studies have found measurable levels of antithrombin in the urine of a majority of patients [228–230]. In one study the plasma antithrombin levels were unchanged [229]; however, another study found that both plasma concentration and the functional activity of antithrombin were reduced [228]. A third study 230 of 48 patients included several with SLE. Nine patients had thrombotic episodes, including 4 with renal vein thrombosis; antithrombin concentrations were below normal in 8, and levels correlated with urinary total protein excretion [230]. Free protein S deficiency can occur in the nephrotic syndrome [231], although the relationship of this phenomenon to thrombosis requires further investigation.

Other coagulation factors also may be lost in the urine in the nephrotic syndrome. Depressed plasma levels of these factors may prolong coagulation tests and/or contribute to a bleeding tendency. For example, the nephrotic syndrome is commonly associated with factor XII (Hageman factor) deficiency and may cause a prolonged APTT [232]. This APTT prolongation is not associated with any bleeding tendency but may contribute to the well-known hypercoagulability of the nephrotic syndrome, since it is now recognized that Hageman factor deficiency is accompanied by a mild thrombotic risk. Acquired factor IX deficiency has also been reported, although generally it has not been found to be severe enough to cause spontaneous bleeding [232]. Deficiency of factor VII may occur rarely [232]. Prothrombin deficiency may occur [233], and in patients who also have LACs hypoprothrombinemia might be severe.

Other coagulation factor abnormalities have been reported in SLE. Markedly increased levels of von Willebrand factor and of fibronectin were found in a study of 73 SLE patients [224], but this finding may reflect the presence of patients with LACs, who have been reported to have high vWF levels and abnormal vWF multimeric patterns [234]. In another series of 28 patients with SLE [235], the mean level of factor VIII coagulant activity was increased, especially in patients on steroids. The causes and significance of these changes are unknown.

It has been reported that SLE patients may have increased levels of fibrinopeptide A [236], a cleavage product of the action of thrombin on fibrinogen. In this study of 19 women with SLE, fibrinopeptide A levels appeared to parallel the extent of inflammation and to provide a sensitive index of disease activity. However, studies of prothrombin fragment F1 + 2 levels, another marker of coagulation activation, have not produced consistent results. Levels of fibrin degradation products are generally normal or only minimally elevated [237], implying only minor degrees of activation of the fibrinolytic system. Changes in components of the fibrinolytic system have been studied in SLE only to a limited extent and with conflicting results. Plasminogen levels have been found to be increased or normal in the majority of patients, but may be decreased in a few [130].

COAGULATION FACTOR INHIBITORS AND BLEEDING IN SYSTEMIC LUPUS ERYTHEMATOSUS

As already mentioned, hypoprothrombinemia has been noted in up to 25% of SLE patients with LACs [20]. The mechanism of the hypoprothrombinemia, usually mild but occasionally severe, is probably the rapid clearing of antigen–antibody complexes [238]. Nonneutralizing antibodies to prothrombin may be present in two-thirds of all LAC-positive SLE patients [239]. However, when accelerated clearance of complexes cannot be compensated and substantial decreases in prothrombin are present, bleeding may occur [239–244]. A markedly prolonged prothrombin time (ratio >1.5) in a patient with a LAC should alert one to the possibility of serious hypoprothrombinemia, since only rarely does a LAC directly produce this

degree of prothrombin prolongation [239]. In one study, 67 of 201 LAC patients had a prolonged prothrombin time [46], but only 44 had a prothrombin ratio greater than 1.25 and only 16 had a ratio greater than 1.5. In another study, 15 of 43 LAC patients had a prothrombin time more than 2 s greater than the control [239]; antibodies against prothrombin were demonstrated in 14. In 10 of the 15 patients the prothrombin time was less than 16.5 s (normal range, 11–13 s); all 10 had immunologic levels of prothrombin above 40%, and none bled abnormally. In 5 patients the prothrombin time exceeded 16.5 s; in 2 the prolonged prothrombin time was due to the presence of a potent lupus anticoagulant. In the other 3 the prolonged prothrombin time was associated with severe hypoprothrombinemia; all 3 patients bled. Despite lack of *in vitro* evidence of prothrombin neutralization, infusion of plasma or concentrates does not increase prothrombin levels. There are several reports of a rapid rise of prothrombin levels after treatment with corticosteroids [238, 245–247]; however, the response to steroids is not always so effective [244]. To establish the presence or absence of hypoprothrombinemia in a patient with a prolonged prothrombin time, both prothrombin activity and antigen should be measured.

Among the many types of antibodies found in patients with SLE are functional inhibitors to coagulation factors, most frequently inhibitors to factor VIII. In a survey of 215 nonhemophiliacs who developed factor VIII inhibitors, nearly all IgG, patients with SLE, rheumatoid arthritis, and other autoimmune disorders accounted for 6, 8, and 5% of the patients, respectively [248]. In these disorders, factor VIII antibodies are unlikely to resolve spontaneously or following steroid therapy. In one large series, the combination of azathioprine or cyclophosphamide and corticosteroids, frequently with an initial large intravenous bolus of factor VIII, resulted in the decline or disappearance of the inhibitor in half the patient [248]. In another study, successful eradication of factor VIII inhibitors was seen in 11 of 12 patients treated with the combination of cyclophosphamide, vincristine, and prednisone [249].

Treatment of bleeding in patients with factor VIII inhibitors is determined by the clinical situation. Soft tissue hemorrhage may be managed conservatively. Life-threatening bleeding demands aggressive therapy, including the use of human factor VIII if inhibitor titers are below 5 Bethesda U/ml, porcine factor VIII if the level is higher and the patient's antibody shows little species cross-reactivity, prothrombin complex concentrates, and activated factor VII preparations. Anamnestic rises in antibody titer have occurred as a result of infusion of human factor VIII. Plasmapheresis has been used successfully to lower inhibitor titers [250–254], but repeated plasmapheresis, over a period of several days, is usually necessary.

Intravenous immunoglobulin has been used in patients with spontaneous factor VIII inhibitors with inconsistent results. Other approaches used with occasional success include l-desamino-8-D-arginine vasopressin (DDAVP) [255–258] and cyclosporine [259].

Other coagulation inhibitors are rare. Acquired factor IX inhibitors have been reported in 2 patients with SLE and in 1 patient with a collagen vascular disorder [260]. Inhibitors against factor XI have been observed in about 30 patients without underlying factor XI deficiency [261, 262]; of these, 10 had SLE and another 4 had rheumatoid arthritis. Bleeding manifestations were variable in this group of patients. Of two acquired factor XII inhibitors reported in the literature, one was in a female with SLE; neither bled [261]. The only factor VII inhibitor reported [261] was in a 66-year-old man with a pulmonary neoplasm, elevated ANA titers, and a positive lupus erythematosus preparation, but without clinical evidence of SLE; little bleeding was noted in this patient. There are no reports of factor V or factor X inhibitors in SLE patients. Two of the nine reported cases of inhibitors against fibrinogen occurred in SLE patients [263].

Among the many manifestations of SLE, thrombosis and bleeding are both common occurrences. Our understanding of the causes of bleeding episodes far exceeds our knowledge of the mechanisms of thrombosis. A better insight into the latter, especially in the setting of LACs and ACAs, will not only aid materially in the care of patients with SLE but should also substantially increase our general ability to treat patients with thrombosis.

References

1. Shapiro, S. S. (1996). The lupus anticoagulant/antiphospholipid syndrome. *Annu. Rev. Med.* **47**, 533.
2. Feinstein, D. L., and Rapaport, S. I. (1972). Acquired inhibitors of blood coagulation. *Prog. Hemost. Thromb.* **1**, 75.
3. Mueller, J. F., Ratnoff, O. D., and Heinle, R. W. (1951). Observations on the characteristics of an unusual circulating anticoagulant. *J. Lab. Clin. Med.* **38**, 254.
4. Conley, C. L., and Hartmann, R. C. (1952). A hemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J. Clin. Invest.* **32**, 621.
5. Love, P. E., and Santoro, S. A. (1990). Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. *Ann. Intern. Med.* **112**, 682.
6. Simioni, P., Prandoni, P., Zanon, E., *et al*. (1996). Deep venous thrombosis and lupus anticoagulant. A case-control study. *Thromb. Haemost.* **76**, 187.

7. Nojima, J., Suehisa, E., Akita, N., *et al*. (1997). Risk of arterial thrombosis in patients with anticardiolipin antibodies and lupus anticoagulant. *Br. J. Haematol.* **96**, 447.
8. Levine, J. S., Branch, D. W., and Rauch, J. (2002). Medical progress: The antiphospholipid syndrome. *N. Engl. J. Med.* **346**, 752.
9. Thiagarajan, P., De Marco, L., and Shapiro, S. S. (1980). Monoclonal immunoglobulin Mλ coagulation inhibitor with phospholipid specificity: Mechanism of a lupus anticoagulant. *J. Clin. Invest.* **66**, 397.
10. Shapiro, S. S., Thiagarajan, P., and De Marco, L. (1981). Mechanism of action of the lupus anticoagulant. *Ann. N. Y. Acad. Sci.* **370**, 359.
11. Harris, E. N., Gharavi, A. K., Boey, M. L., *et al*. (1983). Anticardiolipin antibodies: Detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet* **2**, 1211.
12. Brandt, J. T., Triplett, D. A., Alving, B., and Scharrer, I. (1995). Criteria for the diagnosis of lupus anticoagulants: An update. *Thromb. Haemost.* **74**, 1185.
13. Schleider, M. A., Nachman, R. L., Jaffe, E. A., and Coleman, M. (1976). Clinical study of the lupus anticoagulant. *Blood* **48**, 499.
14. Liu, H. W., Wong, K. L., Lin, C. K., *et al*. (1989). The reappraisal of dilute tissue thromboplastin inhibition test in the diagnosis of lupus anticoagulant. *Br. J. Haematol.* **72**, 229.
15. Exner, T., Rickard, A., and Kronenberg, H. (1978). A sensitive test demonstrating lupus anticoagulant and its behavioural patterns. *Br. J. Haematol.* **40**, 143.
16. Exner, T. (1985). Comparison of two simple tests for the lupus anticoagulant. *Am. J. Clin. Pathol.* **83**, 215.
17. Thiagarajan, P., Pengo, V., and Shapiro, S. S. (1986). The use of the dilute Russell viper venom time for the diagnosis of lupus anticoagulants. *Blood* **68**, 869.
18. Alving, B. M., Baldwin, P. E., Richards, R. L., and Jackson, B. J. (1985). The dilute phospholipid APTT: A sensitive assay for verification of lupus anticoagulants. *Thromb. Haemost.* **54,** 709.
19. Alving, B. M., Barr, C. F., Johansen, L. E., and Tang, D. B. (1992). Comparison between a one-point dilute phospholipid APTT and the dilute Russell viper venom time for verification of lupus anticoagulants. *Thromb. Haemost.* **67**, 672.
20. Shapiro, S. S., and Thiagarajan, P. (1982). Lupus anticoagulants. *Prog. Hemost. Thromb.* **6**, 263.
21. Triplett, D. A., Brandt, J. T., Kaczor, D., and Schaeffer, J. (1983). Laboratory diagnosis of lupus inhibitors: A comparison of the tissue thromboplastin inhibition procedure with a new platelet neutralization procedure. *Am. J. Clin. Pathol.* **79**, 678.
22. Exner, T., Papadopoulos, G., and Koutts, J. (1990). Use of a simplified dilute Russell viper venom time (DRVVT) confirms heterogeneity among "lupus anticoagulants." *Blood Coag. Fibrinol.* **1**, 259.
23. Exner, T., Triplett, D. A., Taberner, D., and Machin, S. J. (1991). Guidelines for testing and revised criteria for lupus anticoagulants. *Thromb. Haemost.* **65**, 320.
24. Mannucci, P. M., Canciani, M. T., and Meucci, D. M. P. (1979). The varied sensitivity of partial thromboplastin and prothrombin time reagents in the demonstration of the lupus-like anticoagulant. *Scand. J. Haematol.* **22**, 423.
25. Brandt, J. T., and Triplett, D. A. (1989). The effect of phospholipid on the detection of lupus anticoagulants by the dilute Russell viper venom time. *Arch. Pathol. Lab. Med.* **113**, 1376.
26. Stevenson, K. J., Easton, A. C., Curry, A., *et al*. (1986). The reliability of activated partial thromboplastin time methods and the relationship to lipid composition and ultrastructure. *Thromb. Haemost.* **55**, 250.
27. Brandt, J. T., Triplett, D. A., Musgrave, K., and Orr, C. (1987). The sensitivity of different coagulation reagents to the presence of lupus anticoagulants. *Arch. Pathol. Lab. Med.* **111**, 120.
28. Guermazi, S., Gorgi, Y., Ayed, K., and Dellagi, K. (1997). Interference de l'exces de facteur VIII sur la detection des lupus anticoagulants par le temps de cephaline active. *Pathol. Biol.* **45**, 28.
29. Eschwege, V., Seddiki, S., and Robert, A. (1996). The tissue thromboplastin inhibition test in the detection of lupus anticoagulants: Importance of a correction factor eliminating the influence of fibrinogen level. *Thromb. Haemost.* **76**, 65.
30. Lo, S. C. L., Oldmeadow, M. J., Howard, M. A., and Firkin, B. G. (1989). Comparison of laboratory tests used for identification of the lupus anticoagulant. *Am. J. Hematol.* **30**, 213.
31. Martin, B. A., Branch, D. W., and Rodgers, G. M. (1996). Sensitivity of the activated partial thromboplastin time, the dilute Russell's viper venom time, and the kaolin clotting time for the detection of the lupus anticoagulant: A direct comparison using plasma dilutions. *Blood Coag. Fibrinol.* **7**, 31.
32. Goudemand, J., Caron, C., De Prost, D., *et al*. (1997). Evaluation of the sensitivity and specificity of a standardized procedure using different reagents for the detection of lupus anticoagulants. *Thromb. Haemost.* **77**, 336.
33. Lesperance, B., David, M., Rauch, J., *et al*. (1988). Relative sensitivity of different tests in the detection of low titer lupus anticoagulants. *Thromb. Haemost.* **60**, 217.
34. Kelsey, P. R., Stevenson, K. J., and Poller, L. (1984). The diagnosis of lupus anticoagulants by the activated partial thromboplastin time—the central role of phosphatidylserine. *Thromb. Haemost.* **52**, 172.
35. Rosove, M. H., Ismail, M., Koziol, B. J., *et al*. (1986). Lupus anticoagulants: Improved diagnosis with a kaolin clotting time using rabbit brain phospholipid in standard and high concentrations. *Blood* **68**, 472.
36. Rauch, J., Tannenbaum, M., Tannenbaum, H., *et al*. (1986). Human hybridoma lupus anticoagulants distinguish between lamellar and hexagonal phase lipid systems. *J. Biol. Chem.* **261**, 9672.
37. Rauch, J., Meng, Q.-H., and Tannenbaum, H. (1987). Lupus anticoagulant and antiplatelet properties of human hybridoma autoantibodies. *J. Immunol.* **139**, 2598.

38. Rauch, J., Tannenbaum, M., and Janoff, A. S. (1989). Distinguished plasma lupus anticoagulants from anti-factor antibodies using hexagonal (II) phase phospholipids. *Thromb. Haemost.* **62**, 892.
39. Horbach, D. A., v Oort, E., Donders, R. C. J. M., *et al*. (1996). Lupus anticoagulant is the strongest risk factor for both venous and arterial thrombosis in patients with systemic lupus erythematosus. *Thromb. Haemost.* **76**, 916.
40. Meyer, O., Cyna, L., Bourgeois, P., *et al*. (1987). A. Profile and cross-reactivities of antiphospholipid antibodies in systemic lupus erythematosus and syphilis. *Clin. Rheumatol.* **6**, 369.
41. Derksen, R. H., Hasselaar, P., Blokzijl, L., *et al*. (1988). Coagulation screen is more specific than the anticardiolipin antibody ELISA in defining a thrombotic subset of lupus patients. *Ann. Rheum. Dis.* **47**, 364.
42. Alarcon-Segovia, D., Deleze, M., Oria, C. V., *et al*. (1989). Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus: A prospective analysis of 500 consecutive patients. *Medicine* **68**, 353.
43. Fort, J. G., Cowchock, F. S., Abruzzo, J. L., and Smith, I. B. (1987). Anticardiolipin antibodies in patients with rheumatic diseases. *Arthritis Rheum*. **30**, 752.
44. Keane, A., Woods, R., Dowding, V., *et al*. (1987). Anticardiolipin antibodies in rheumatoid arthritis. *Br. J. Rheumatol.* **26**, 346.
45. Harris, E. N., Chan, J. K. H., and Asherson, R. A. (1986). Thrombosis, recurrent fetal loss, and thrombocytopenia: Predictive value of the anticardiolipin antibody test. *Arch. Intern. Med.* **146**, 153.
46. Gastineau, D. A., Kazmier, F. J., Nichols, W. L., and Bowie, E. J. W. (1985). Lupus anticoagulant: An analysis of the clinical and laboratory features of 219 cases. *Am. J. Hematol.* **19**, 265.
47. Lechner, K., and Pabinger-Fasching, I. (1985). Lupus anticoagulants and thrombosis. *Haemostasis* **15**, 254.
48. Vaarala, O., Palosuo, T., Kleemola, M., and Aho, K. (1986). Anticardiolipin response in acute infections. *Clin. Immunol. Immunopathol.* **41**, 3.
49. Canoso, R. T., and de Oliveira, R. M. (1988). Chlorpromazine-induced anticardiolipin antibodies and lupus anticoagulant: Absence of thrombosis. *Am. J. Hematol.* **27**, 272.
50. Mueh, J. R., Herbst, K. D., and Rapaport, S. I. (1980). Thrombosis in patients with the lupus anticoagulant. *Ann. Intern. Med.* **92**, 156.
51. Walker, T. S., Triplett, D. A., Javed, N., and Musgrave, K. (1988). Evaluation of lupus anticoagulants: Antiphospholipid antibodies, endothelium-associated immunoglobulin, endothelial prostacyclin secretion, and antigenic protein S levels. *Thromb. Res.* **51**, 267.
52. Wahl, D. G., Guillemin, F., de Maistre, E., *et al*. (1997). Risk for venous thrombosis related to antiphospholipid antibodies in systemic lupus erythematosus—a meta-analysis. *Lupus* **6**, 467.
53. Boey, M. L., Colaco, C. B., Gharavi, A. E., *et al*. (1983). Thrombosis in systemic lupus erythematosus: Striking association with the presence of circulating lupus anticoagulant. *Br. Med. J.* **287**, 102.
54. Glueck, H. I., Kant, K. S., and Weiss, M. A. (1985). Thrombosis in systemic lupus erythematosus: Relation to the presence of circulating anticoagulants. *Arch. Intern. Med.* **145**, 1389.
55. Averbuch, M., Kolfman, B., and Levo, Y. (1987). Lupus anticoagulant, thrombosis and thrombocytopenia in systemic lupus erythematosus. *Am. J. Med. Sci.* **293**, 2.
56. Fijnheer, R., Horbach, D. A., Donders, R. C. J. M., *et al*. (1996). Factor V Leiden, antiphospholipid antibodies and thrombosis in systemic lupus erythematosus. *Thromb. Haemost.* **76**, 514.
57. Elias, M., and Eldor, A. (1984). Thromboembolism in patients with the "lupus"-type circulating anticoagulant. *Arch. Intern. Med.* **144**, 510.
58. McHugh, N. J., Maymo, J., Skinner, R. P., *et al*. (1988). Anticardiolipin antibodies, livedo reticularis, and major cerebrovascular and renal disease in systemic lupus erythematosus. *Ann. Rheum. Dis.* **47**, 110.
59. Cohen, A. J., Philips, T. M., and Kessler, C. M. (1986). Circulating coagulation inhibitors in the acquired immunodeficiency. *Ann. Intern. Med.* **104**, 175.
60. Canoso, R. T., Zon, L. I., and Groopman, J. E. (1987). Anticardiolipin antibodies associated with HTLV-III infection. *Br. J. Haematol.* **65**, 495.
61. Coll, J., Yazbeck, H., Garces, J. M., *et al*. (1990). Anticardiolipin antibodies and *Pneumocystis carinii* pneumonia in patients with AIDS. *J. Infect. Dis.* **21**, 120.
62. Bernard, C., Exquis, B., Reber, G., and deMoerloos, P. (1990). Determination of anticardiolipin and other antibodies in HlV-1-infected patients. *J. AIDS* **3**, 536.
63. Naimi, N., Plancherel, C., Bosser, C., *et al*. (1990). Anticardiolipin antibodies in HIV-negative and HIV-positive haemophiliacs. *Blood Coag. Fibrinol.* **1**, 5.
64. Abuaf, N., Laperche, S., Rajoely, B., *et al*. (1997). Autoantibodies to phospholipids and the coagulation proteins in AIDS. *Thromb. Haemost.* **77**, 856.
65. Carreras, L. O., and Vermylen, J. G. (1982). Lupus anticoagulant and thrombosis—possible role of inhibition of prostacyclin formation. *Thromb. Haemost.* **48**, 38.
66. Carreras, L. O., Defreyn, G., Machin, S. J., *et al*. (1981). Arterial thrombosis, intrauterine death and "lupus" anticoagulant. Detection of immunoglobulin interfering with prostacyclin formation. *Lancet* **1**, 244.
67. Hasselaar, P., Blokzijl, L., Derksen, R. H. W. M., and deGroot, P. G. (1987). The effect of cardiolipin-positive sera from SLE patients on platelet and endothelial prostanoid synthesis (abstract). *Thromb. Haemost.* **58**, 391.
68. Rustin, M. H. A., Bull, H. A., Dowd, P. M., *et al*. (1987). Presence of the lupus anticoagulant in patients with systemic lupus erythematosus does not cause inhibition of prostacyclin production (abstract). *Thromb. Haemost.* **58**, 390.
69. Schorer, A. K., and Watson, K. V. (1987). The "lupus anticoagulant" induces functional changes in endothelial cells and platelets (abstract). *Thromb. Haemost.* **58**, 232.

70. Violi, F., Valesini, G., Iuliano, L., *et al*. (1987). Anticardiolipin antibodies and prostacyclin synthesis. *Thromb. Haemost.* **57**, 374.
71. Petraiuolo, W., Bovill, E., and Hoak, J. (1988). The lupus anticoagulant does not inhibit the release of prostacyclin from human endothelial cells. *Thromb. Res.* **50**, 847.
72. Esmon, N. L., Smirnov, M. D., and Esmon, C. T. (1997). Thrombogenic mechanisms of antiphospholipid antibodies. *Thromb. Haemost.* **78**, 79.
73. Marciniak, E., and Romond, E. H. (1989). Impaired catalytic function of activated protein C: A new *in vitro* manifestation of lupus anticoagulant. *Blood* **74**, 2426.
74. Ames, P. R. J., Tommasino, C., Iannaccone, L., *et al*. (1996). Coagulation activation and fibrinolytic imbalance in subjects with idiopathic antiphospholipid antibodies—a crucial role for acquired free protein S deficiency. *Thromb. Haemost.* **76**, 190.
75. Reverter, J.-C., Tassies, D., Font, J., *et al*. (1996). Hypercoagulable state in patients with antiphospholipid syndrome is related to high induced tissue factor expression on monocytes and to low free protein S. *Arterioscler. Thromb. Vasc. Biol.* **16**, 1319.
76. Angles-Cano, E., Sultan, Y., and Clauvel, J.-P. (1979). Predisposing factors to thrombosis in systemic lupus erythematosus: Possible relation to endothelial damage. *J. Lab. Clin. Med.* **94**, 312.
77. Moroz, L. A., MacLean, L. D., and Langleben, D. (1986). Abnormalities in the cellular phase of blood fibrinolytic activity in systemic lupus erythematosus and in venous thromboembolism. *Thromb. Res.* **43**, 595.
78. Borrell, M., Fontcuberta, J., Muniz, E., *et al*. (1987). Fibrinolytic activity and other coagulation proteins in patients with lupus anticoagulant (abstract). *Thromb. Haemost.* **58**, 393.
79. Francis, R. B., Jr., McGehee, W. G., and Feinstein, D. I. (1988). Endothelial-dependent fibrinolysis in subjects with the lupus anticoagulant and thrombosis. *Thromb. Haemost.* **59**, 412.
80. Panzer, S., Gschwandtner, M. E., Hutter, D., *et al*. (1997). Specificities of platelet autoantibodies in patients with lupus anticoagulants in primary antiphospholipid syndrome. *Ann. Hematol.* **74**, 239.
81. Macchi, L., Rispal, P., Clofent-Sanchez, G., *et al*. (1997). Anti-platelet antibodies in patients with systemic lupus erythematosus and the primary antiphospholipid syndrome: Their relationship with the observed thrombocytopenia. *Br. J. Haematol.* **98**, 336.
82. Roubey, R. A. S. (1994). Autoantibodies to phospholipid-binding plasma proteins: A new view of lupus anticoagulants and other "antiphospholipid" antibodies. *Blood* **84**, 2854.
83. Arvieux, J., Rousiel, B., Jacob, M. C., and Colomb, M. G. (1991). Measurement of antiphospholipid antibodies by ELISA using beta$_2$ glycoprotein I as an antigen. *J. Immunol. Methods* **143**, 223.
84. Viard, J. P., Amoura, Z., and Bach, J. F. (1992). Association of anti beta$_2$ glycoprotein I antibodies with lupus type circulating anticoagulant and thrombosis in systemic lupus erythematosus. *Am. J. Med.* **93**, 181.
85. Amiral, J., Larrivaz, I., Cluzeau, D., and Adam, M. (1994). Standardization of immunoassays for antiphospholipid antibodies with beta$_2$GPI and role of other phospholipid cofactors. *Thromb. Haemost.* **24**, 191.
86. Balestrieri, G., Tincani, A., Spatola, L., *et al*. (1995). Anti-β_2-glycoprotein I antibodies: A marker of antiphospholipid syndrome? *Lupus* **4**, 122.
87. Cabiedes, J., Cabral, A. R., and Alarcon-Segovia, D. (1995). Clinical manifestations of the antiphospholipid syndrome in patients with systemic lupus erythematosus associate more strongly with anti-β_2-glycoprotein-I than with antiphospholipid antibodies. *J. Rheumatol.* **22**, 1899.
88. Martinuzzo, M. E., Forastiero, R. R., and Carreras, L. O. (1995). Anti β_2 glycoprotein I antibodies: Detection and association with thrombosis. *Br. J. Haematol.* **89**, 397.
89. Roubey, R. A. S., Maldonado, M. A., and Byrd, S. N. (1996). Comparison of an enzyme-linked immunosorbent assay for antibodies to β_2-glycoprotein I and a conventional anticardiolipin immunoassay. *Arthritis Rheum.* **39**, 1606.
90. Guerin, J., Feighery, C., Sim, R. B., and Jackson, J. (1997). Antibodies to β_2-glycoprotein I—a specific marker for the antiphospholipid syndrome. *Clin. Exp. Immunol.* **109**, 304.
91. Oosting, J. D., Derksen, H. W. M., Entjes, H. T. I., *et al*. (1992). Lupus anticoagulant activity is frequently dependent on the presence of β_2-glycoprotein I. *Thromb. Haemost.* **67**, 499.
92. Permpikul, P., Rao, L. V. M., and Rapaport, S. (1994). Functional and binding studies on the roles of prothrombin and beta 2 glycoprotein I in the expression of lupus anticoagulant. *Blood* **83**, 2878.
93. Galli, M., Finazzi, G., Bevers, E. M., and Barbui, T. (1995). Kaolin clotting time and dilute Russell's viper venom time distinguish between prothrombin-dependent and β_2-glycoprotein-I-dependent antiphospholipid antibodies. *Blood* **86**, 617.
94. Takeya, H., Mori, T., Gabazza, E. C., *et al*. (1997). Anti-β2-glycoprotein I (β2GPI) monoclonal antibodies with lupus anticoagulant-like activity enhance the β2GPI binding to phospholipids. *J. Clin. Invest.* **99**, 2260.
95. Bevers, E. M., Galli, M., Barbui, T., *et al*. (1991). Lupus anticoagulant IgGs are not directed to phospholipids only, but to a complex of lipid-bound human prothrombin. *Thromb. Haemost.* **66**, 629.
96. Oosting, J. D., Derksen, R. H. W. M., Bobbinck, I. W. G., *et al*. (1993). Antiphospholipid antibodies directed against combination of phospholipids with prothrombin, protein C or protein S. An explanation for the pathogenic mechanism. *Blood* **81**, 2618.
97. Sugi, T., and McIntyre, J. A. (1995). Autoantibodies to phosphatidylethanolamine (PE) recognize a kininogen–PE complex. *Blood* **86,** 3083.
98. Long, M., Broze, G., Thiagarajan, P., *et al*. (1996). TFPI binds to anionic phospholipids via a -KRKRKK- sequence near its C-terminus and elicits antibodies in patients with SLE. *Blood* **88**(Suppl. 1), 522a (abstract).

99. de Groot, P. G., Oosting, J., and Derksen, R. H. W. M. (1993). Antiphospholipid antibodies: Specificity and pathophysiology. *Ballieres Clin. Haematol.* **6**, 691.
100. Matsuda, J., Saitoh, N., Gohchi, K., *et al*. (1994). Anti-annexin V antibody in systemic lupus erythematosus patients with lupus anticoagulant and/or anticardiolipin antibody. *Am. J. Hematol.* **47**, 56.
101. Del Papa, N., Raschi, E., Catelli, L., *et al*. (1997). Endothelial cells as a target for antiphospholipid antibodies: Role of anti-beta 2 glycoprotein I antibodies. *Am. J. Reprod. Immunol.* **38**, 212.
102. Hunt, B. J., and Khamashta, M. A. (2000). Antiphospholipid antibodies and the endothelium. *Curr. Rheumatol. Rep.* **2**, 252.
103. Meroni, P. L., Raschi, E., Camera, M., Testoni, C., *et al*. (2000). Endothelial activation by aPL: A potential pathogenetic mechanism for the clinical manifestations of the syndrome. *J. Autoimmun.* **15**, 237.
104. Pierangeli, S. S., Espinola, R. G., Liu, X., and Harris, E. N. (2001). Thrombogenic effects of antiphospholipid antibodies are mediated by intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and P-selectin. *Circ. Res.* **88**, 245.
105. Martinuzzo, M. E., Forastiero, R. R., and Carreras, L. O. (1996). Increased plasma thrombomodulin in different subgroups of patients with antiphospholipid and anti β_2 glycoprotein I antibodies. *Thromb. Haemost.* **75**, 971.
106. Cucurull, E., and Gharavi, A. E. (1997). Thrombomodulin: A new frontier in lupus research? *Clin. Exp. Rheumatol.* **15**, 1.
107. Hanly, J. G., and Smith, S. A. (2000) Anti-β_2-glycoprotein I autoantibodies, annexin V binding and the antiphospholipid syndrome. *Clin. Exp. Immunol.* **120**, 537.
108. Rand, J. H. (2002). Molecular pathogenesis of the antiphospholipid syndrome. *Circ. Res.* **90**, 29.
109. Rand, J. H., Wu, X. X., Guller, S., *et al*. (1994). Reduction of annexin V (placental anticoagulant protein-I) on placental villi of women with antiphospholipid antibodies and recurrent spontaneous abortion. *Am. J. Obstet. Gynecol.* **171**, 1566.
110. Rand, J. H., Wu, X. X., Guller, S., *et al*. (1997). Antiphospholipid immunoglobulin G antibodies reduce annexin V levels on syncytiotrophoblast apical membranes and in culture media of placental villi. *Am. J. Obstet. Gynecol.* **177**, 918.
111. Rand, J. H., Wu, X. X., Andree, H. A. M., *et al*. (1998). Antiphospholipid antibodies accelerate plasma coagulation by inhibiting annexin V binding to phospholipids: A "lupus procoagulant" phenomenon. *Blood* **92**, 1652.
112. Willems, G. M., Janssen, M. P., and Comfurius, P. (2000). Competition of annexin V and anticardiolipin antibodies for binding to phosphatidylserine containing membranes. *Biochemistry* **39**, 982.
113. Bevers, E. M., Janssen, M. P., and Willems, G. M. (2000). No evidence for enhanced thrombin formation through displacement of annexin V by antiphospholipid antibodies. *Thromb. Haemost.* **83**, 792.
114. Galli, M., Finazzi, G., and Barbui, T. (1997). Antiphospholipid antibodies: Predictive values of laboratory tests. *Thromb. Haemost.* **78**, 75.
115. Galli, M., Finazzi, G., Norbis, F., *et al*. (1999). The risk of thrombosis in patients with lupus anticoagulants is predicted by their specific coagulation profile. *Thromb. Haemost.* **81**, 695.
116. Galli, M., Dlott, J., Norbis, F., Ruggeri, L., *et al*. (2000). Lupus anticoagulants and thrombosis: Clinical association of different coagulation and immunologic tests. *Thromb. Haemost.* **84**, 1012.
117. Hamsten, A., Norberg, R., Bjorkholm, M., *et al*. (1986). Antibodies to cardiolipin in young survivors of myocardial infarction: An association with recurrent cardiovascular events. *Lancet* **1**, 113.
118. Deleze, M., Alarcon-Segovia, D., Valdes-Macho, E., *et al*. (1989). Relationship between antiphospholipid antibodies and recurrent fetal loss in patients with systemic lupus erythematosus and apparently healthy women. *J. Rheumatol.* **16**, 768.
119. Triplett, D. A., Brandt, J. Y., Musgrave, K. A., and Orr, C. A. (1988). The relationship between lupus anticoagulants and antibodies to phospholipid. *JAMA* **259**, 550.
120. Ishii, Y., Nagasawa, K., Mayumi, T., and Niho, Y. (1990). Clinical importance of persistence of anticardiolipin antibodies in systemic lupus erythematosus. *Ann. Rheum. Dis.* **49**, 387.
121. Pengo, V., Biasiolo, A., Brocco, T., *et al*. (1996). Autoantibodies to phospholipid-binding proteins in patients with thrombosis and phospholipid-reactive antibodies. *Thromb. Haemost.* **75**, 721.
122. Nojima, J., Kuratsune, H., and Suehisa, E. (2001). Antiprothrombin antibodies combined with lupus anticoagulant activity is an essential risk factor for venous thromboembolism in patients with systemic lupus erythematosus. *Br. J. Haematol.* **114**, 647.
123. Vaarala, O., Puurunen, M., Manttari, M., *et al*. (1996). Antibodies to prothrombin imply a risk of myocardial infarction in middle-aged men. *Thromb. Haemost.* **75**, 456.
124. Forastiero, R. R., Martinuzzo, M. E., Cerrato, G. S., *et al*. (1997). Relationship of anti β_2-glycoprotein I and antiprothrombin antibodies to thrombosis and pregnancy loss in patients with antiphospholipid antibodies. *Thromb. Haemost.* **78**, 1008.
125. Palosuo, T., Virtamo, J., Haukka, J., *et al*. (1997). High antibody levels to prothrombin imply a risk of deep venous thrombosis and pulmonary embolism in middle-aged men. *Thromb. Haemost.* **78**, 1178.
126. Swadzba, J., De Clerck, L. S., Stevens, W. J., *et al*. (1997). Anticardiolipin, anti-β_2-glycoprotein I, antiprothrombin antibodies, and lupus anticoagulant in patients with systemic lupus erythematosus with a history of thrombosis. *J. Rheumatol.* **24**, 1710.
127. Nojima, J., Kuratsune, H., Suehisa, E., *et al*. (2001). Association between the prevalence of antibodies to β_2-glycoprotein I, prothrombin, protein C, protein S, and annexin V in patients with systemic lupus erythematosus

and thrombotic and thrombocytopenic complications. *Clin. Chem.* **47**, 1008.
128. Galli, M. (2000). Which antiphospholipid antibodies should be measured in the antiphospholipid syndrome? *Haemostasis* **30**(Suppl. 2), 57.
129. Galli, M., and Barbui, T. (2001). Prevalence of different anti-phospholipid antibodies in systemic lupus erythematosus and their relationship with the antiphospholipid syndrome. *Clin. Chem.* **47**, 985.
130. Hasselaar, P., Derksen, R. H. W. M., Blokzijl, L., *et al*. (1989). Risk factors for thrombosis in lupus patients. *Ann. Rheum. Dis.* **48**, 933.
131. Bertina, R. M. (1999). Molecular risk factors for thrombosis. *Thromb. Haemost.* **82**, 601.
132. Lane, D. A., and Grant, P. J. (2000). Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* **95**, 1517.
133. Federman, D. G., and Kirsner, R. S. (2001). An update on hypercoagulable disorders. *Arch. Intern. Med.* **161**, 1051.
134. Dahlback, B. (1994). Inherited resistance to activated protein C, a major cause of venous thrombosis, is due to a mutation in the Factor V gene. *Haemostasis* **24**, 139.
135. Svensson, P. J., and Dahlback, B. (1994). Resistance to activated protein C as a basis for venous thrombosis. *N. Engl. J. Med.* **330**, 517.
136. Bertina, R. M., Koeleman, B. P., Koster, T., *et al*. (1994). Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* **369**, 64.
137. Poort, S. R., Rosendaal, F. R., Reitsma, P. H., and Bertina, R. M. (1996). A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* **88**, 3698.
138. Kohler, H. P., and Grant, P. J. (2000). Plasminogen-activator inhibitor type 1 and coronary artery disease. *N. Engl. J. Med.* **342**, 1792.
139. Humphries, S. E., Lane, A., Dawson, S., and Green, F. R. (1992). The study of gene–environment interactions that influence thrombosis and fibrinolysis. Genetic variation at the loci for factor VII and plasminogen activator inhibitor-1. *Arch. Pathol. Lab. Med.* **116**, 1322.
140. Kawai, Y., Matsumoto, Y., Watanabe, K., *et al*. (1996). Hemodynamic forces modulate the effects of cytokines on fibrinolytic activity of endothelial cells. *Blood* **87**, 2314.
141. Cattaneo, M. (1999). Hyperhomocysteinemia, atherosclerosis and hrombosis. *Thromb. Haemost.* **81**, 165.
142. den Heijer, M., and Keijzer, M. B. (2001). Hyperhomocysteinemia as a risk factor for venous thrombosis. *Clin. Chem. Lab. Med.* **39**, 710.
143. Hanson, N. Q., Aras, O., Yang, F., and Tsai, M. Y. (2001). C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: Incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. *Clin. Chem.* **47**, 661.
144. Dekou, V., Whincup, P., Papacosta, O., *et al*. (2001). The effect of the C677T and A1298C polymorphisms in the methylenetetrahydrofolate reductase gene on homocysteine levels in elderly men and women from the British regional heart study. *Atherosclerosis* **154**, 659.
145. van der Put, N. M., Gabreels, F., Stevens, E. M., *et al*. (1998). A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural tube defects? *Am. J. Hum. Genet.* **62**, 1044.
146. Rozen, R. (2000). Genetic modulation of homocysteinemia. *Semin. Thromb. Haemost.* **26**, 255.
147. Booth, G. L., and Wang, E. E. (2000). Preventive health care, 2000 update: Screening and management of hyperhomocysteinemia for the prevention of coronary artery disease events. *Can. Med. Assoc. J.* **163**, 21.
148. Hansen, K. E., Kong, D. F., Moore, K. D., and Ortel, T. L. (2001). Risk factors associated with thrombosis in patients with antiphospholipid antibodies. *J. Rheumatol.* **28**, 2018.
149. Galli, M., Finazzi, G., Duca, F., *et al*. (2000). The G1691→A mutation of factor V, but not the G20210→A mutation of II or the C677→T mutation of methylenetetrahydrofolate reductase genes, is associated with venous thrombosis in patients with lupus anticoagulants. *Br. J. Haematol.* **108**, 865.
150. Ames, P. R., Tommasino, C., D'Andrea, G., *et al*. (1998). Thrombophilic genotypes in subjects with idiopathic antiphospholipid antibodies—prevalence and significance. *Thromb. Haemost.* **79**, 46.
151. Cappucci, G., Margaglione, M., and Ames, P. (2001). Comparative prevalence of antiphospholipid antibodies and thrombophilic genotypes in consecutive patients with venous thrombosis. *Blood Coag. Fibrinol.* **12**, 659.
152. Forastiero, R., Martinuzzo, M., Adamczuk, Y., *et al*. (2001). The combination of thrombophilic genotypes is associated with definite antiphospholipid syndrome. *Haematologica* **86**, 735.
153. Tassies, D., Espinosa, G., Muno-Rodriguez, F. J., *et al*. (2000). The 4G/5G Polymorphism of the type 1 plasminogen activator inhibitor gene and thrombosis in patients with antiphospholipid syndrome. *Arthritis Rheum.* **43**, 2349.
154. Yasuda, S., Tsutsumi, A., Atsumi, T., *et al*. (2002). Gene polymorphisms of tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with antiphospholipid antibodies. *J. Rheumatol.* **29**, 1192.
155. Bentolila, S., Ripoll, L., Drouet, L., *et al*. (1997). Lack of association between thrombosis in primary antiphospholipid syndrome and the recently described thrombophilic 3′-untranslated prothrombin gene polymorphism. *Thromb. Haemost.* **78**, 1415.
156. Bertolaccini, M. L., Atsumi, T., Hunt, B. J., *et al*. (1998). Prothrombin mutation is not associated with thrombosis in patients with antiphospholipid syndrome. *Thromb. Haemost.* **80**, 202.
157. Khamashta, M. A., Cuadrado, M. J., Mujic, F., *et al*. (1995). The management of thrombosis in the antiphospholipid-antibody syndrome. *N. Engl. J. Med.* **332**, 993.
158. Rosove, M. H., and Brewer, P. M. (1992). Antiphospholipid thrombosis: Clinical course after the first thrombotic event in 70 patients. *Ann. Intern. Med.* **117**, 303.

159. Derksen, R. H., de Groot, P. G., Kater, L., and Nieuwenhuis, H. K. (1993). Patients with antiphospholipid antibodies and venous thrombosis should receive long term anticoagulant treatment. *Ann. Rheum. Dis.* **52**, 689.
160. Ahn, S. S., Kalunian, K., Rosove, M., and Moore, W. S. (1988). Postoperative thrombotic complications in patients with the lupus anticoagulant: Increased risk after vascular procedures. *J. Vasc. Surg.* **7**, 749.
161. Eldrup-Jorgensen, J., Brace, L., Flanigan, P., *et al*. (1989). Lupus-like anticoagulants and lower extremity arterial occlusive disease. *Circulation* **80**, III-54.
162. Espinoza, L. R., and Hartmann, R. C. (1986). Significance of the lupus anticoagulant. *Am. J. Hematol.* **22**, 331.
163. Prandoni, P., Simioni, P., and Girolami, A. (1996). Antiphospholipid antibodies, recurrent thromboembolism, and intensity of warfarin anticoagulation. *Thromb. Haemost.* **75**, 859.
164. Pauzner, R., Rosner, E., and Many, A. (1986). Circulating anticoagulant in systemic lupus erythematosus: Clinical manifestations. *Acta Haematol.* **76**, 90.
165. Asherson, R. A., Chan, J. K. H., Harris, E. N., *et al*. (1985). Anticardiolipin antibody, recurrent thrombosis, and warfarin withdrawal. *Ann. Rheum. Dis.* **44**, 823.
166. Hart, R. G., Miller, V. T., Coull, B. M., and Bril, V. (1984). Cerebral infarction associated with lupus anticoagulants—preliminary report. *Stroke* **15**, 114.
167. Douketis, J. D., Crowther, M. A., Julian, J. A., *et al*. (1999). The effects of low-intensity warfarin on coagulation activation in patients with antiphospholipid antibodies and systemic lupus erythematosus. *Thromb. Haemost.* **82**, 1028.
168. Ginsberg, J. S., Wells, P. S., Brill-Edwards, P., *et al*. (1995). Antiphospholipid antibodies and venous thromboembolism. *Blood* **86**, 3685.
169. Rance, A., Emmerich, J., and Fiessinger, J. N. (1997). Anticardiolipin antibodies and recurrent thromboembolism. *Thromb. Haemost.* **77**, 221.
170. Ginsburg, K. S., Liang, M. H., Newcomer, L., *et al*. (1992). Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. *Ann. Intern. Med.* **117**, 997.
171. Erkan, D., Merrill, J. T., Yazici, Y., *et al*. (2001). High thrombosis rate after fetal loss in antiphospholipid syndrome: Effective prophylaxis with aspirin. *Arthritis Rheum.* **44**, 1466.
172. Rai, R., Cohen, H., Dave, M., and Regan, L. (1997). Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies) *Br. Med. J.* **314**, 253.
173. Jan Out, H., deGroot, P. G., and Hasselaar, P. (1989). Fluctuations of anticardiolipin antibody levels in patients with systemic lupus erythematosus: A prospective study. *Ann. Rheum. Dis.* **48**, 1023.
174. Drenkard, C., Sanchez-Guerrero, J., and Alarcon-Segovia, D. (1989). Fall in antiphospholipid antibody at time of thrombo-occlusive episodes in systemic lupus erythematosus. *J. Rheumatol.* **16**, 614.
175. Shergy, W. J., Kredich, D. W., and Pisetsky, D. S. (1988). The relationship of anticardiolipin to disease manifestations in pediatric systemic lupus erythematosus. *J. Rheumatol.* **15**, 1389.
176. Isenberg, D. A., Colaco, C. B., and Dudeney, C. (1986). The relationship of anti-DNA antibody idiotypes and anticardiolipin antibodies to disease activity in systemic lupus erythematosus. *Medicine* **65**, 46.
177. Derksen, R. H. W. M., Biesma, D., Bouma, B. N., *et al*. (1986). Discordant effects of prednisone on anticardiolipin antibodies and the lupus anticoagulant. *Arthritis Rheum.* **29**, 1295.
178. Margolius, A., Jr., Jackson, D. P., and Ratnoff, O. D. (1961). Circulating anticoagulants. A study of 40 cases and a review of the literature. *Medicine* **40**, 145.
179. Lubbe, W. F., Butler, W. S., Palmer, S. J., and Liggins, G. C. (1983). Fetal survival after prednisone suppression of maternal lupus anticoagulant. *Lancet* **1**, 1361.
180. Hedfors, E., Lindahl, G., and Lindblad, S. (1987). Anticardiolipin antibodies during pregnancy. *J. Rheumatol.* **14**, 160.
181. Sturfelt, G., Nived, O., Norberg, R., *et al*. (1987). Anticardiolipin antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **30**, 382.
182. Derksen, R. H. W. M., Hasselaar, P., Blokzijl, and deGroot, P. G. (1987). Lack of efficacy of plasma exchange in removing antiphospholipid antibodies. *Lancet* **2**, 222.
183. Lockshin, M. D., Qamar, S., Goei, S., and Druzin, M. (1986). Anticardiolipin antibody in SLE pregnancy: Prognostic value and response to therapy abstract. *Arthritis Rheum.* **29**, S44.
184. Harris, E. N., Gharavi, A. K., and Hughes, G. R. V. (1985). Antiphospholipid antibodies. *Clin. Rheum. Dis.* **11**, 3.
185. Frances, C., Tribout, B., Boisnic, S., *et al*. (1989). Cutaneous necrosis associated with the lupus anticoagulant. *Dermatologica* **178**, 194.
186. Petri, M. (1996). Hydroxychloroquine use in the Baltimore Lupus Cohort: Effects on lipids, glucose and thrombosis. *Lupus* **5**(Suppl. 1), S16.
187. Erkan, D., Yazici, Y., Peterson, M. G., *et al*. (2002). A cross-sectional study of clinical thrombotic risk factors and preventive treatments in antiphospholipid syndrome. *Rheumatology* **41**, 924.
188. Edwards, M. H., Pierangeli, S., Liu, X., *et al*. (1997). Hydroxychloroquine reverses thrombogenic properties of antiphospholipid antibodies in mice. *Circulation* **96**, 4380.
189. Kent, M., Alvarez, F., Vogt, E., *et al*. (1997). Monoclonal antiphosphatidylserine antibodies react directly with feline and murine central nervous system. *J. Rheumatol.* **24**, 1725.
190. Ziporen, L., Shoenfeld, Y., Levy, Y., and Korczyn, A. D. (1997). Neurological dysfunction and hyperactive behavior associated with antiphospholipid antibodies. A mouse model. *J. Clin. Invest.* **100**, 613.
191. Cronin, M. E., Biswas, R. M., Van der Straeton, C., *et al*. (1988). IgG and IgM anticardiolipin antibodies in

patients with lupus with anticardiolipin antibody-associated clinical syndromes. *J. Rheum.* **15**, 795.
192. Brey, R. L., Hart, R. G., Sherman, D. G., and Tegeler, C. H. (1990). Antiphospholipid antibodies and cerebral ischemia in young people. *Neurology* **40**, 1190.
193. Czlonkowska, A., Meurer, M., Palasik, W., *et al*. (1992). Antiphospholipid antibodies, a disease marker for ischemic cerebrovascular events in a younger patient population? *Acta Neurol. Scand.* **86**, 304.
194. Baca, V., Garcia-Ramirez, R., Ramirez-Lacayo, M., *et al*. (1996). Cerebral infarction and antiphospholipid syndrome in children. *J. Rheumatol.* **23**, 1428.
195. Nagaraja, D., Christopher, R., and Manjari, T. (1997). Anticardiolipin antibodies in ischemic stroke in the young: Indian experience. **150**, 137.
196. Levine, S. R., Kim, S., Deegan, M. J., and Welch, K. M. A. (1987). Ischemic stroke associated with anticardiolipin antibodies. *Stroke* **18**, 1101.
197. Levine, S. R., Deegan, M. J., Futrell, N., and Welch, K. M. A. (1990). Cerebrovascular and neurologic disease associated with antiphospholipid antibodies: 48 cases. *Neurology* **40**, 1181.
198. Pope, J. M., Canny, C. L. B., and Bell, D. A. (1990). Cerebral ischemic events associated with endocarditis, retinal vascular disease, and lupus anticoagulant. *Am. J. Med.* **90**, 299.
199. Kushner, M., and Simonian, N. (1989). Lupus anticoagulants, anticardiolipin antibodies, and cerebral ischemia. *Stroke* **20**, 225.
200. Ford, P. M., Brunet, D., Lillicrap, D. P., and Ford, S. E. (1990). Premature stroke in a family with lupus anticoagulant and antiphospholipid antibodies. *Stroke* **21**, 66.
201. Fisher, M., and McGehee, W. (1986). Cerebral infarct, TIA, and lupus inhibitor. *Neurology* **36**, 1234.
202. Landi, G., Calloni, M. V., Sabbandini, M. G., *et al*. (1983). Recurrent ischemic attacks in two young adults with lupus anticoagulant. *Stroke* **14**, 377.
203. Levine, S. R., Crofts, J. W., Lesser, G. R., *et al*. (1988). Visual symptoms associated with the presence of a lupus anticoagulant. *Ophthalmology* **95**, 686.
204. UK-TIA Study Group. (1987). The UK-TIA aspirin trial: Interim results. *Br. Med. J.* **296**, 316.
205. Gent, M. (1989). The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. *Lancet* **1**, 1215.
206. Brey, R. L., and Levine, S. R. (1996). Treatment of neurologic complications of antiphospholipid antibody syndrome. *Lupus* **5**, 473.
207. Rai, R. S., Clifford, K., Cohen, H., and Regan, L. (1995). High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriages and antiphospholipid antibodies. *Hum. Reprod.* **10**, 3301.
208. Farquharson, R. G., Pearson, J. F., and John, L. (1984). Lupus anticoagulant and pregnancy management. *Lancet* **2**, 228.
209. Branch, D., Scott, J., Kochenour, N., and Hershgold, E. (1985). Obstetric complications associated with the lupus anticoagulant. *N. Engl. J. Med.* **313**, 1322.
210. Lubbe, W. F., and Liggins, G. C. (1985). Lupus anticoagulant and pregnancy. *Am. J. Obstet. Gynecol.* **153**, 322.
211. Lima, F., and Khamashta, M. A., Buchanan, N. M. M., *et al*. (1996). A study of sixty pregnancies in patients with the antiphospholipid syndrome. *Clin. Exp. Rheumatol.* **14**, 131.
212. Rosove, M. H., Tabsh, K., Wasserstrum, N., *et al*. (1990). Heparin therapy for pregnant women with lupus anticoagulant or anticardiolipin antibodies. *Obstet. Gynecol.* **75**, 630.
213. Clifford, K., Rai, R., Watson, H., and Regan, L. (1994). An informative protocol for the investigation of recurrent miscarriage: Preliminary experience of 500 consecutive cases. *Hum. Reprod.* **9**, 1328.
214. Kutteh, W. H., and Ermel, L. D. (1996). A clinical trial for the treatment of antiphospholipid antibody-associated recurrent pregnancy loss with lower dose heparin and aspirin. *Am. J. Reprod. Immunol.* **35**, 402.
215. Yetman, D. L., and Kutteh, W. H. (1996). Antiphospholipid antibody panels and recurrent pregnancy loss: Prevalence of anticardiolipin antibodies compared with other antiphospholipid antibodies. *Fertil. Steril.* **66**, 540.
216. Rai, R., Cohen, H., Dave, M., and Regan, L. (1997). Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). *Br. Med. J.* **314**, 253.
217. Sammaritano, L. R. (2001). Update on the management of the pregnant patient with antiphospholipid antibody. *Curr. Rheumatol. Rep.* **3,** 213.
218. Empson, M., Lassere, M., Craig, J. C., and Scott, J. R. (2002). Recurrent pregnancy loss with antiphospholipid antibody: A systematic review of therapeutic trials. *Obstet. Gynecol.* **99**, 135.
219. Cowchock, S. (1996). Prevention of fetal death in the 0antiphospholipid antibody syndrome. *Lupus* **5**, 467.
220. Stratta, P., Canavese, C., Schinco, P. C., *et al*. (1997). Intravenous immunoglobulin contains detectable amounts of antiphospholipid antibodies. *Br. J. Haematol.* **96**, 872.
221. Branch, D. W., Peaceman, A. M., Druzin, M., *et al*. (2000). A multicenter, placebo-controlled pilot study of intravenous immune globulin treatment of antiphospholipid syndrome during pregnancy. *Am. J. Obstet. Gynecol.* **182**, 122.
222. Branch, D. W., Porter, T. F., Paidas, M. J., *et al*. (2001). Obstetric uses of intravenous immunoglobulin: Successes, failures, and promises. *J. Allergy Clin. Immunol.* **108**(Suppl.), S133.
223. van Wijnen, M., Stam, J. G., van 't Veer, C., *et al*. (1996). The interaction of protein S with the phospholipid surface is essential for the activated protein C-independent activity of protein S. *Thromb. Haemost.* **76**, 397.
224. Byron, M. A., Allington, M. J., Chapel, H. M., *et al*. (1987). Indications of vascular endothelial cell dysfunction in systemic lupus erythematosus. *Ann. Rheum. Dis.* **46**, 741.
225. Preston, F. E., Malla, R. G., Cooper, P., and Greaves, M. (1988). Reduced free protein S in patients with the lupus anticoagulant (abstract). *Clin. Exp. Rheumatol.* **6**, 210.
226. Tomas, J. F., Alberca, I., Tabernero, M. D., *et al.* (1998). Natural anticoagulant proteins and antiphospholipid

antibodies in systemic lupus erythematosus. *J. Rheumatol.* **25**, 57.
227. Costallat, L. T., Ribeiro, C. C., and Annichino-Bizzacchi, J. M. (1998). Antithrombin, protein C and protein S and antiphospholipid antibodies in systemic lupus erythematosus. *Sangre* **43**, 345.
228. Panicucci, F., Sagripanti, A., Vispi, M., *et al*. (1983). Comprehensive study of haemostasis in nephrotic syndrome. *Nephron* **33**, 9.
229. Vaziri, N. D., Paule, P., and Toohey, J. (1984). Acquired deficiency and urinary excretion of antithrombin III in nephrotic syndrome. *Arch. Intern. Med.* **144**, 1803.
230. Kauffmann, R. H., Veltkamp, J. J., van Tilburg, N. H., and van Es, L. A. (1978). Acquired antithrombin III deficiency and thrombosis in the nephrotic syndrome. *Am. J. Med.* **65**, 607.
231. Vigano-D'Angelo, S., D'Angelo, A., Kaufman, C. E., *et al*. (1987). Protein S deficiency occurs in the nephrotic syndrome. *Ann. Intern. Med.* **107**, 42.
232. Vaziri, N. D. (1983). Nephrotic syndrome and coagulation and fibrinolytic abnormalities. *Am. J. Nephrol.* **3**, 1.
233. Vaziri, N. D., Toohey, J., Paule, P., *et al*. (1984). Urinary excretion and deficiency of prothrombin in nephrotic syndrome. *Am. J. Med.* **77**, 433.
234. Schinco, P., Borchiellini, A., Tamponi, G., *et al*. (1997). Lupus anticoagulant and thrombosis: Role of von Willebrand factor multimeric forms. *Clin. Exp. Rheumatol.* **15**, 5.
235. Angles-Cano, E., Sultan, Y., and Clauvel, J.-P. (1979). Predisposing factors to thrombosis in systemic lupus erythematosus. *J. Lab. Clin. Med.* **94**, 312.
236. Hardin, J. A., Cronlund, M., Haber, E., and Bloch, K. J. (1978). Activation of blood clotting in patients with systemic lupus erythematosus: Relationship to disease activity. *Am. J. Med.* **65**, 430.
237. Regan, M. G., Lackner, H., and Karpatkin, S. (1974). Platelet function and coagulation profile in lupus erythematosus: Studies in 50 patients. *Ann. Intern. Med.* **81**, 462.
238. Bajaj, S. P., Rapaport, S. I., Fierer, D. S., *et al*. (1983). A mechanism for the hypoprothrombinemia of the acquired hypoprothrombinemia-lupus anticoagulant syndrome. *Blood* **61**, 684.
239. Fleck, R. A., Rapaport, S. I., and Rao, L. V. M. (1988). Anti-prothrombin antibodies and the lupus anticoagulant. *Blood* **72**, 512.
240. Baudo, F., Redaelli, R., and Pezzetti, L. (1990). Prothrombin antibody coexistent with lupus anticoagulant (LA): Clinical study and immunochemical characterization. *Thromb. Res.* **7**, 279.
241. Lee, M. T., Nardi, M. A., Hu, G., *et al*. (1996). Transient hemorrhagic diathesis associated with an inhibitor of prothrombin with lupus anticoagulant in a $1\frac{1}{2}$-year-old girl. *Am. J. Hematol.* **51**, 307.
242. Amiral, J., Aronis, S., Adamtziki, E., *et al*. (1997). Association of lupus anticoagulant with transient antibodies to prothrombin in patient with hypoprothrombinemia. *Thromb. Res.* **86**, 73.
243. Becton, D. L., and Stine, K. C. (1997). Transient lupus anticoagulants associated with hemorrhage rather than thrombosis: The hemorrhagic lupus anticoagulant syndrome. *J. Pediatr.* **130**, 998.
244. Vivaldi, P., Rossetti, G., Galli, M., and Finazzi, G. (1997). Severe bleeding due to acquired hypoprothrombinemia-lupus anticoagulant syndrome. *Haematologia* **82**, 345.
245. Bajaj, S. P., Rapaport, S. I., Barclay, S., and Herbst, K. D. (1985). Acquired hypoprothrombinemia due to non-neutralizing antibodies to prothrombin: mechanism and management. *Blood* **65**, 6.
246. Natelson, E. A., Cyprus, G. S., and Hettig, R. A. (1976). Absent factor II in systemic lupus erythematosus. *Arthritis Rheum.* **19**, 79.
247. Lillquist, K. B., Dyerberg, J., and Krogh-Jensen, M. (1978). The absence of factor II in a child with systemic lupus erythematosus. *Acta Paediatr. Scand.* **67**, 533.
248. Green, P., and Lechner, K. (1981). A suryey of 215 nonhemophilic patients with inhibitors to factor VIII. *Thromb. Haemost.* **45**, 200.
249. Lian, E. C.-Y., Larcada, A. F., and Chiu, A. Y. O. Z. (1989). Combination immunosuppressive therapy after factor VIII infusion for acquired factor VIII inhibitor. *Ann. Intern. Med.* **110**, 774.
250. Francesconi, M., Korninger, C., Thaler, E., *et al*. (1982). Plasmapheresis: its value in the management of patients with antibodies to factor VIII. *Haemostasis* **11**, 79.
251. Paracchini, M. L., Rocchini, G. M., Renoldi, P., *et al*. (1984). Acquired factor VIII inhibitor in a non-haemophilic patient: Successful treatment with plasma exchange associated with factor VIII concentrate and immunosuppressors. *Haemostasis* **14**, 249.
252. Hambley, H., Watkins, R., Tansey, P. A., *et al*. (1985). Plasmapheresis and factor VIII:C inhibitors. *Lancet* **1**, 274.
253. Sultan, Y., Maisonneuve, P., Bismuth, A., and Steg, A. (1983). Successful management of a patient with an acquired factor VIII inhibitor. *Transfusion* **23**, 62.
254. Pintado, T., Taswell, H. F., and Bowie, E. J. W. (1975). Treatment of life-threatening hemorrhage due to acquired factor VIII inhibitor. *Blood* **46**, 535.
255. Naorose-Abidi, S. M., Bond, L. R., Chitolie, A., *et al*. (1988). Desmopressin therapy in patients with acquired factor VIII inhibitors. *Lancet* **1**, 366.
256. Chistolini, A., Ghirardina, A., Tirindelli, M. C., *et al*. (1987). Inhibitor to factor VIII in a non-haemophilic patient: Evaluation of the response to DDAVP and the in vitro kinetics of factor VIII. A case report. *Nouv. Rev. Fr. Hematol.* **29**, 2221.
257. Vincente, V., Alberca, I., Gonzalez, and Borrasca, A. L. (1985). DDAVP in a non-haemophilic patient with an acquired factor VIII inhibitor. *Br. J. Haematol.* **60**, 585.
258. de la Fuente, B., Panek, S., and Hoyer, L. W. (1985). The effect of l-deamino-8-D-arginine vasopressin (DDAVP) in a non-haemophilic patient with an acquired type II factor VIII inhibitor. *Br. J. Haematol.* **59**, 127.
259. Hart, H. C., Kraaijenhagen, R. J., Kerckhaert, J. A. M., *et al*. (1988). A patient with a spontaneous factor VIII:C autoantibody: Successful treatment with cyclosporine. *Transplant. Proc.* **20**, 323.

260. Shapiro, S. S., and Hultin, M. (1975). Acquired inhibitors to the blood coagulation factors. *Semin. Thromb. Haemost.* **1**, 336.
261. Shapiro, S. S., and Rajagopalan, V. (1996). Hemorrhagic disorders associated with circulating inhibitors. In "Disorders of Hemostasis" (O. D. Ratnoff and C. D. Forbes, Eds.), p. 208. Saunders, Philadelphia.
262. Reece, E. A., Clyne, L. P., Romero, R., *et al*. (1984). Spontaneous factor XI inhibitors: Seven additional cases and a review of the literature. *Arch. Intern. Med.* **144**, 525.
263. Galanakis, D. K., Ginzler, E. M., and Fikrig, S. M. (1978). Monoclonal IgG anticoagulants delaying fibrin aggregation in two patients with systemic lupus erythematosus (SLE). *Blood* **52**, 1037.

43

THE ANTIPHOSPHOLIPID SYNDROME: PATHOGENESIS AND IMPLICATIONS FOR SYSTEMIC LUPUS ERYTHEMATOSUS

Joan T. Merrill

ABSTRACT

Anti-phospholipid antibodies are prevalent in lupus patients and have been implicated in life-threatening thrombotic events. Unfortunately, detecting the presence of these antibodies does not predict the likelihood of thrombosis for an individual nor does it predict when such an event may occur. Numerous reports indicate that isolated patient antibodies interfere directly with various elements of the coagulation cascade. Nevertheless clinicians are left in doubt as to when and for whom anticoagulant therapy is useful. There is an obvious need to determine the coagulation events which trigger the sudden thrombotic episodes that characterize this syndrome, and whether there are measurable clinical parameters which can be used as warning signals for impending thrombosis. This chapter reviews current literature in support of a pathogenic model for thrombosis involving a complex equilibrium between autoantibodies, endothelium, platelets, and coagulation proteins, particularly those which modulate the anticoagulant, protein S. An overall model for thrombotic risk, which takes into account the possibility that there might be multiple potential targets for anti-phospholipid antibodies in the coagulation cascade is now possible. Benign and/or transient autoantibodies which may develop in most people as a result of infection could, in susceptible individuals, spread and persist to the point that a potentially pathologic dysequilibrium exists in the clotting cascade. Even then, serious consequences are rare, awaiting the justaposition of at least two conditions: triggering of a significant thrombotic event and turnover of vascular phospholipids (through apoptosis or inflammation). This creates a primed environment for problems: stress on the system such that the input of anticoagulant proteins becomes most critical, and optimal binding conditions for antibodies that interfere with the function(s) of these proteins. Markers for thrombotic risk, then, might be found among blood tests which provide subtle evidence for vascular oxidation or inflammation, tests which reveal some of the unique functional changes predictive of autoantibody-mediated vascular imbalance, or tests which subcategorize the autoantibodies to each important coagulation protein to detect their pathologic epitope(s).

INTRODUCTION

The heterogenous immunoglobulins known as anti-phospholipid antibodies (APLA) or lupus "anticoagulants" (LA) are prevalent in lupus patients and have been implicated in life-threatening thrombotic events [1–3]. Unfortunately, detecting the presence of these

antibodies in an individual does not predict the likelihood of thrombosis nor does it predict when such an event may occur [4–6]. A pathogenic role for these antibodies is supported by the observation that high titer and IgG isotype confer an increased risk of thrombosis [7]. Additionally, numerous reports indicate that isolated patient antibodies interfere directly with various elements of the coagulation cascade [8–28]. Nevertheless, attempts to correlate specific antibody characteristics with a hypercoagulable state in an individual patient [29] have been unsuccessful to date. Because of this, a clinician is left in doubt as to when and for whom anticoagulant therapy is useful.

Given such widespread uncertainty combined with the potential complications of anticoagulant medications, patients are generally not treated until after some significant morbidity has occurred. Finally, because of the apparent high rate of reoccurrence [30] most patients remain on aggressive anticoagulant therapy indefinitely, regardless of individual need, which simply cannot be predicted. There is an obvious need to determine the coagulation events which trigger sudden thrombotic episodes, and whether there are measurable clinical parameters which can be used as warning signals for impending thrombosis. A remarkable effort has been underway in many laboratories around the world to do just that.

An improved understanding of the pathogenesis of the antiphospholipid syndrome might lead not only to better predictive tests and more rational timing of anticoagulant therapy, but perhaps eventually to novel, more specific and safer therapies, aimed at the specific underlying coagulation disorder(s). The current chapter will describe experimental work which may provide signposts to improved future therapy for patients with this disorder, and will also examine currently available methods of diagnosis, and those anticoagulant and immunosuppressive therapies which either have been tested in preliminary studies or are in more widespread use at the time of this writing. The aim will be to review both the limited state of the art and options which may become available in the near future to manage this difficult complication of systemic lupus.

Primary Secondary Antiphospholipid Syndrome: Is There a Fundamental Difference or Is the Antiphospholipid Syndrome a *Forme Fruste* of SLE?

Clinically, it is not possible to distinguish the manifestations of primary and secondary antiphospholipid syndrome. An intuitive common practice has been to use the accumulated data from patients with or without SLE in discussing or determining therapy for an individual with this syndrome. The rationality of this practice has been borne out by data from two large European cohorts, suggesting that the broad range of coagulative complications are nearly identical [31, 32]. Furthermore, the overall prevalence of thrombosis and thrombocytopenia appears to be the same in patients with or without full-blown SLE [31, 32], and in an additional large clinical study of 110 antiphospholipid patients, there was no significant difference found in the neuroradiologic findings for those with or without SLE [33]. On the other hand, certain of the less well-established antiphospholipid-associated features, including heart valve disease, hemolytic anemia, low C4, and neutropenia, have been found to be more common in those antiphospholipid patients who meet criteria for SLE [32].

As will be discussed later, the antiphospholipid syndrome, like SLE, is characterized by the emergence of a broad range of autoantibodies, which are associated with its clinical features, and which are common to those patients with the primary or secondary syndromes. It seems logical, then, to speculate that the primary antiphospholipid syndrome is nothing more than a partial manifestation of SLE. Anti-nuclear antibodies have been detected in up to 33% of patients with primary antiphospholipid syndrome, and about 8% of these patients have relatives with SLE [34]. Furthermore, patients with primary antiphospholipid syndrome may develop systemic lupus at a later date, although one study, following 165 patients for a median of 78 months, found the emergence of systemic lupus to be relatively rare (<2%) in that time span [35]. On the other hand, when evolution from primary antiphospholipid syndrome to lupus has been documented, it has appeared to be a relatively late phenomenon, occurring at least 10 years after the initial diagnosis [35, 36].

In animal models, the induction of anti-phospholipid antibodies is associated with other lupus-like autoimmune phenomena. Aron *et al*. [37] inoculated both rabbits and mice with β_2-glycoprotein I, a major target antigen for anti-phospholipid antibodies, and found production of anti-phospholipid antibodies, anti-nuclear antibodies, and antibodies to DNA, which could be absorbed by phospholipid micelles, suggesting cross-reactivity between the antibodies to phospholipid and DNA. In a different mouse model, antibodies to heparan sulfate were detected which cross-reacted with DNA, cardiolipin, and RNA polymerase [38]. In addition, antibodies directed against anti-DNA antibodies may be found in some patients with primary antiphospholipid syndrome [39], suggesting an evolutionary relationship between anti-phospholipid and other lupus-related autoantibodies.

Although the immunogenetics of the antiphospholipid syndrome may be complex, a familial tendency to the development of anti-cardiolipin antibodies, lupus anticoagulant, and clinical manifestations of the antiphospholipid syndrome appears to be linked to HLA DR4, DR7, and Drw53 [40–42], whether the disorder is primary or a manifestation of SLE [40]. Not surprisingly, the DR4 association is more likely to involve Anglo Saxons and the DR7 is associated with people of Latin origin [41, 42]. It should be noted, on the other hand, that inheritance of these alleles confers only a modest increased relative risk of developing the antiphospholipid syndrome [41].

Finally, lupus activity per se may contribute to the clinical outcome of patients with the antiphospholipid syndrome. In one study of 19 lupus patients with a history of antiphospholipid syndrome and a diagnosis dating back 5 or more years, lupus activity was found to have been relatively active in the 9 with recurrent thrombotic events as compared to the 10 patients who had no further thrombosis [43]. Lupus flare was also noted to correlate with thrombotic events in some of these patients. Several additional studies have correlated complement activation to thrombosis in the antiphospholipid syndrome [44–49].

Given clinical, pathologic, and genetic similarities between the primary and secondary antiphospholipid syndrome, the current chapter will review physiologic models and treatment strategies for this coagulation disorder without differentiating further between the primary and secondary forms. Furthermore, whether or not it ever becomes possible to distinguish some fundamental difference between idiopathic and lupus-related coagulopathy, the strong association between SLE, SLE flare, and the antiphospholipid syndrome may provide a clue to an underlying pathogenic mechanism common to both the primary and secondary syndromes. Evidence in support of this possibility will be one focus of the following discussion.

PATHOGENESIS OF THE ANTIPHOSPHOLIPID SYNDROME: OVERVIEW OF A COMPLEX DISORDER

It has become increasingly apparent that various anti-phospholipid antibodies have the capability of binding to and potentially interfering with many of the critical elements and structures that regulate hemostasis, including endothelial cells, platelets, monocytes, humoral components, and circulating coagulation factors [50]. It is now thought that the majority of the pathogenic antibodies do not bind phospholipid alone, but rather to various proteins attached to platelet, endothelial, or other phospholipid surfaces [8–28]. Central to the antiphospholipid syndrome, by virtue of their high prevalence, are autoantibodies which specifically bind to either the plasma anticoagulant β_2-glycoprotein I [51], or to prothrombin, the latter of which seems to account for most of the lupus anticoagulant activity [52]. Other phospholipid-binding proteins which have been found to be targets for anti-phospholipid antibodies are listed in Table 1, and by reviewing this extensive list of hemostatic regulating proteins, it becomes obvious that anti-phospholipid antibodies have the potential to disrupt coagulation from multiple directions. Therefore, unlike many of the autoantibodies associated with SLE, a direct role for circulating antiphospholipid antibodies in the disruption of homeostasis now appears not only possible, but likely.

TABLE 1 Phospholipid-Binding Proteins: Targets for Anti-phospholipid Antibodies

β_2 glycorprotein-I
Prothrombin
Annexin V
Protein S
Protein C
Kininogens
Apolipoprotein A1
Platelet adhesion proteins
Endothelial surface proteins
Other coagulation factors
Other cholesterol transport proteins

An Explanation for the Association between "Lupus Anticoagulants" and a Thrombotic Clinical Syndrome: Autoantibodies May Either Enhance or Inhibit Coagulation, Depending on the Assay Used to Detect This

Lupus anticoagulants were originally identified as circulating plasma factors capable of prolonging the activated partial thromboplastin time, and quixotically associated with a hypercoagulable state. The lupus anticoagulant later became a clinically useful marker for the antiphospholipid syndrome, and has been thought to be more specific for the syndrome than conventional ELISA detection of antibodies to cardiolipin [53]. This seems counterintuitive, since the functional outcome being measured would predict a clinical tendency to bleed rather than thrombosis. There are several possible explanations for this: (1) Lupus anticoagulant func-

tion may be an artifact of *in vitro* testing and the same antibodies may have different functional effects *in vivo*. (2) Lupus anticoagulants may be detectable plasma markers for the presence of structurally similar antibodies which have very different procoagulant effects. Lupus anticoagulants may be closely related but not identical to these pathologic antibodies, and have a tendency to appear in tandem with them during the evolution of a polyclonal immune response.

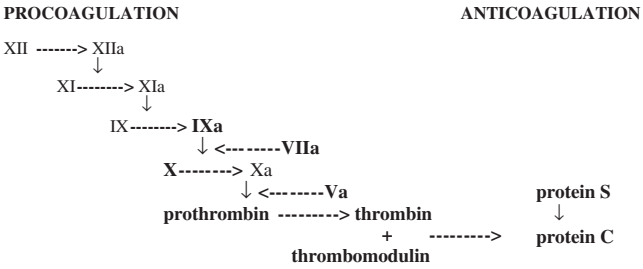


FIGURE 1 Phospholipid-based interactions in the coagulation cascade. Pertinant reactions which occur on a phospholipid background are shown in bold.

An early hypothesis was that most if not all lupus anticoagulants were antibodies with immunologic specificity for the anionic phospholipids which provide active sites in the calcium-mediated binding of vitamin K-dependent coagulation factors. Phospholipids are components in several steps of the coagulation process (see Fig. 1) including the activation of factor X by VIIa and IXa, the prothrombin activation complex, and the proteolytic inactivation of factor VIIa and factor Va by activated protein C. As illustrated in Fig. 2, potential phospholipid-based targets of autoantibody-mediated procoagulant activity might include sites of protein C activation and/or its activity in the proteolysis of factors VIIIa and Va, since inhibition of these elements would be predicted to result in a coagulant effect [54–56].

Assays for activated partial thromboplastin time (APTT) reflect APLA interference with interactions occurring on the left (or procoagulant) side of the picture, so that the net effect of the antibodies in these *in vitro* clotting tests is that of anticoagulation. These anticoagulant effects do not usually have clinical significance *in vivo*. On the other hand, since activated protein C inhibits factors VIIIa and Va, it would follow

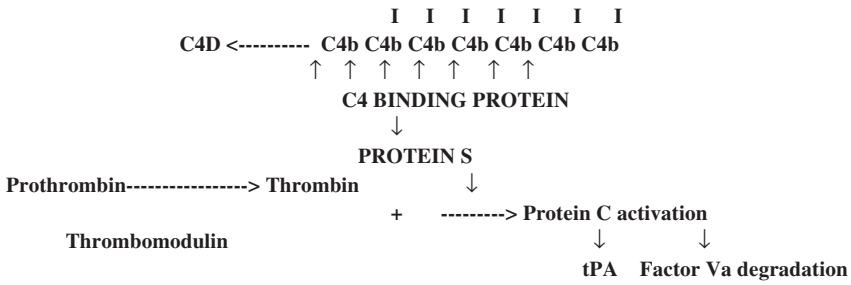


FIGURE 2 Protein S regulation: An interface between the complement system and coagulation. The rate of factor Va degradation by protein C is proportional to the concentration of activated phospholipid-bound protein C. The cofactor activity of protein S requires a one-to-one stoichiometric complex of activated protein C and protein S. In plasma, protein S associated noncovalently in a calcium-dependent manner with C4BP, also approaching a one-to-one stoichiometry (K_d < 3 nM/liter). The C4BP is composed of a small central body with 7 thin projections of molecular weight 70,000. Each of these tentacles can bind and remove C4b from the site of the C3 convertase, making C4b accessible to degradation by factor I, with C4D as a measurable product.

Each C4BP molecule also contains one shorter arm or β chain which binds and inactivates protein S. With physiologic calcium concentrations all available β chain sites are bound such that levels of free or active protein S depend on a molar excess of protein S over available C4BP molecules. Given the high affinity of protein S for phospholipid, protein S may help to localize C4BP to membranes, at which point C4BP remains capable of binding and inactivating C4b. The large C4BP may then sterically hinder more protein S and protein C from binding to the membrane, and inactivates the protein S that is bound to it as well. β2-glycoprotein I may fill the protein S binding site on C4BP as a further control in this system. To the extent that the concentration of C4BP with available protein S binding site is greater than the total protein S concentration minus the concentration of activated protein C, a functional protein S deficiency can occur. We hypothesize that this is what happens in states of disordered complement activation, and in the presence of antibodies which hinder β2-glycoprotein I/phospholipid from performing as regulatory elements.

that interference with protein C interactions by antiphospholipid antibodies might have a thrombogenic effect. Effects of anti-phospholipid antibodies on protein C activity, however, would not be picked up by a standard APTT or the dilute Russell viper venom time (DRVVT) assay, commonly used to detect lupus anticoagulants.

In fact, plasma containing lupus anticoagulant activity, as well as purified antibodies with lupus anticoagulant function have been shown to inhibit anticoagulant proteins [56–62]. It may be that lupus anticoagulants have a dual effect on coagulation *in vivo*. The net outcome of antibody interference and coagulation cascade regulatory adjustments might usually result in hemostatic balance, sporadically in thrombosis, and only rarely in a hemorrhagic consequence, such as is seen clinically in the antiphospholipid syndrome. This net outcome would be determined at any point in time by the dynamic equilibrium between the dysregulating forces of the antibodies and the robustness of complex compensatory adjustments in the coagulation cascade.

On the other hand, there is some evidence to suggest that the lupus anticoagulant function is indeed a laboratory artifact, without physiologic meaning. For example, when 22 IgG fractions which prolonged APTT in a standard assay were subsequently tested using an endothelial cell-mediated prothrombinase reaction only 4 (18%) inhibited prothrombinase in this more physiologic environment [9]. Furthermore, it is now appreciated that most lupus anticoagulants are not specific for phospholipids. About one-third have been found to depend on the anticoagulant, β_2-glycoprotein I for binding and the other two-thirds appear to target prothrombin [63]. Takeya *et al*. have demonstrated that monoclonal antibodies specific for the third and fourth domains of β_2-glycoprotein I , which also have lupus anticoagulant activity, appear to increase the binding between β_2-glycoprotein I and phospholipids [53]. Presumably, increased β_2-glycoprotein I on an activated hemostatic surface would enhance its anticoagulant functions. The association between β_2-glycoprotein I and thrombosis would not be explained by lupus anticoagulants under these circumstances, but the association between lupus anticoagulants and thrombosis might be as bystanders: Antibodies to nearby epitopes on β_2-glycoprotein I, which appear by immunogenic spreading in association with lupus anticoagulants, might interfere with the anticoagulant functions of β_2-glycoprotein I and be the thrombosis-inducing culprits.

A lupus anticoagulant, then, might be defined as any antibody against either prothrombin or β_2-glycoprotein I that either enhances their anticoagulant function(s) or inhibits a procoagulant interaction of these molecules in an *in vitro* assay. The increased specificity of such antibodies for the antiphospholipid syndrome may be the consequence of alternative net effects *in vivo* or result from immunologic spreading giving rise to associated antibodies that bind to tandem structures. Lupus anticoagulants may bind epitopes near to or even overlapping those with procoagulant effects, and thereby be more likely to develop together with procoagulant antibodies than are the broad range of polyclonal antiphospholipid antibodies.

Anti-phospholipid Antibodies Primarily Target Either β_2-Glycoprotein I or Prothrombin: Is There a Role for Phospholipid?

It has become generally accepted that the lipid-binding anticoagulant protein, β_2-glycoprotein I (apolipoprotein H) is necessary for the binding of many anti-phospholipid antibodies in an *in vitro* ELISA assay [64, 65]. It now appears that at least a significant proportion of patients have APLA which bind directly to epitopes on β_2-glycoprotein I, although whether this is a universal phenomenon remains controversial [51, 66–68]. Autoantibodies to β_2-glycoprotein I tend to be low affinity, monoreactive antibodies, specific for this molecule even in the absence of phospholipid as long as there is a negatively charged environment. Anti-β_2-glycoprotein I antibodies are further directed to epitopes which have been preserved across species, and therefore may have some functional significance [64]. One study has demonstrated good correlation between anti-cardiolipin antibody and anti-β_2-glycoprotein I antibody level, light chain association, and IgG subclass, suggesting the possibility that to a large extent the same antibodies are being measured by both assays [52].

Another common protein antigen for anti-phospholipid antibodies is prothrombin [65, 69]. Antibodies to prothrombin may account for more lupus anticoagulant activity than antibodies to β_2-glycoprotein I [63]. The actual prevalence of antibodies to the other lipid binding proteins listed in Table 1 remains to be determined. One study of 22 patients with thrombotic history and IgG anti-phospholipid antibodies found high levels of anti-β_2-glycoprotein I in all 22, anti-prothrombin antibodies in 11 (50%), anti-protein S antibodies in 12, and anti-protein C antibodies in 4 patients [65].

Antibodies which bind to β_2-glycoprotein I, like lupus anticoagulants, appear to correlate better with morbidity in the antiphospholipid syndrome than the general range of anti-phospholipid antibodies do [52, 65, 68, 70, 71]. In at least one study, anti-β_2-glycoprotein I correlated with morbidity better than anti-prothrombin anti-

bodies [65], although in a different study involving 139 patients, both anti-β_2-Glycoprotein I and anti-prothrombin antibodies were significantly associated with the development of deep venous thrombosis ($p = 0.009$ for both) [71].

β_2-Glycoprotein I may have multiple anticoagulant functions, so it appears to be a particularly likely candidate for providing functional epitope(s) to pathologic anti-phospholipid antibodies. For example, β_2-glycoprotein I has been shown to inhibit contact activation of the intrinsic blood coagulation pathway [72], the prothrombinase reaction [73], and lipoprotein lipase-induced factor XII activation [74]. β_2-Glycoprotein I may also be involved in monocyte–endothelial interactions [75]. One report has suggested that β_2-glycoprotein I neutralizes the anticoagulant activity of activated protein C [76], although evidence from our laboratory and others suggests the opposite: that it interferes with inhibition of the protein C cofactor, protein S, serving as an additional anticoagulant cofactor in this system [77, 78].

Given mounting evidence, then, that pathologic anti-phospholipid antibodies are directed against epitopes on β_2-glycoprotein I and possibly other lipid-binding proteins, are phospholipids irrelevant in the antiphospholipid syndrome? The answer is clearly no, since unique phospholipid environments may provide critical modulation of protein structures upon which anticoagulant functions and/or antibody binding depend [66, 67]. Roubey *et al*. have demonstrated that β_2-glycoprotein I-dependent anti-phospholipid antibodies are low affinity antibodies which require bivalent binding to densely packed β_2-glycoprotein I, in order to achieve optimal detection in an ELISA [79]. There is relatively poor binding of these antibodies to β_2-glycoprotein I in fluid phase and markedly improved binding when β_2-glycoprotein I is complexed to phospholipid micelles. In fact, assays which detect autoantibody interactions with β_2-glycoprotein I in the absence of phospholipid depend on the use of γ-irradiated polystyrene plates which, like phospholipid, provide a background surface for the antigen that contains a negative charge. Since there is no γ-irradiated plastic in the vasculature, it can be argued that the standard anticardiolipin assay, in which β_2-glycoprotein I is complexed to phospholipid, may provide a very similar, and more physiologic test for these autoantibodies than the β_2-glycoprotein I-specific test. Similar dependence on either phospholipid or negatively charged polystyrene has been described in the detection of antibodies with specificity for prothrombin [80].

Further evidence of the phospholipid-dependence of β_2-glycoprotein I antibodies has been provided by Hunt and Krilis, who identified a truncated form of β_2-glycoprotein I which was clipped at Lys317/Thr318, a potential thrombin cleavage site. Autoantibodies from patients with autoimmune disease were found to be unreactive with this shortened protein, and did not adhere to cardiolipin [81, 82]. Subsequently, the same group identified a region in the fifth domain of β_2-glycoprotein I which contains both the phospholipid binding site and a region recognized by anti-cardiolipin antibodies [82].

Phospholipids can play critical roles in the detection of autoantibodies which are specific for lipid-binding proteins in two ways: either by modifying the environment to allow increased density of antigen or by modifying the antigen to promote a favorable conformation for antibody binding. In the first case, the improved detection of antibody in the context of a phospholipid environment may or may not be related to the pathogenicity of the antibody. In the second case, it seems more likely that the conformation selecting for pathologic antibodies is related to an important functional conformation of the molecule.

Roubey *et al.* have demonstrated that improved detection of antibodies to β_2-glycoprotein I using oxidized polystyrene plates may be associated with increased density of β_2-glycoprotein I that seems to pack more efficiently on a negatively charged surface, providing a better template for bivalent antibody interactions [79]. In similar studies using prothrombin, Galli *et al*. found increased detection of antibodies to prothrombin when the background was phosphatidylserine instead of oxidized plastic, and in this case the difference did not appear to be due to increased antigen density [80]. Pierangeli *et al*. have furthermore observed that phospholipids may be critical to the lupus anticoagulant function of anti-prothrombin antibodies [66].

It has been suggested by the molecular studies of Ichikawa and co-workers that β_2-glycoprotein I exposes an otherwise cryptic epitope in the presence of phospholipid [83, 84]. Additional evidence for phospholipid-induced modulation of β_2-glycoprotein I conformation was provided by spectroscopic studies. Cardiolipin, which forms a hexagonal crystal lattice in both anhydrous and aqueous environments and β_2-glycoprotein I, which contains 46% β-pleated sheet structure in its purified form, are both significantly altered when bound together. In the case of β_2-glycoprotein I, the β-pleated sheet organization decreases from 46 to 23% [85].

It seems apparent, then, that phospholipids may be intrinsic to the interactions of anticoagulant proteins and autoantibodies both by anchoring and increasing the surface density of at least some of these proteins and

by altering their conformation in ways that may be important both to their hemostatic or anticoagulant functions and to the ability of pathologic antibodies to bind them. It would follow that different phospholipid membrane environments, created by various disease states or degrees of immune activation, might have profound effects on the pathogenicity of anti-phospholipid antibodies. For example, the structure and length of fatty acid chains in phospholipids may play a critical role in the binding of human sera in an antiphospholipid ELISA, with preferential binding of autoantibodies to C18:1 phosphatidlyglycerol [38], and anti-phospholipid antibodies may have increased affinity for lysophosphatidylethanolamine as opposed to phosphatidylethanolamine [86]. A large literature suggesting complexity in the phospholipid preferences of clinical sera containing anti-phospholipid antibodies remains to be tied into the evolving recognition that many if not most of these antibodies are recognizing specific lipid-binding proteins in the context of these phospholipid specificities. As reviewed by Rauch and Janoff, preliminary data suggest that kininogens mediate the binding of antibodies to phosphatidylethanolamine, prothrombin, and/or annexin V may be the targets of anti-phosphatidylserine antibodies, and erythrocyte-binding antibodies may recognize a complex antigen involving phosphatidylcholine [87].

Anionic phospholipids, the most common favorable background to promote the binding of antiphospholipid sera, are normally absent from the extracellular surface of cell membranes, but do redistribute from the inner to the outer leaflets during either cell activation or in the early stages of programmed cell death (apoptosis) [88]. Anti-phospholipid antibodies have been found to bind specifically to apoptotic but not viable thymocytes in a β_2-glycoprotein I-dependent manner [89]. Furthermore, the exposed negatively charged phospholipids, such as phosphatidylserine are thought to be potent surface procoagulants, a phenomenon that is ameliorated by the phosphatidylserine-binding protein, annexin V, which has been found bound directly to the outer surface of apoptotic blebs [88].

The findings suggest that the controversy surrounding whether anti-phospholipid antibodies recognize only phospholipid-binding proteins, or sometimes bind to phospholipid alone or a complex antigen may be spurious. *In vivo*, coagulation-regulating proteins are in close association with phospholipids and they may be tightly complexed during hemostatic events. By targeting either phospholipid or the proximate cofactor proteins, heterogeneous antibodies may interfere with the roles of both in coagulation.

Pathogenic Mechanism for the Antiphospholipid Syndrome: Autoantibodies Interfere with Protein Regulators of Hemostasis, Presented to the Immune System on a Phospholipid Template, Which Itself May Be Activated to Contribute to a Procoagulant State

Endothelial Cell Membranes as a Phospholipid Template and a Procoagulant Surface

Sera containing anti-phospholipid antibodies appear to have a high incidence of reactivity with endothelial cells [90–95]. In one study, 47 of 76 anti-phospholipid antibody positive samples bound endothelial cells and this was significantly more frequent in sera from patients with a history of thrombosis than in those without thrombotic history [90]. In a different experiment 23 of 43 SLE patients had anti-phospholipid antibodies and a higher prevalence of endothelium-reactive antibodies [95]. Similarly when 15 patients with antiphospholipid syndrome were compared to 15 with SLE and no thrombotic history, 13 (87%) of the sera from patients with antiphospholipid syndrome had endothelial cell antibodies and only 7 (47%) with SLE alone reacted with endothelial cells [92]. Anti-endothelial cell antibodies were also detected in 20 of 30 patients (67%) with anti-phospholipid antibodies and associated thrombotic history, but in none of 30 patients with thrombosis but no associated anti-phospholipid antibodies [91]. In the latter study there was also no association found between endothelial-binding antibodies and other autoantibodies including ANA, anti-dsDNA and anti-neutrophilic cytoplasmic antibodies.

Some data have suggested the hypothesis, as formulated by Meroni *et al*. [25], that a significant proportion of antibodies binding endothelial cells are actually anti-β_2 glycoprotein I antibodies [92–94]. Monoclonal anti-β_2 glycoprotein I antibodies have been found to bind endothelial cells [93, 94] in a β_2 glycoprotein I-dependent manner [94], and this was associated with increased IL6, adhesion molecule upregulation, and secretion of 6 keto-PGF1α, suggesting activation of endothelium by antibodies in a direction likely to contribute to a procoagulant state.

Other evidence of endothelial activation by endothelium-binding autoantibodies includes increased release of von Willebrands factor [90, 95], higher levels of prothrombin fragments and tissue type plasminogen activator [95], and the observation that membrane lipid turnover may be triggered by these antibodies such that anionic phospholipids are detectable on the surface of the bilayer [93].

β_2-Glycoprotein I is probably not the only endothelium-associated antigen targeted by anti-phospholipid antibodies. A 70-kDa protein has been immune-precipitated from endothelial cells by endothelium-reactive IgG fractions of 5 of 6 patients with anti-phospholipid antibodies [90]. There have been many reports of interference by anti-phospholipid antibodies with anticoagulant proteins that act by binding phospholipids on the surface of endothelial cells *in vivo* [9]. For example, anti-phospholipid antibodies (or plasma containing lupus anticoagulants) have demonstrated effects on the anticoagulant activity of protein C. This has been confirmed using lupus anticoagulant fractions from patients with APLA by a number of laboratories, studying the formation of the thrombin–thrombomodulin complex as well as protein C function using a chromogenic substrate and an assay of factor Va degradation [15, 56–61]. Amer *et al*. demonstrated that one anticoagulant could interfere with an activated protein C-mediated clotting time assay without inhibiting protein C amidolytic function [58]. Lo *et al*. [62] demonstrated inhibition of the anticoagulant activity of activated protein C using plasma from 20/21 patients with APLA. Marciniak and Romond [56] found decreased rates of protein C-mediated factor Va degradation in 15 plasma samples from patients with lupus anticoagulants as compared to controls, and furthermore plasma with LA activity decreased or abolished the factor Va degradation when mixed with normal plasma. All patients in this study were receiving no immunosuppressive or anticoagulant therapy and there were no acute thrombotic episodes. This suggests that the inhibition of protein C activity is a chronic function of these antibodies, occurring in clinically compensated patients, and not necessarily related to the events which lead to acute thrombosis. On the other hand, a chronic deficit in protein C function might still be a predisposing factor for thrombosis which then increases in importance when compensatory mechanisms fail.

Endothelial-Associated Cells as Phospholipid Templates and Procoagulant Factors

Other endothelium-associated structures may play a role as phospholipid-containing templates. Simantov *et al*. found that IgG from patients with anti-phospholipid antibodies increased monocyte adhesion to endothelial cells, dependent on β_2-glycoprotein I but not LPS, Fc interactions with monocyte receptors, or immune complexes [75]. Production of tissue factor by monocytes, a measurement of procoagulant activity, may be induced by anti-phospholipid antibodies or lupus anticoagulants [20, 21]. Pathology of placentas in patients with antiphospholipid-associated fetal loss suggests thrombosis of uteroplacental arteries as well as ischemic damage to placental villi [96]. Annexin V, a phospholipid-binding protein with potent anticoagulant effects has been found to be markedly reduced on placental villi in these patients, and appeared to be decreased in cultured trophoblast cells or endothelial cells in the presence of anti-phospholipid antibodies [97].

Platelets as Phospholipid Templates and Regulators of Hemostatsis

Anti-phospholipid antibodies are known to bind to platelet membrane structures [24, 98–102] and this may result in either thrombocytopenia or hemostatic activation [24]. An association between anti-phospholipid antibodies and immune-mediated thrombocytopenia (ITP) has also been established [98]. In ITP, antibodies to phospholipid membrane-bound glycoproteins have been pathogenically linked to the development of thrombocytopenia, and in one study, 27 of 68 sera with anti-phospholipid antibodies were also found to react to the same glycoproteins [98]. In a different report, anti-platelet glycoprotein antibodies were detected in 15 of 22 plasmas containing lupus anticoagulant activitiy [99]. Antibodies to platelet glycoproteins do not appear to select patients with a history of thrombosis, although there does seem to be a significant correlation with thrombocytopenia [98, 99].

Activation of platelets by a thrombotic stimulus causes a turnover in membrane phospholipids to expose anionic phospholipids on the surface. β_2-glycoprotein I binds preferentially to activated platelets, and β_2-glycoprotein I-dependent anti-phospholipid antibodies have been found to increase their adhesion to platelet surfaces sixfold when the platelets are activated with thrombin and a calcium ionophore [100]. β_2-glycoprotein I also enhances thromboxane A2 biosynthesis by platelets in response to anti-phospholipid antibodies [101], suggesting an additional procoagulant role for the antigen–antibody complex.

Thrombocytopenia occurs in 20–40% of patients with the antiphospholipid syndrome [103] but severe thrombocytopenia and hemmorhage are relatively rare. The actual incidence of platelet function abnormalities in association with anti-phospholipid antibodies is unknown, but decreased platelet aggregation has been observed at our institution on several occasions (J. T. Merrill, unpublished data). Conversely, one investigation of 21 lupus anticoagulant positive samples noted accelerated platelet aggregation by a calcium independant microscopic assay (not using a standardized platelet aggregometer) [104]. It may be that polyclonal antibodies with proaggregation and antiaggregation effects on platelets cancel each other out. Interestingly,

according to data compiled by the Italian Registry of Antiphospholipid Antibodies [31], the rate of clinical thrombosis is significantly decreased in patients with severe thrombocytopenia, although not in those with mild reductions in platelet counts.

Other Phospholipid-Rich Structures as Phospholipid Templates: Cholesterol Transport Vesicles Support an Atherogenic Mechanism for Anti-phospholipid Antibodies

Monoclonal anti-cardiolipin antibodies from the NZW/BXSB mouse model of antiphospholipid syndrome, have been found to cross-react with oxidized low-density lipoprotein (LDL), and a significant correlation was found between binding to cardiolipin and to oxidized LDL [26]. Oxidized LDL may be important in atherogenesis as a mediator of foam cell formation and cell cytotoxicity. Shoenfeld's group has studied the ability of LDL to increase experimentally induced antiphospholipid syndrome in mice. In this model, mice develop an antiphospholipid-like syndrome after receiving an intradermal injection of anti-cardiolipin antibodies [105]. Mice which were subsequently infused with oxidized LDL developed a more severe clinical picture, consistant with worsening antiphospholipid syndrome, including decreased platelet couts, prolonged PTT, and higer fetal resorption rates.

Low levels of apolipoprotein A1, the major protein constituent of high-density lipoprotein [106–108] is also associated with increased risk for atherosclerosis. Accelerated cerebrovascular and heart disease is a complication of systemic lupus, and Lahita *et al*. reported low levels of apolipoprotein A1 in lupus patients, correlated with the presence of anti-phospholipid antibodies [109]. We subsequently isolated a gene for apolipoprotein A1 from a mouse cDNA library screened with serum from a lupus patient who had a CVA [28]. Antibodies against plasma lipoproteins have previously been reported in patients with immune diseases [110–112]. Since natural antibodies binding specifically to apolipoprotein A1 had been described in a murine model [113], attempts were made to identify similar autoantibodies in patients with SLE using standard ELISA and Western blot techniques, but these were at first unsuccessful. Others had observed that apolipoprotein A1 has markedly altered epitope expression in different aqueous and charged phospholipid environments [114–120]. Accordingly, γ-irradiated, oxidized ELISA plates, such as those that were successful in identifying antibodies to β_2-glycoprotein I, were used as an alternative antigen-binding surface, and high-titer antibodies were then identified in the sera of 5/30 patients with lupus [28]. We next collected 520 samples during a 3-year period from 175 patients, including 114 with American College of Rheumatology (ACR) criteria for SLE and 35 with primary antiphospholipid syndrome. These were tested for antibodies to apolipoprotein A1 and positive sera were retested for binding to apolipoprotein incorporated into reconstructed nascent or mature high-density lipoprotein (HDL).

Autoantibodies specific for apolipoprotein A1 were found in 32.5% of patients with SLE and 22.9% of patients with primary antiphospholipid syndrome, associated with concomitant presence of anti-phospholipid (anti-β_2 glycoprotein-I) antibodies [121]. When reconstructed nascent and mature HDL molecules were compared as antigen-containing environments, positive sera reacted best to apolipoprotein A1 embedded in mature HDL molecules. These data confirmed a high prevalence of specific antibodies to apolipoprotein A1 in patients with systemic lupus, and suggest a relatively high affinity of these antibodies for apolipoprotein A1 in mature HDL-like proteoliposomes. Furthermore, there appears to be some at least circumstantial association between the presence of anti-apolipoprotein A1 antibodies and anti-phospholipid antibodies, as suggested both by the relatively high prevalence of anti-apolipoprotein A1 in patients with primary antiphospholipid syndrome and the fact that the majority of patients with anti-apolipoprotein A1 also have anti-phospholipid antibodies.

It can be speculated that an unchecked autoimmune process might give rise to polyclonal antibodies with evolving specificities that move from one protein target to another along a continuous, negatively charged phospholipid surface in a manner similar to the antibody spreading which has been described in several animal models [122, 123]. It is interesting, therefore, that autoantibodies specific both for delipidated β_2 glycoprotein-I and delipidated apolipoprotein A1 are best detected by the use of oxidized, γ-irradiated plates which mimic a negatively charged phospholipid environment.

As has been found with β_2-glycoprotein I, some evidence supports a model in which the most immunogenic epitopes of apolipoprotein A1 are exposed only in the phospholipid milieu of HDL. It is known that different regions of apolipoprotein A1 differ in antigenicity [124] and that apolipoprotein A1 has altered epitope expression in aqueous and charged phospholipid environments [114–120]. Curtiss and Smith [125] described a region at residues 90–105 of apolipoprotein A1 exposed when the protein was incorporated into HDL, but not in aqueous phase. Banka *et al*. [126] found two monoclonal antibodies, derived from immunization of mice with HDL, that were directed against an overlapping peptide of this same region of apolipo-

protein A1 and interfered with its function as the LCAT cofactor.

Phospholipids, then, may play a key role in exposure of cryptic immunogenic and functional epitopes of apolipoprotein A1, which may have relevance both to *in vivo* movement of cholesterol and during the detection of autoantibodies *in vitro* [115–120, 126].

Endothelial activation, increased monocyte adhesion (see previous section) as well as antibodies to oxidized LDL and to apolipoprotein A1 have now been associated with the antiphospholipid syndrome. Taken together these data support the hypothesis that autoantibodies arising in association with anti-phospholipid antibodies may contribute to a wider spectrum of intravascular dysfunction than those inducing the sudden, dramatic thrombosis that characterizes the antiphospholipid syndrome. This may help to explain the reported associations of anti-phospholipid antibodies and myocardial infarction [45, 127] or the premature atherosclerosis that has been described in patients with SLE.

Given the known importance of phospholipids to the function of coagulation proteins, and given these insights into the role of phospholipids in promoting structural modifications to β_2-glycoprotein I and apolipoprotein A1, it can be appreciated that phospholipid may have a widespread, profound role in hemostatic regulation, both functionally and immunologically.

There Are Many Autoantibodies, but Few Are Thrombotic

Antibody spreading has been defined as a natural consequence of the humoral immune response to autoantigens [122, 123]. Given the demonstration that autoantibody specificities can and do spread over associated structures on contiguous surfaces, it seems logical that in a dysregulated autoimmune state such as SLE, anti-phospholipid antibodies might similarly develop unchecked and spread their targets over a wide range of closely associated lipid-binding proteins on interacting phospholipid surfaces of endothelial cells, platelets, and circulating lipoproteins. It would then follow that the culmination of such an evolution might be complete dysregulation of the coagulation mechanism, such as is seen in the minority of patients who develop the malignant antiphospholipid syndrome.

A workable disease model for this syndrome, however, must take into account the apparent contradiction between the wide scope of the antibodies, their comprehensive capabilities for doing harm, and the seemingly unpredictable, sporadic nature of the coagulopathy in most patients. The disease model should furthermore aim to explain the intermittent nature of the thrombosis in a manner that might lead to better predictive testing, and/or provide a strategy to spare therapy for those who do not need it.

Phospholipids Tie Together a Complex Disease Model

Figure 2 demonstrates that it may be possible to develop a simplified, combined model which integrates the concept of phospholipid–protein modulation with the spreading of autoantibodies to develop fine specificities for disparate hemostasis-regulating proteins embedded in endothelial, platelet, and cholesterol transport membranes along continuous phospholipid structures. The net pathogenicity of the antibodies, whether in the short term (hypercoagulable state) or long term (atherosclerosis) would depend on multiple factors. These include the functionality of the specific proteins involved, the epitope(s) bound by specific antibodies, antibody avidity and affinity, which in turn may be influenced by the local density of antigen and antibody or the structure and net charge of the phospholipid environment in which a given antigen is embedded. Finally, both the immune and coagulation systems are finely tuned mechanisms containing many checks and balances. It seems intuitively obvious that pathologic thrombosis results only when multiple dominoes have fallen. In order to differentiate between the low grade prethrombotic state that may chronically exist in this illness and factors which predict risk for acute life-threatening events, it seems logical to focus on clinical clues suggesting risk for sudden collapse in some critical aspect of coagulation feedback regulation.

Pathogenesis of the Antiphospholipid Syndrome: Clues from the Association with SLE. The Coagulopathy Is Sporadic and SLE Is a Disease Characterized By Sporadic Complement Activity

Optimally a disease model would also shed light on various unexplained clinical features of the antiphospholipid syndrome, such as its link to SLE and the fact that in some patients there is a link between thrombotic events and SLE flare [43], in other patients, a link only to pregnancy. In fact, these unanswered questions can be considered as clues to the pathogenesis of the antiphospholipid syndrome, and will be used to develop a new model for thrombosis which specifically addresses them. Although it is likely that the antiphospholipid syndrome is, even in its simplest construct, multifactorial, the current focus will be on a potential coagulation mechanism which is of particular interest because it

involves the most prevalent antiphospholipid antigen, β_2-glycoprotein I, because it can address each of the questions listed earlier, and because there has been increasing clinical evidence in support of this model in human patients.

The coagulation system is recognized as a finely tuned network of checks and balances. APLA may persist for a long time prior to a clotting event. Because the clinical syndrome occurs intermittently, it seems likely that critical components of the coagulation disorder itself are sporadic. In patients in whom antibodies are persistent, but thrombosis is sporadic, acute thrombotic events may be the result of factors which arise intermittently to impair regulatory elements. Under normal circumstances, such regulatory elements might successfully compensate for additional, more chronic deficits induced by anti-phospholipid antibodies. At the time of a thrombotic event, these compensatory mechanisms might fail to operate because the checks and balances of the coagulation cascade become transiently impaired. This model is consistent with the understanding that the coagulation system exists in a complex dynamic equilibrium with internal controls against dysregulation.

Coagulopathy associated with anti-phospholipid antibodies may occur in the presence or absence of systemic lupus erythematosus, but the striking association between SLE and APLA syndrome (APLAS) should be taken into account in considering the pathogenesis of either the primary or secondary syndrome. Since SLE is characterized by intermittent activation and dysregulation of the complement system, it is tempting to speculate that a complement disorder underlies the acute coagulopathy and that this is even a factor which connects lupus and nonlupus patients with antiphospholipid syndrome. In this model, a major clotting event would only occur in the context of events which led to concomitant activation of both complement and coagulation. In the case of a lupus patient this may be a systemic dysregulation of complement activity. In the case of a woman with no disorder other than recurrent fetal loss, it might be a more local problem occurring in the specialized context of the pregnant state in the altered intravascular milieu of the uteroplacental arteries.

Complement activation has been reported in association with the antiphospholipid syndrome and may independently induce hypercoagulability as well [43–49]. Complement activation has been associated with acute manifestations of the antiphospholipid syndrome both in at least one convincing animal study and in human subjects, clinically [43, 44, 46] and with *in vitro* procoagulant activities of anti-phospholipid antibodies [102]. In one study, a direct association of acute antiphospholipid syndrome and complement activation was detected, when the complement membrane attack complex (Sc5b-9) was measured in 26 patients with either TIA or complete cerebrovascular accident. Nine of 13 anti-phospholipid antibody positive patients had abnormal complement levels as opposed to only 1 of 13 anti-phospholipid negative patients with brain ischemia ($p = 0.0018$) [44]. We found elevated complement split products, including C4D, in 6/6 patients with SLE, APLA, and recent thromboembolism (J. T. Merrill, unpublished data). In light of this, it is of particular interest that a logical link between the complement and coagulation systems has more recently been associated with acute manifestations of the antiphospholipid syndrome (see later).

C4b-Binding Protein: A link between Protein C, Its Cofactor Protein S, and the Complement System

Activity of the key anticoagulant feedback modulator protein C, can be inhibited *in vitro* by anti-phospholipid antibodies [59–61]. On the other hand, protein C activity can be enhanced 10-fold by normal plasma levels of its cofactor, protein S. A dynamic equilibrium in the amount of circulating, active protein S may be a critical regulator of the activity and anticoagulant functions of protein C, serving as a final modulation loop in a complex chain of coagulation checks and balances.

Reports of a transient and reversible acquired protein S deficiency at the time of thrombosis in patients with anti-phospholipid antibodies are therefore particularly interesting [128–134]. In several studies, acquired free protein S deficiency was associated with both anti-phospholipid antibodies and procoagulant laboratory measures in patients with SLE and/or the antiphospholipid syndrome [21, 131, 135–137]. On the other hand, some attempts to correlate more chronic deficits of protein S function with the presence of anti-phospholipid antibodies and/or a distant history of clotting events have resulted in either no association or changes with questionable clinical significance [138, 139]. It should be stressed that the phenomenon being sought could either be transient, occurring only at the time of a clotting event, or local, occurring only at the vascular site where the occlusion occurs, and therefore not measurable in a peripheral venous blood sample.

Functional deficiency of free protein S results from enhanced binding of protein S by its circulating plasma inhibitor, C4BP [128]. Given the association of the antiphospholipid syndrome with systemic lupus it is intriguing that C4BP is also a suppressor of the complement system [140, 141]. Each C4BP molecules may

contain up to seven complement-binding subunits and only one shorter arm or β chain which binds and inactivates protein S. It is apparent that C4BP circulates in two forms, a high molecular weight form, containing the protein S-binding β chain (β+) and a lower molecular weight form without this protein-S binding subunit (β–). Levels of total C4BP have been documented to rise in acute phase responses, and more specifically in SLE flare [141]. One study suggests that most of the increase in C4BP is made up of (β–) isoforms, and that in a controlled acute phase response, protein S rises with molar equivalence to the (β+) form [142], essentially providing compensation for the complement activation and allowing normal levels of free protein S to persist. It is unclear how applicable these data are to severe inflammatory states since the average increase in total C4BP in that study was only to 162% and in some acute phase responses levels may rise to 400% [142]. More importantly it has been well documented that in some inflammatory conditions, including acute phase responses, sepsis, disseminated intravascular coagulation (DIC) and systemic lupus, and in specialized states such as pregnancy or during the use of hormonal therapies, free protein S levels do decrease, relative to an increase in C4BP [128–130, 141, 143–147]. One study of 24 patients with severe infection found that the free protein S was significantly decreased relative to an increase of C4BP with evidence of hypercoagulability as well [144]. This kind of functional protein S deficiency is usually transient, but is associated with risk for thrombosis [128].

We hypothesize that a unique disturbance of the normal checks and balances of the complement system occurs in patients with the antiphospholipid syndrome and alters the binding equilibrium between C4BP and protein S. This may become a particular problem at a critical juncture of a lupus flare or in the special environment of the uteroplacental interface in certain pregnant women. In fact, in normal pregnancy, complement deposition reflective of classic pathway activation is found locally in the uteroplacental arteries [148–151]. Furthermore, protein S declines and C4BP levels increase reaching a peak in the second trimester when abortion is most frequent in the antiphospholipid syndrome [152, 153].

Some evidence suggests the possibility of a circulating plasma factor which regulates and opposes the inhibitory binding of protein S by C4BP [154]. Clinical support for this hypothesis was provided by one description of a patient who was found to have abnormally increased binding of protein S by C4BP in the presence of calcium [155]. This was corrected by the addition of normal human plasma, strongly suggesting that the patient had a deficiency of a plasma constituent which inhibits calcium dependent association of protein S with its inhibitor. Intriguingly, one study of 73 patients with anti-phospholipid antibodies found deficiencies of free protein S but not increased C4BP in patients with lupus anticoagulants, indicating abnormal uptake of protein S by C4BP which was unrelated to its plasma levels [135]. This might be explained by interference of these antibodies with a normal plasma regulator of protein S–C4BP binding.

As discussed earlier, the phospholipid-binding anticoagulant protein β_2-glycoprotein I is a likely candidate as a primary antigen for pathologic anti-phospholipid antibodies [70]. The structure of β_2-glycoprotein I is that of repetitive sushi domains, homologous to members of the complement control superfamily, including consensus sequences strongly homologous with C4BP [156]. Antibodies to β_2-glycoprotein I have been found to inhibit factor Va degradation by activated protein C and its cofactor protein S, suggesting a role for β_2-glycoprotein I in promoting the protein C–protein S anticoagulant function [157]. In a preliminary report, Walker found that β_2 glycoprotein-I interferes with the binding of protein S to C4BP [158], although the functional significance of this binding in fluid phase plasma was not known. Based on these data, we undertook experiments to determine whether β_2-glycoprotein I interferes with binding and functional inhibition of protein S by C4BP [78, 159]. We found that fluid phase β_2-glycoprotein I interferes with capture of protein S by immobilized C4BP and that β_2-glycoprotein I reverses inhibition of protein S anticoagulant function by C4BP in a dose-responsive manner. This effect was reversed, in an epitope specific manner by one of four monoclonal antibodies specific for human β_2-glycoprotein I [159]. Subsequently, after we and others found a high prevalence of antibodies specific for protein S in patients with lupus and/or the antiphospholipid syndrome [162–164], we found that dual reactivity of antibodies to both protein S and β_2-glycoprotein I described an interesting subset of patients with a strong correlation to a history of thrombotic events [164]. We and others have also noted an anecdotal relationship between free protein S deficiency and the presence of these autoantibodies [162, 164]

Taken together, these data suggest a model to explain at least a key subset of sporadic thrombosis associated with anti-phospholipid antibodies. Anti-β_2-glycoprotein I antibodies interfere with its ability to counter the inhibition of protein S by C4BP. Antibodies to protein S may impair this relationship in a synergistic manner. This chronic imbalance is normally compensated by a controlled release of different C4BP subunits. Alteration of systemic or local complement equilibrium in lupus, pregnancy, or other as yet undefined physiologic

states leads to excess C4BP containing protein S-binding subunits and/or because of antibody interference with β_2-glycoprotein I, a functional protein S deficiency is either initiated or worsens to a critical point, resulting in thrombosis.

In this model, even though complement activation might be a local vascular phenomenon, increased circulating total C4BP and/or β_2-glycoprotein I might be identifiable and measurable in patients. Problems which may arise in their detection include the possibility that antibodies to β_2-glycoprotein I may quickly remove it from the circulation and thereby decrease its apparent concentration and the finding that the degree of protein S binding to C4BP may be in delicate balance depending on the medium in which it is measured, in particular the calcium concentration [160, 161]. This may apply to the binding and measurement of β_2-glycoprotein as well. Further studies of these relationships are ongoing in a number of laboratories and it can be hoped that with further work in this area, greater insight into the acute pathogenesis of the antiphospholipid syndrome may emerge.

IMPLICATIONS FOR LUPUS

Pathophysiologic Implications for Lupus

What do insights about anti-phospholipid antibody pathophysiology contribute to our understanding of lupus? This intriguing question can be approached from the point of view of what is special about these antibodies. Unlike most autoantibodies, the antigenic targets of anti-phospholipid antibodies are not intracellular proteins. The targets themselves are ubiquitous bloodstream proteins that would be considered normally accessible to immune surveillance. Given this indisputable fact, the mechanistic evidence reviewed earlier provides a unique kind of circumstantial confirmation of two important hypotheses that have emerged in the study of lupus pathophysiology: (1) autoantibody spreading (2) the existence of cryptic epitopes. Circumstantial support for the former is provided by the fact that once patients develop autoantibodies to one, relatively accessible bloodstream protein, the immune response would be more likely to spread to a ligand for that protein (complementary structure) than to an unrelated structure. We have confirmed a circumstantial association between antibodies to protein S and its ligand, β_2-glycoprotein I but not to prothrombin, another antiphospholipid target, which is consistent with this model [164]. Direct evidence that there are cryptic epitopes for phospholipid-binding proteins have also been reviewed previously [84, 85, 120, 121]. Circumstantial evidence in support of this model is provided by the fact that patients only develop pathologic thromboses sporadically and intermittantly. If antigenic determinants were freely accessible in their most avid form at all times, it would seem less likely that this would be the case.

Clinical Implications for Lupus: Integrating an Evolving Pathophysiologic Model into Current (Albeit Limited) Approaches to Predicting Thrombotic Risk for Patients

A better understanding of pathologic anti-phospholipid antibodies and the epitopes they bind may someday lead to safer and more specific therapies for this disorder. However, the current standard of care is simply to recognize the association between these antibodies and recurrent thrombosis and to initiate anticoagulation therapy after a serious thrombotic risk has been confirmed. Given the limited therapeutic options at this time, and the limited scope of predictive testing despite new insights into likely pathogenic models, therapeutic issues primarily revolve around when and for whom to initiate global anticoagulant treatment and how aggressive the treatment should be. Critical to these decisions is the ability to utilize the available tests wisely in determining an accurate diagnosis and establishing thrombotic risk to the patient. Since pathologic epitopes, functional studies for potential thrombotic risk, are neither widely available nor clinically validated, we must rely on a less well-integrated mix of clinical clues which may be, at best, indirect markers for patients at risk. However, by keeping the concepts discussed previously in mind, and reviewing the clinical risk factors that have been more or less validated to date, there seems to be compelling reason at this time not to rely today on a single test at a single time point or a single, relatively minor event to rule the diagnosis of this syndrome in or out, or in predicting future risk for a given patient.

Clinical Predictors of Outcome

Assays to Detect Anti-phospholipid Antibodies: What to Order and How to Interpret It

Despite laudable international efforts to introduce conformity into both ELISA and lupus anticoagulant assays, current widely available tests to detect antiphospholipid antibodies are not well standardized. Nevertheless the published literature has relied almost completely on these assays as the only means of distinguishing antiphospholipid patients, and it is this literature upon which current understanding of the epidemiologic features of the disorder rests.

The association of thrombotic risk with high titer IgG anti-cardiolipin or anti-phoshpholipid antibodies, especially if combined with a positive assay for lupus anticoagulant, has been confirmed in several different studies [7, 164–167]. In one 4-year follow-up of 321 women with anti-phospholipid antibodies, patients with IgM or lower level IgG anti-cardiolipin antibodies were not at risk for antiphospholipid-related disorders [166]. However, 12 of 129 patients who had either IgM or low IgG at the start of this study, developed higher levels of IgG or lupus anticoagulants during the 4-year period. Of these, half experienced at least one thrombotic complication [166]. This indicates that lupus patients or other patients at risk should be retested for anti-phospholipid antibodies over time if negative, particularly if they develop new or recurrent clinical symptoms suggestive of the antiphospholpid syndrome.

This brings up the question of whether it makes sense to test each patient by multiple techniques to increase the sensitivity of these assays, as has been suggested repeatedly in the literature [34, 80, 168–171]. In one study of 1513 sera from 399 patients [169], 60% of the samples containing anti-phospholipid antibodies reacted to phospholipids other than cardiolipin, the most commonly used phospholipid in commercial assays. Considering only the more pathologic IgG subtypes, reactivity to phosphatidylserine was more prevalent than reactivity to cardiolipin. Reactivity to phosphatidylethoanolamine was also relatively common [169]. In another report of 141 sera tested using both anionic and zwitterionic phospholipids 79 phospholipid-reactive sera were found. Of these 11 reacted with noncardiolipin phospholipid, of which 7 patients had a history of ether recurrent fetal loss or thrombotic events [168]. This confirms the need to use more than just the anticardiolipin assay in obtaining a diagnosis for high risk patients. In one intriguing study, IgG from women with clinical features suggestive of the antiphospholipid syndrome but negative at one point in time in both anti-phospholipid antibodies and lupus anticoagulant assays, were tested in a murine passive immunization model, causing significantly more fetal loss in the mice than control IgG [170]. Either low titer anti-cardiolipin or anti-phosphatidylserine antibodies were subsequently found in some, but not all of these women.

It seems likely that the laboratory analysis of antiphospholipid antibodies will continue to evolve over the coming years. Bilayer phospholipids of varying composition may be used increasingly to provide a more physiologic substrate in the detection of these antibodies in combination with various lipid-binding proteins [172]. Given the multiple protein antigens now associated with the antiphospholipid syndrome, and the emergence of clinical tests to detect anti-β_2-glycoprotein I and antiprothrombin antibodies, assays to detect antibodies for a wider range of lipid-binding proteins such as protein S, thrombomodulin, tissue factor, or annexin V may also become available in the near future, although their ultimate usefulness as add on testing in whole protein assays remains to be determined. It is now understood that some lupus anticoagulants are directed against β_2-glycoprotein, some against prothrombin, and some antibodies against β_2-glycoprotein and prothrombin are not lupus anticoagulants [80]. Whether or not antibodies predictive of pathology can be definitively ruled in or out based on some combination of these assays remains to be determined, but it seems unlikely. Major advances in the development of specific and sensitive assays for the diagnosis of the antiphospholipid syndrome await more comprehensive understanding of the coagulation disorder(s) and the specific epitopes involved on the coagulation proteins that may be involved.

Some commercial antiphospholipid assays incorporate mixed phospholipids and β_2-glycoprotein into one ELISA. Since it is now thought that different phospholipid environments may differentially affect detection of anti-β_2-glycoprotein antibodies, it is not clear that the net effect of this mixture would increase the range or sensitivity of this assay. It seems logical, however, to use up to three different assays from the more easily available common testing methods first, such as an anticardiolipin and/or antiphosphatidylserine test plus lupus anticoagulant assays (here, too, sensitivity may be increased employing three different LA tests, including one employing hexagonal phase phospholipids). Where available, an anti-β_2-glycoprotein immunoassay should be employed using γ-irradiated ELISA plates, which, by eliminating phospholipid-specific antibodies associated with infections, may be more specific for the autoimmune syndrome. To further analyze the sera of patients for whom there is a high suspicion of this syndrome but the previously mentioned tests are negative, it might be worth obtaining a more comprehensive testing battery, particularly if a laboratory can be found capable of testing for antibodies to β_2-glycoprotein against different phospholipid backgrounds.

Positivity in a specific anti-β_2-glycoprotein I immunoassay has been more closely associated with the clinical manifestations of the antiphospholipid syndrome than positivity in conventional anticardiolipin ELISA [101, 173]. Unlike the anticardiolipin assay, there appears to be less chance of false-positive results for syphilis patients using the assay specific for β_2-glycoprotein I [16, 174]. This assay, which at any given point in time appears to be specific but not sensitive in detection of the antiphospholipid syndrome [101], has become commercially available.

Anti-phospholipid antibodies produced by syphilis patients can be distinguished from autoimmune anti-phospholipid antibodies by several methods. In one report, 20 SLE patients and 16 syphilis-infected patients with high titer IgG anti-phospholipid antibodies were further tested. Antibodies from syphilis patients had lower avidity, predominance of κ light chains, and IgG1 or IgG3 subclasses, whereas antibodies from lupus patients had higher avidity, λ light chains, and IgG2 or IgG4 subclasses [175]. In a different study, a similar finding distinguished sera reacting only to cardiolipin (predominance of IgG3 and IgG1) from those which reacted to anticardiolipin plus β_2-glycoprotein I (IgG2, λ light chain predominance) [52]. It therefore appears that syphilis patients are more likely to have true anti-phospholipid antibodies, further characterized by κ light chains and IgG1 or IgG3 subclasses. Autoimmune patients, especially those at risk for the antiphospholipid syndrome are more likely to have β_2-glycoprotein I-dependent anti-phospholipid antibodies, characterized by λ light chains, IgG2, or possibly IgG4. However, unlike syphilis-infected individuals and patients infected with other viruses, anti-phospholipid antibodies arising in the context of Parvovirus B19 exposure do not appear to be similarly distinguishable from autoimmune anti-phospholipid antibodies [176]. The clinical significance of this finding remains unclear.

It has not yet been possible to identify distinguishing characteristics of antibodies which can predict which patients with SLE and anti-phospholipid antibodies are at increased risk for thrombosis. For example, one attempt to differentiate anti-phospholipid antibodies from SLE patients with fetal death on the basis of isotype, subclass, lupus anticoagulant activity, phospholipid specificity, and avidity, found no associations [29].

In summary, the lack of standardization in the tests currently available to clinicians, coupled with evidence that patient results may change over time using these assays, indicates that patients at risk should be tested using more than one technique and retested over time if negative, particularly if they develop new or recurrent clinical symptoms suggestive of the antiphospholipid syndrome. It seems obvious, however, that to avoid excessive false-positive diagnoses, the use of more than one assay in the detection of the antiphospholipid syndrome should only be used for patients at apparent high clinical risk, and in the absence of other explanations for thrombosis. Multiple assays may not only increase the sensitivity of detection, but positive results on multiple assays can be considered confirmatory, and to some extent prognostic. For example, three assays positive for anti-phospholpid antibodies at conception has been associated with a major risk for obstetric complications [171].

The antiphospholipid syndrome was identified as a poor prognostic factor for survival in one cohort of 667 patients, and a stepwise cox multivariate analysis suggested that this was primarily attributable to either thrombocytopenia or arterial occlusions [177]. A different group of 360 patients with lupus anticoagulants with or without anti-phospholipid antibodies were followed in a 4-year prospective study. Thirty-four developed thrombotic complications, suggesting an overall incidence of 2.5%/patient year. Two independent risk factors identified were history of previous thrombosis or presence of an IgG anti-cardiolipin antibody with a binding strength of greater than 40 international units [178]. Of some concern, 4 patients in the original group of 360 developed non-Hodgkins lymphoma.

Other Considerations to Help Establish Thrombotic Risk

Immunologic tests such as the assays discussed earlier do not establish thrombotic risk for patients until some morbidity, usually a life-threatening thrombotic event, has occurred. There are a few additional tests which might indicate the likelihood of thrombotic risk prior to clinically obvious disease, but it has not as yet been determined whether these should be used either routinely in lupus patients with high titer anti-phospholipid antibodies or to initiate anticoagulation earlier than might occur under the current standard of care. In certain clinical situations these tests might also be helpful in establishing a diagnosis when the history suggests the possibility of thrombosis but remains equivocal.

Pregnancy Loss

Spontaneous abortion in the antiphospholipid syndrome is strongly associated with fetal loss, as opposed to earlier first trimester loss, but first trimester loss is also well described in this syndrome. In one study of patients with recurrent pregnancy loss [179], the specificity of fetal death for patients with anti-phospholipid antibodies was 76%. Conversely, the specificity of two or more early first trimester abortions for the ability to select patients with anti-phospholipid antibodies was 6%. Nevertheless, in the same study, although more than 80% of women with anti-phospholipid antibodies had at least one fetal death by history, only 50% of overall anti-phospholipid antibody-related abortion involved fetal death. This leaves a clinician in doubt about what to do in a second pregnancy when a lupus patient with high titer IgG anti-cardiolipin antibodies suffers a first trimester pregnancy loss. It is apparently not warranted to initiate heparin in a subsequent pregnancy on the basis of this insufficient evidence [180].

However, this is often a highly charged issue for patients, who request to know if anything more can be done diagnostically.

One strategy, if the loss is late enough and tissue available, is to have the placenta examined. Given the fact that villous infarction is rare in first trimester abortion, finding this clear-cut clue in the placenta from a patient with anti-phospholipid antibodies might alter the strategy for the next pregnancy [181]. One study also indicates that IgG anti-cardiolipin antibodies can be eluted from placentae of patients with the antiphospholipid syndrome but not from controls [182]. Not surprisingly, β_2-glycoprotein I is also found bound to both normal and control placentae, located in the syncytiotrophoblast [182].

There is evidence that placental pathology in the antiphospholipid syndrome is repeated in subsequent pregnancies [183]. Therefore, when antiphospholipid positive lupus patients with past early first trimester loss reach term or near term in a subsequent pregnancy, placental pathology should not be overlooked due to the happy outcome, especially with low birth weight infants. Similarly, when a subsequent pregnancy reaches the second trimester in a patient for whom there is some but not definitive suspicion of the antiphospholipid syndrome, fetal size should be closely monitored. One study also suggests that abnormal resistance of uterine arteries at 18–24 weeks gestation (measured by velocimetry) may predict pregnancies at increased risk for obstetric complications [171].

Cerebrovascular Monitoring

Optimally, patients with high titer IgG antiphospholipid antibodies and established transient ischemic attacks (TIAs) should be anticoagulated before serious cerebrovascular accidents occurred. However, in practice, TIAs may or may not be clear-cut from a patients description, if they are described at all. A thorough history is obviously of primary importance, but even so, there is a large gray zone between the description of fearful, but equivocal symptoms, and making a commitment to lifelong, or at least indefinitely long anticoagulation. CT or MRI neuroimaging findings in patients with possible TIAs may also be difficult to interpret. Results range from cerebral atrophy to focal infarcts, which appear to be fairly common and are of uncertain significance [184]. On the other hand angiography of these patients may demonstrate more dramatic abnormalities, including clear-cut stenosis and subclinical occlusions [184]. There is one report suggesting that single photon emission computerized tomography (SPECT) may be useful in demonstrating decreased cerebral blood flow in patients with the antiphospholipid syndrome [185], although its usefulness as a predictive test remains unclear. A relative decrease in total cerebral circulation and increased asymmetry of arterial blood flow has also been reported, using ultrasonic duplex scanning to distinguish between patients with SLE who did or did not have concomitant antiphospholipid syndrome [186]. Again, the usefulness of this interesting approach in predictive testing remains to be evaluated. Lockshin's group has found strong evidence for a cardioembolic etiology in antiphospholipid-related stroke [187], so the importance of obtaining an echocardiogram for patients with known or suspected TIA cannot be overstressed. In an additional study either anti-cardiolipin antibody or lupus anticoagulant were found in all 35 of 41 patients with Sneddon's syndrome (defined as cerebrovascular disease and livedo reticularis). Thirty-four percent of these patients had evidence of ischemic heart disease and 13/32 of these patients were found to have mitral valve thickening, again suggesting a link between the heart and the brain. Although arterial occlusion accounts for most reports of cerebrovascular accidents in the antiphospholipid syndrome, hemorrhagic venous infarction (in the context of dural sinus thrombosis) has also been described [188], stressing the importance of confirming a pure thrombotic event prior to initiating anticoagulation, even in patients with this syndrome.

Monitoring the Kidney

There is evidence to suggest that the kidney may be a more frequent target organ in the antiphospholipid syndrome than may have been previously appreciated [189]. Clinical manifestations may easily be confused with lupus nephritis, including proteinuria, hypertension, and/or acute renal failure. The pathology is clearly thrombotic, however, stressing the importance of renal biopsy in patients with SLE and apparent renal disease, since the first line of therapy for thrombotic kidney disease is probably anticoagulation rather than immune suppression.

Drug-Induced Anti-phospholipid Antibodies

Anti-phospholipid antibodies have now been found in association with medications known to induce lupus-like syndromes [190, 191]. Our laboratory found a high incidence of anti-phospholipid antibodies in patients taking procainamide, whether or not there was clinically evident drug-related lupus [190]. These antibodies have characteristics similar to autoimmune anti-phospholipid antibodies, as opposed to the infectious type [190, 191], including direct binding to β_2-glycoprotein I in the oxidized plate system [191]. It remains to be determined whether these antibodies increase risk for thrombosis in a population that may already be at high risk for heart and vascular disease.

The Epidemiology of Recurrence: Is Risk Predictable?

It is widely accepted that recurrent thrombosis in the antiphospholipid syndrome has a tendency to mimic the original thrombotic event, with arterial thrombosis following arterial occlusion and venous following venous [192]. Although clinical observation appears to follow these observations to a significant extent, the study cited involved only 20 patients, and none were followed during subsequent pregnancies. Clinical experience confirms the impression that thrombotic patterns tend to be repetitive to the point that some patients, followed for many years, specifically suffer recurrent deep venous thrombosis (DVT) or, alternatively, recurrent pregnancy loss, but no other sequelae of the syndrome. Several studies performed in an attempt to predict risk factors for pregnancy, however, confirm the intuitive impression that there are multifactorial outcomes in this multifactorial disease, citing, along with the known risks of high IgG anti-phospholipid antibodies and history of previous miscarriage, the additional factors of thrombocytopenia and/or other previous thrombotic events as predictors of adverse pregnancy outcome [31, 178, 193].

References

1. Hughes, G. R., Harris, E. N., and Gharavi, A. E. (1986). The anticardiolipin syndrome. *J. Rheumatol.* **13**, 486–489.
2. Alarcon-Segovia, D., Cardiel, M. H., and Reyes, E. (1989). Antiphospholipid arterial vasculopathy. *J. Rheumatol.* **16**, 762–767.
3. Boey, M. L., Colaco, C. B., Gharavi, A. E., *et al.* (1983). Thrombosis in systemic lupus erythematosus: Striking association with the presence of circulating lupus anticoagulant. *Br. Med. J.* **287**, 1021–1023.
4. Buchanen, R. C., Wardlow, J. R., and Riglar, A. (1989). Antiphospholipid antibodies in the connective tissue diseases: Their relation to the antiphospholipid syndrome and forme fruste disease. *J. Rheumatol.* **16**, 757–761.
5. Harris, E. N. (1989). Anticardiolipin antibodies and autoimmune diseases. *Curr. Opin. Rheumatol*. **1**, 215–220.
6. Hendra, T. J., Baguley, E., and Harris, E. N. (1989). Anticardiolipin antibody levels in diabetic subjects with and without coronary artery disease. *Postgrad. Med. J.* **65**, 140–143.
7. Harris, E. N. (1992). Serologic detection of antiphospholipid antibodies. *Stroke* **23**, SI3.
8. Bevers, E. M., Galli, M., Barbui, T., *et al.* (1991). Lupus anticoagulant IgG's [LA] are not directed to phospholipids only but to a complex of lipid-bound human prothrombin. *Thromb. Haemost*. **66**, 629–632.
9. Oosting, J. D., Derksen, R. H., Bobbink, I. W., Hackeng, T. M., Bouma, B. N., and de Groot, P. G. (1993). Antiphospholipid antibodies directed against a combination of phospholipids with prothrombin, protein C or protein S: An explanation for their pathogenic mechanism? *Blood* **81**, 2618–2625.
10. Oosting, J. D., Derksen, R. H. W. M., Entjes, T. I., *et al.* (1992). Lupus anticoagulant activity is frequently dependent on the presence of β2-glycoprotein-1. *Thromb. Haemost*. **67**, 499–502.
11. Triplett, D. A. (1992). Antiphospholipid antibodies: Proposed mechanisms of action. *Am. J. Reprod. Immunol*. **28**, 211–215.
12. Malia, R. G., Kitchen, S., Graves, M., and Preston, F. E. (1990). Inhibition of activated protein C and its cofactor protein S by antiphospholipid antibodies. *Br. J. Haematol.* **76**, 101–107.
13. Killeen, A. A., Meyer, K. C., Vogt, J. M., and Edson, J. R. (1987). Kallikrein inhibition and C1-esterase inhibitor levels in patients with the lupus inhibitor. *Am. J. Clin. Pathol.* **88**, 223–228.
14. Keeling, D. M., Campbell, S. J., and Mackie, I. J. (1991). The fibrinolytic response to venous occlusion and the natural anticoagulants in patients with antiphospholipid antibodies both with and without systemic lupus erythematosus. *Br. J. Haematol.* **77**, 354–357.
15. Triplett, D. A. (1993). Antiphospholipid antibodies and thrombosis. A consequence, coincidence or cause? *Arch. Pathol. Lab. Med*. **117**, 78–88.
16. Roubey, R. A. S., Pratt, C. W., Buyon, J. P., and Winfield, J. B. (1992). Lupus anticoagulant activity of autoimmune antiphospholipid antibodies is dependent on beta 2-glycoprotein 1. *J. Clin. Invest*. **90**, 1100–1104.
17. Shibata, S., Harpel, P. C., Gharavi, A., Rand, J., and Fillit, H. (1994). Autoantibodies to heparin from patients with antiphospholipid antibody syndrome inhibit formation of antithrombin III–thrombin complexes. *Blood* **83**, 2389–2391.
18. Fillit, H., Shibata, S., Sasaki, T., Spiera, H., Kerr, L. D., and Blake, M. (1993). Autoantibodies to the protein core of vascular basement membrane heparan sulfate proteoglycan in systemic lupus erythematosus. *Autoimmunity* **14**, 243–249.
19. Aron, A. L., Gharavi, A. E., and Shoenfelfd, Y. (1995). Mechanisms of action of antiphospholipid antibodies in the antiphospholipid syndrome. *Int. Arch. Allergy Immunol.* **106**, 8–12.
20. Martini, F., Farsi, A., Gori, A. M., Boddi, M., Fedi, S., Domeneghetti, M. P., Passaleva, A., Prisco, D., and Abbate, R. (1996). Antiphospholipid antibodies [aPL] increase the potential monocyte procoagulant activity in patients with systemic lupus erythematosus. *Lupus* **5**, 206–211.
21. Reverter, J. C., Tassies, D., Font, J., Monteagudo, J., Escolar, G., Ingelmo, M., and Ordinas, A. (1996). Hypercoagulable state in patients with antiphospholipid syndrome is related to high induced tissue factor expression on monocytes and to low free protein S. *Arterioscler. Thromb. Vasc. Biol*. **16**, 1319–1326.
22. Wisbey, H. L., and Klestov, A. C. (1996). Thrombocytopenia corrected by warfarin in antiphospholipid syndrome. *J. Rheumatol*. **23**, 769–771.

23. Barquinero, J., Ordi-Ros, J., Selva, A., Perez-Peman, P., Vilardell, M., and Khamashta, M. (1994). Antibodies against platelet-activating factor in patients with antiphospholipid antibodies. *Lupus* **3**, 55–58.
24. Machin, S. J. (1996). Platelets and antiphospholipid antibodies. *Lupus* **5**, 386–387.
25. Meroni, P. L., Papa, N. D., Beltrami, B., Tincani, A., Balestrieri, G., and Krilis, S. A. (1996). Modulation of endothelial cell function by antiphospholipid antibodies. *Lupus* **5**, 448–450.
26. Mizutani, H., Kurata, Y., Kosugi, S., Shiraga, M., Kashiwagi, H., Tomiyama, Y., Kanakura, Y., Good, R. A., and Matsuzawa, Y. (1995). Monoclonal anticardiolipin autoantibodies established from the [New Zealand white × BXSB]F1 mouse model of antiphospholipid syndrome cross-react with oxidized low-density lipoprotein. *Arthritis. Rheum*. **38**, 1382–1388.
27. Sorice, M., Arcieri, P., Griggi, T., Circella, A., Misasi, R., Lenti, L., Di Nucci, G. D., and Mariani, G. (1996). Inhibition of protein S by autoantibodies in patients with acquired protein S deficiency. *Thromb. Haemost*. **75**, 555–559.
28. Merrill, J. T., Rivkin, E., Shen, C., and Lahita, R. G. (1995). Selection of a gene for apolipoprotein A1 using autoantibodies from a patient with SLE. *Arthritis. Rheum*. **38**, 1655–1659.
29. Qamar, T., Levy, R. A., Sammaritano, L., Gharavi, A. E., and Lockshin, M. D. (1990). Characteristics of high-titer IgG antiphospholipid antibody in systemic lupus erythematosus patients with and without fetal death. *Arthritis. Rheum*. **33**, 501–504.
30. Rosove, M. H., and Brewer, P. M. (1992). Antiphospholipid thrombosis: Clinical course after the first thrombotic event in 70 patients. *Ann. Intern. Med*. **117**, 303–308.
31. Finazzi, G. (1997). The Italian Registry of Antiphospholipid Antibodies. *Haematologica* **82**, 101–105.
32. Vianna, J. L., Khamashta, M. A., Ordi-Ros, J., Font, J., Cervera, R., Lopez-Soto, A., Tolosa, C., Franz, J., Selva, A., and Ingelmo, M. (1994). Comparison of the primary and secondary antiphospholipid syndrome: A European multicenter study of 114 patients. *Am. J. Med*. **96**, 3–9.
33. Provenzale, J. M., Barboriak, D. P., Allen, N. B., and Ortel, T. L. (1996). Patients with antiphospholipid antibodies: CT and MR findings of the brain. *Am. J. Roentgenol*. **167**, 1573–1578.
34. Vivancos, J., Lopez-Soto, A., Font, J., Balasch, J., Cervera, R., Reverter, J. C., Carmona, F., and Ingelmo, M. (1994). Primary antiphospholipid syndrome: Clinical and biological study of 36 cases. *Med. Clin*. **102**, 561–565.
35. Mujic, F., Cuadrado, M. J., Lloyd, M., Khamashta, M. A., Page, G., and Hughes, G. R. (1995). Primary antiphospholipid syndrome evolving into systemic lupus erythematosus. *J. Rheumatol.* **22**, 1589–1592.
36. Derksen, R. H., Gmelig-Meijling, F. H., and de Groot, P. G. (1996). Primary antiphospholipid syndrome evolving into systemic lupus erythematosus. *Lupus* **5**, 77–80.
37. Aron, A. L., Cuellar, M. L., Brey, R. L., Mckeown, S., Espinoza, L. R., Shoenfeld, Y., and Gharavi, A. E. (1995). Early onset of autoimmunity in MRL/++ mice following immunization with beta 2 glycoprotein I. *Clin. Exp. Immunol*. **101**, 78–81.
38. Shibata, S., Harpel, P., Bona, C., and Fillit, H. (1993). Monoclonal antibodies to heparan sulfate inhibit the formation of thrombin–antithrombin III compexes. *Clin. Immunol. Immunopathol*. **67**, 264–272.
39. Villarreal, G. M., Alarcon-Segovia, D., Villa, A. R., Cabral, A. R., and Shoenfeld, Y. (1991). Presence of a 16/6 related human anti-DNA common idiotype (SA1) in the anticardiolipin antibodies of patients with primary antiphospholipid syndrome. *J. Rheumatol.* **18**, 1537–1541.
40. Sebastiani, G. D., Galeazzi, M., Morozzi, G., and Marcolongo, R. (1996). The immunogenetics of the antiphospholipid syndrome, anticardiolipin antibodies, and lupus anticoagulant. *Semin. Arthritis. Rheum*. **25**, 414–420.
41. Wilson, W. A. (1996). Histocompatibility genes in antiphospholipid syndrome. *Lupus* **5**, 259–262.
42. Sebastiani, G. D., Galeazzi, M., Morozzi, G., and Marcolongo, R. (1996). The immunogenetics of the antiphospholipid syndrome, anticardiolipin antibodies and lupus anticoagulant (review). *Semin. Arthritis. Rheum*. **25**, 414–420.
43. Asherson, R. A., Baguley, E., Pal, C., and Hughes, G. R. (1991). Antiphospholipid syndrome: Five year follow up. *Ann. Rheum. Dis.* **50**, 805–810.
44. Davis, W. D., and Brey, R. L. (1992). Antiphospholipid antibodies and complement activation in patients with cerebral ischemia. *Clin. Exp. Rheumatol*. **10**, 455–460.
45. Norberg, R., Nived, O., and Strurfeld, G. (1987). Anticardiolipin and complement activation: Relation to clinical symptoms. *J. Rheumatol*. **14**, 149–153.
46. Holers, V. M., Girardi, G., Mo, L., Guthridge, J. M., Molina, H., Pierangeli, S. S., Espinola, R., Xiaowei, L. E., Mao, D., Vialpando, C. G., and Salmon, J. E. (2002). Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J. Exp. Med.* **195**, 211–220.
47. Fukumura, H., Hayoshi, K., and Ishikawa, S. (1987). Complement-induced thrombus formation on the surface of poly (*N*-vinylpyrrolidone)-grafted polyethylene. *Biomaterials* **8**, 74–79.
48. Unander, A. M., Norberg, R., Hahn, L., and Arfors, L. (1987). Anticardiolipin antibodies and complement in 99 women with habitual abortion. *Am. J. Obstet. Gynecol.* **156**, 114–119.
49. Cheng, H. M., and Yap, S. F. (1988). Anticardiolipin and complement. *Arthritis. Rheum*. **31**, 1211–1212.
50. Galli, M. (1996). Non beta 2-glycoprotein I cofactors for antiphospholipid antibodies. *Lupus* **5**, 388–392.
51. Galli, M., Comfurius, P., and Maassen, C. (1990). Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. *Lancet* **335**, 1544–1547.
52. Arvieux, J., Roussel, B., Ponard, D., and Colomb, M. G. (1994). IgG2 subclass restriction of anti-beta 2 glycoprotein 1 antibodies in autoimmune patients. *Clin. Exp. Immunol*. **95**, 310–315.
53. Takeya, H., Mori, T., Gabazza, E. C., Kuroda, K., Deguchi, H., Matsuura, E., Ichikawa, K., Koike, T., and Suzuki, K.

(1997). Anti-beta2-glycoprotein I (beta2GPI) monoclonal antibodies with lupus anticoagulant-like activity enhance the beta2GPI binding to phospholipids. *J. Clin. Invest*. **99**, 2260–2268.
54. Walker, F. J. (1981). Regulation of activated protein C by protein S. The role of phospholipid in factor Va inactivation. *J. Biol. Chem*. **256**, 11128–11131.
55. Stenflo, J. (1984). Structure and function of protein C. *Semin. Thromb. Haemost.* **10**, 109–121.
56. Marciniak, E., and Romond, E. (1989). Impaired catalytic function of activated protein C: A new in vitro manifestation of lupus anticoagulant. *Blood* **74**, 2426–2432.
57. Tsakiris, D. A., Settas, L., Makris, P. C., and Marbet, C. A. (1990). Lupus anticoagulant, antiphospholipid antibodies and thrombophilia. Relation to protein C-protein S-thrombomodulin. *J. Rheumatol*. **17**, 705–707.
58. Amer, L., Kisiel, W., Searles, R., and Williams, R. C., Jr. (1990). Impairment of the protein C anticoagulant pathway in a patient with systemic lupus erythematosus, anticardiolipin antibodies and thrombosis. *Thromb. Res*. **57**, 247–258.
59. Freyssinet, J. M., Wiesel, M. L., and Gauchy, J. (1986). An IgM lupus anticoagulant that neutralizes the enhancing effect of phospholipid on purified endothelial thrombomodulin activity—a mechanism for thrombosis. *Thromb. Haemost*. **55**, 309–313.
60. Cariou, G., Tobelem, G., Soria, C., and Caen, J. (1986). Inhibition of protein C activation by endothelial cells in the presence of lupus anticoagulant (letter). *N. Engl. J. Med.* **314**, 1193–1194.
61. Cariou, R., Tobelem, G., and Belluci, S. (1988). Effect of lupus anticoagulant on antithrombogenic properties of endothelial cells—inhibition of thrombomodulin-dependent protein C activation. *Thromb. Haemost*. **60**, 54–58.
62. Lo, S. C., Salem, H. H., and Howard, M. A. (1990). Studies of natural anticoagulant proteins and anticardiolipin antibodies in patients with the lupus anticoagulant. *Br. J. Haematol.* **76**, 380–386.
63. Exner T. (1994). Some recent developments with lupus anticoagulants. *Blood Coag. Fibrinol*. **5**, 281–289.
64. Tincani, A., Spatola, L., Prati, E., Allegri, F., Ferremi, P., Cattaneo, R., Meroni, P., and Balestrieri, G. (1996). The anti-beta2-glycoprotein I activity in human antiphospholipid syndrome sera is due to monoreactive low-affinity autoantibodies directed to epitopes located on native beta2-glycoprotein I and preserved during species' evolution. *J. Immunol.* **157**, 5732–5738.
65. Pengo, V., Biasiolo, A., Brocco, T., Tonetto, S., and Ruffatti, A. (1996). Autoantibodies to phospholipid-binding plasma proteins in patients with thrombosis and phospholipid-reactive antibodies. *Thromb. Haemost*. **75**, 721–724.
66. Pierangeli, S. S., Goldsmith, G. H., Branch, D. W., and Harris, E. N. (1997). Antiphospholipid antibody: Functional specificity for inhibition of prothrombin activation by the prothrombinase complex. *Br. J. Haematol*. **97**, 768–774.
67. Gharavi, A. E., Sammaritano, L. R., Bovastro, J. L., Jr., and Wilson, W. A. (1995). Specificities and characteristics of beta 2 glycoprotein 1-induced antiphospholipid antibodies. *J. Lab. Clin. Med.* **125**, 775–778.
68. Pierangeli, S. S., Harris, E. N., Davis, S. A., and DeLorenzo, G. (1992). Beta 2-glycoprotein 1 enhances cardiolipin binding activity but is not the antigen for antiphospholipid antibodies. *Br. J. Haematol*. **82**, 565–570.
69. Amiral, J., Adam, M., Cluzeau, D., Vissac, A. M., and Maillet, T. (1996). Different target specificities of phospholipid-dependent antibodies. *Ann. Med. Interne* **147**, 18–21.
70. Viard, J. P., Amoura, Z., and Bach, J. F. (1991). Anti-beta 2 glycoprotein I antibodies in systemic lupus erythematosus: A marker of thrombosis associated with a circulating anticoagulant. *Comptes Rend L'Acad. Sci.* **313**, 607–612.
71. Puurunen, M., Vaarala, O., Julkunen, H., Aho, K., and Palosuo, T. (1996). Antibodies to phospholipid-binding plasma proteins and occurrence of thrombosis in patients with systemic lupus erythematosus. *Clin. Immunol. Immunopathol*. **80**, 16–22.
72. Schousboe, I. (1985). β2-glycoprotein-1: A plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. *Blood* **66**, 1086–1091.
73. Pierangeli, S. S., Dean, J., Goldsmith, G. H., Branch, D. W., Gharavi, A., and Harris, E. N. (1996). Studies on the interaction of placental anticoagulant protein I, beta 2 glycoprotein I, and antiphospholipid antibodies in the prothrombinase reaction and in the solid phase anticardiolipin assays. *J. Lab. Clin. Med.* **128**, 194–201.
74. McNally, T., Mackie, I. J., Isenberg, D. A., and Machin, S. J. (1996). Beta 2 glycoprotein-I inhibits factor XII activation on triglyceride rich lipoproteins: The effect of antibodies from plasma of patients with the antiphospholipid syndrome. *Thromb. Haemost*. **76**, 220–225.
75. Simantov, R., LaSala, J. M., Lo, S. K., Gharavi, A. E., Sammaritano, L. R., Salmon, J. E., and Silverstein, R. L. (1995). Activation of cultured vascular endothelial cells by antiphospholipid antibodies. *J. Clin. Invest*. **96**, 2211–2219.
76. Canfield, W. M., and Kisiel, W. (1982). Evidence of normal functional levels of activated protein C inhibitor in combined Factor V/Vlll deficiency disease. *J. Clin. Invest*. **70**, 1260–1266.
77. Walker, F. (1993). Does β2-glycoprotein-1 inhibit the interaction between protein S and C4B-binding protein? *Thromb. Haemost*. **64**, 930 (abstract).
78. Shen, C., Lahita, R. G., Dorsett, E., and Merrill, J. T. (1995). Protein S deficiency in the antiphospholipid syndrome: Role of β_2-glycoprotein I. *Arthritis. Rheum.* **38**, S57 (abstract).
79. Roubey, R. A. S., Eisenberg, R. A., Harper, M. F., and Winfield, J. B. (1995). Anticardiolipin autoantibodies recognize beta 2-glycoprotein 1 in the absence of phospholipid. Importance of Ag density and bivalent binding. *J. Immunol*. **154**, 954–960.

80. Galli, M., Beretta, G., Daldossi, M., Bevers, E. M., and Barbui, T. (1997). Different anticoagulant and immuological properties of anti-prothrombin antibodies in patients with antiphospholipid antibodies. *Thromb. Haemost.* **77**, 486–491.
81. Hunt, J. E., Simpson, R. J., and Krilis, S. A. (1993). Identification of a region of beta 2-glycoprotein I critical for lipid binding and anti-cardiolipin cofactor activity. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2141–2145.
82. Hunt, J., and Krilis, S. (1994). The fifth domain of beta 2-glycoprotein I contains a phospholipid binding site (Cys281–Cys288) and a region recognized by anticardiolipin antibodies. *J. Immunol.* **152**, 653–659.
83. Ichikawa, K., Khamashta, M. A., Koike, T., Matsuura, E., and Hughes, G. R. (1994). Beta 2-glycoprotein I reactivity of monoclonal anticardiolipin antibodies from patients with the antiphospholipid syndrome. *Arthritis. Rheum.* **37**, 1453–1461.
84. Matsuura, I., Igarashi, M., Igarashi, Y., Katahira, T., Nagae, H., Ichikawa, K., Triplett, D., and Koike, T. (1995). Molecular studies on phospholipid binding sites and cryptic epitopes appearing on beta 2 glycoprotein-1 structure recognized by anticardiolipin antibodies. *Lupus* **4**, S13–S17.
85. Borchman, D., Harris, E. N., Pierangelli, S. S., and Lamba, O. P. (1995). Interactions and molecular structure of cardiolipin and beta 2-glycoprotein I (beta 2-GP1). *Clin. Exp. Immunol.* **102**, 373–378.
86. Qamar, T., Gharavi, A. E., Levy, R. A., and Lockshin, M. D. (1990). Lysophosphatidylethanolamine is the antigen to which apparent antibody to phosphatidylethanolamine binds. *J. Clin. Immunol.* **10**, 200–203.
87. Rauch, J., and Janoff, A. S. (1996). Antibodies against phospholipids other than cardiolipin: Potential roles for both phospholipid and protein. *Lupus* **5**, 498–502.
88. Casciola-Rosen, L., Rosen, A., Petri, M., and Schlissel, M. (1996). Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: Implications for coagulation events and antigenic spread in systemic lupus erythematosus. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1624–1629.
89. Price, B. E., Rauch, J., Shia, M. A., Walsh, M. T., Lieberthal, W., Gilligan, H. M., O'Laughlin, T., Koh, J. S., and Levine, J. S. (1996). Anti-phospholipid antibodies bind to apoptotic, but not viable, thymocytes in a beta 2-glycoprotein I-dependent manner. *J. Immunol.* **157**, 2201–2208.
90. McCrae, K. R., DeMichele, A., Samuels, P., Roth, D., Kuo, A., Meng, Q. H., Rauch, J., and Cines, D. B. (1991). Detection of endothelial cell-reactive immunoglobulin in patients with anti-phospholipid antibodies. *Br. J. Haematol.* **79**, 595–605.
91. Cervera, R., Khamashta, M. A., Font, J., Ramirez, G., D'Cruz, D., Montalban, J., Lopez-Soto, A., Asherson, R. A., Ingelmo, M., and Hughes, G. R. (1991). Antiendothelial cell antibodies in patients with the antiphospholipid syndrome. *Autoimmunity* **11**, 1–6.
92. Navarro, M., Cervera, R., Teixido, M., Reverter, J. C., Font, J., Lopez-Soto, A., Monteagudo, J., Escoar, G., and Ingelmo, M. (1996). Antibodies to endothelial cells and to beta 2-glycoprotein I in the antiphospholipid syndrome: Prevalence and isotype distribution. *Br. J. Rheumatol.* **35**, 523–528.
93. Dueymes, M., Levy, Y., Ziporen, L., Jamin, C., Piette, J. C., Shoenfeld, Y., and Youinou, P. (1996). Do some antiphospholipid antibodies target endothelial cells? *Ann. Intern. Med.* **147**, 22–23.
94. Del Papa, N., Guidali, L., Sala, A., Buccellati, C., Khamashta, M. A., Ichikawa, K., Koike, T., Balestrieri, G., Tincani, A., Hughes, G. R., and Meroni, P. L. (1997). Endothelial cells as target for antiphospholipid antibodies. Human polyclonal and monoclonal anti-beta 2-glycoprotein I antibodies react in vitro with endothelial cells through adherent beta 2-glycoprotein I and induce endothelial activation. *Arthritis. Rheum.* **40**, 551–561.
95. Ferro, D., Pittoni, V., Quintarelli, C., Basili, S., Saliola, M., Caroselli, C., Valesini, G., and Violi, F. (1997). Coexistence of anti-phospholipid antibodies and endothelial perturbation in systemic lupus erythematosus patients with ongoing prothrombotic state. *Circulation* **95**, 1425–1432.
96. Out, H. J., Kooijman, C. D., Bruinse, H. W., and Derksen, R. H. (1991). Histopathological findings in placentae from patients with intra-uterine fetal death and antiphospholipid antibodies. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **41**, 179–186.
97. Rand, J. H., Wu, X. X., Andree, H. A., Lockwood, C. J., Guller, S., Scher, J., and Harpel, P. C. (1997). Pregnancy loss in the antiphospholipid-antibody syndrome—a possible thrombogenic mechanism *N. Engl. J. Med.* **337**, 154–160.
98. Galli, M., Daldossi, M., and Barbui, T. (1994). Anti-glycoprotein Ib/IX and Iib/IIIa antibodies in patients with antiphospholipid antibodies. *Thromb. Haemost.* **71**, 571–575.
99. Panzer, S., Gschwandtner, M. E., Hutter, D., Spitzauer, S., and Pabinger, I. (1997). Specificities of platelet autoantibodies in patients with lupus anticoagulants in primary antiphospholipid syndrome. *Ann. Hematol.* **74**, 239–242.
100. Vazquez-Mellado, J., Llorente, L., Richaud-Patin, Y., and Alarcon-Segovia, D. (1994). Exposure of anionic phospholipids upon platelet activation permits binding of beta 2 glycoprotein I and through it that of IgG antiphospholipid antibodies. Studies in platelets from patients with antiphospholipid syndrome and normal subjects. *J. Autoimmun.* **7**, 335–348.
101. Amengual, O., Atsumi, T., Khamashta, M. A., Koike, T., and Hughes, G. R. (1996). Specificity of ELISA for antibody to beta 2-glycoprotein I in patients with antiphospholipid syndrome. *Br. J. Rheumatol.* **35**, 1239–1243.
102. Stewart, M. W., Etches, W. S., and Gordon, P. A. (1997). Antiphospholipid antibody-dependent C5b-9 formation. *Br. J. Haematol.* **96**, 451–457.
103. Galli, M., Finazzi, G., and Barbui, T. (1996). Thrombocytopenia in the antiphospholipid syndrome: Pathophysiology, clinical relevance and treatment. *Ann. Med. Interne* **147**, 24–27.

104. Wiener, H. M., Vardinon, N., and Yust, I. (1991). Platelet antibody binding and spontaneous aggregation in 21 lupus anticoagulant patients. *Vox Sang* **61**, 111–121.
105. George, J., Blank, M., Hojnik, M., Bar-Meir, E., Koike, T., Matsuura, E., Lorber, M., Aviram, M., and Shoenfeld, Y. (1997). Oxidized low-density lipoprotein (Ox-LDL) but not LDL aggravate the manifestations of experimental antiphospholipid syndrome (APS). *Clin. Exp. Immunol*. **108**, 227–233.
106. Forte, T. M., and McCall, M. R. (1994). The role of apolipoprotein A1-containing lipoproteins in atherosclerosis (review). *Curr. Opin. Lipidol*. **5**, 354–364.
107. Fruchart, J. C., and Ailhaud, G. (1992). Apolipoprotein A1-containing lipoprotein particles: Physiological role, quantification, and clinical significance. *Clin. Chem*. **38**, 793–797.
108. Meng, Q. H., Bergeron, J., Sparks, D. L., and Marcel, Y. L. (1995). Role of apolipoprotein A1 in cholesterol transfer between lipoproteins. Evidence for involvement of specific apoA1 domains. *J. Biol. Chem.* **270**, 8588–8596.
109. Lahita, R. G., Rivkin, E., Cavanagh, I., and Romano, P. (1993). Low levels of total cholesterol, high density lipoprotein, and apolipoprotein A1 in association with anticardiolipin antibodies in patients with SLE. *Arthritis. Rheum*. **36**, 1566–1574.
110. Kilgore, L. L., Patterson, B. W., Parenti, D. M., and Fisher, W. R. (1985). Immune complex hyperlipidemia induced by an apolipoprotein-reactive immunoglobulin A paraprotein from a patient with multiple myeloma. *J. Clin. Invest*. **76**, 225–232.
111. Kihara, S. Y., Matsuzawa, Y., Kubo, M., Nozaki, S., Funahashi, T., Yamashita, S., Sho, N., and Tarui, S. (1989). Autoimmune hyperchylomicronemia. *N. Engl. J. Med*. **320**, 1225–1259.
112. Corsini, A., Roma, P., Sommariva, D., Fumagalli, R., and Catapano, A. L. (1986). Autoantibodies to the low density lipoprotein receptor in a subject affected by severe hypercholesterolemia. *J. Clin. Invest*. **78**, 940–946.
113. Imai, H., Suzuki, S., Uchida, K., Kikuchi, K., Sugiyama, H., Kohno, H., Umeda, M., and Inoue, K. (1994). Natural antibody against apolipoprotein A1. Detection and characterization of the monoclonal antibody established from normal unimmunized BALB/c mice. *J. Immunol*. **153**, 2290–2301.
114. Wong, L., Curtiss, L. K., Huang, J., Mann, C. J., Maldonado, B., and Roheim, P. S. (1992). Altered epitope expression of human interstitial fluid apolipoprotein A1 reduces its ability to activate LCAT. *J. Clin. Invest*. **90**, 2370–2375.
115. Jonas, A., Kezdy, K. E., and Wald, J. H. (1989). Defined apolipoprotein A1 conformations in reconstructed HDL discs. *J. Biol. Chem*. **264**, 4818–4824.
116. Jonas, A., Wald, J. H., Toohill, K. L. H., Krul, E. S., and Kezdy, K. E. (1990). Apolipoprotein A1 structure and lipid properties in homogeneous, reconstructed spherical and discoidal HDL. *J. Biol. Chem*. **265**, 22123–22129.
117. Sparks, D. L., Phillips, M. C., and Lund-Katz, S. (1992). The conformation of apolipoprotein A1 in discoidal and spherical recombinant HDL particles. *J. Biol. Chem.* **267**, 25823–25829.
118. Sparks, D. L., Lund-Katz, S., and Phillips, M. C. (1992). The charge and structural stability of apolipoprotein A1 in discoidal and spherical recombinant HDL particles. *J. Biol. Chem.* **267**, 25830–25838.
119. Marcel, Y. L., Provost, P. R., Koa, H., Raffai, E., Dac, N. V., Fruchart, J. C., and Rassart, E. (1991). The epitopes of apolipoprotein A1 define distinct structural domains including a mobile middle region. *J. Biol. Chem*. **266**, 3644–3653.
120. Collet, X., Perret, B., Simard, G., Raffai, E., and Marcel, Y. L. (1991). Differential effects of lecithin and cholesterol on the immunoreactivity and conformation of apolipoprotein A1 in HDL. *J. Biol. Chem*. **266**, 9145–9152.
121. Dinu, A. R., Merrill, J. T., Shen, C., and Lahita, R. G. (1998). High prevalence of antibodies to apolipoprotein A1 in a lupus population. *Lupus* in submission.
122. Scofield, R. H., Henry, W. E., Kurien, B. T., James, J. A., and Harley, J. B. (1996). Immunization with short peptides from the sequence of the systemic lupus erythematosus-associated 60-kDa Ro autoantigen results in anti-Ro ribonucleoprotein autoimmunity. *J. Immunol*. **156**, 4059–4066.
123. James, J. A., Gross, T., Scofield, R. H., and Harley, J. B. (1995). Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B′-derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. *J. Exp. Med*. **181**, 453–461.
124. Curtiss, L. K. (1985). In "Hybridoma Technology in the Biosciences and Medicine" (T. A. Springer, Ed.), pp. 291–308. Plenumm, New York.
125. Curtiss, L. K., and Smith, R. S. (1988). Localization of two epitopes of apolipoprotein A1 that are exposed on human HDL using monoclonal antibodies and synthetic peptides. *J. Biol. Chem.* **263**, 13779–13800.
126. Banka, C. L., Bonnett, D. J., Black, A. S., Smith, R. S., and Curtiss, L. K. (1991). Localization of an apolipoprotein A1 epitope critical for activation of LCAT. *J. Biol. Chem*. **266**, 23866–23892.
127. Vaarala, O. (1996). Antiphospholipid antibodies and atherosclerosis. *Lupus* **5**, 456–457.
128. Moreb, J., and Kitchens, C. S. (1989). Acquired functional protein S deficiency, cerbral venous thrombosis and coumarin skin necrosis in association with antiphospholipid syndrome: Report of two cases. *Am. J. Med*. **87**, 207–210.
129. Sthoeger, Z. M., Sthoeger, D., and Mellnick, S. D. (1991). Transient anticardiolipin antibodies, functional protein S deficiency, and deep vein thrombosis: *Am. J. Hematol*. **36**, 206–207.
130. Ruiz Arguelles, C. J., Ruiz Arguelles, A., and Alarcon Segovia, D. (1991). Natural anticoagulants in systemic lupus erythematosus. Deficiency of protein S bound to C4bp associates with recent history of venous thromboses, antiphospholipid antibodies and the antiphospholipid syndrome. *J. Rheumatol*. **10**, 552–558.

131. Ginsberg, J. S., Demers, C., Brill-Edwards, P., Bona, R., Johnston, M., Wong, A., and Denburg, J. A. (1995). Acquired free protein S deficiency is associated with antiphospholipid antibodies and increased thrombin generation in patients with systemic lupus erythematosus. *Am. J. Med*. **98**, 379–383.
132. Amster, M. S., Conway, J., Zeid, M., and Pincus, S. (1993). Cutaneous necrosis resulting from protein S deficiency and increased antiphospholipid antibody in a patient with systemic lupus erythematosus. *J. Am. Acad. Dermatol*. **29**, 853–857.
133. Wattiaux, M. J., Herve, R., Robert, A., Cabane, J., Housset, B., and Imbert, J. C. (1994). Coumarin-induced skin necrosis associated with acquired protein S deficiency and antiphospholipid antibody syndrome. *Arthritis. Rheum*. **37**, 1096–1100.
134. Hill, V. A., Whittaker, S. J., Hunt, B. J., Liddell, K., Spittle, M. F., and Smith, N. P. (1994). Cutaneous necrosis associated with the antiphospholipid syndrome and mycosis fungoides. *Br. J. Dermatol*. **130**, 92–96.
135. Forastiero, R. R., Kordich, L., Basilotta, E., and Carreras, L. O. (1994). Differences in protein S and C4b-binding protein levels in different groups of patients with antiphospholipid antibodies. *Blood Coag. Fibrinoly*. **5**, 609–616.
136. Ames, P. R., Tommasino, C., Iannaccone, L., Brillante, M., Cimino, R., and Brancaccio, V. (1996). Coagulation activation and fibrinolytic imbalance in subjects wtih idiopathic antiphospholipid antibodies—a crucial role for acquired free protein S deficiency. *Thromb. Haemost.* **76**, 190–194.
137. Parke, A. L., Weinstein, R. E., and Bona, R. D. (1992). The thrombotic diathesis associated with the presence of antiphospholipid antibodies may be due to low levels of free protein S. *Am. J. Med*. **93**, 49–56.
138. Walker, T. S., Triplett, D. A., Javed, N., and Musgrave, K. (1988). Evaluation of lupus anticoagulants: Antiphospholipid antibodies, endothelium associated immunoglobulin, endothelial prostacyclin secretion, and antigenic protein S levels. *Thromb. Res*. **51**, 267–281.
139. Matsuda, J., Gohchi, K., Gotoh, M., Tsukamoto, M., and Saitoh, N. (1994). Plasma concentrations of total/free and functional protein S are not decreased in systemic lupus erythematosus patients with lupus anticoagulant and/or antiphospholipid antibodies. *Ann. Hematol*. **69**, 311–315.
140. Scharfstein, J., Rerreira, A., Gigli, I., and Nussenzweig, V. (1978). Human C4-binding protein. Isolation and characterization. *J. Exp. Med*. **148**, 207–222.
141. Barnum, S. R., and Dahlback, B. (1990). C4b Binding Protein, a regulatory component of the classical pathway of complement, is an acute phase protein and is elevated in systemic lupus erythematosus. *Complement Inflamm*. **7**, 71–77.
142. Garcia de Frutos, P. G., Alim, R. I. M., Hardig, Y., Zoller, B., and Dahlback, B. (1994). Differential regulation of α and β chains of C4b Binding Protein during acute phase response resulting in stable plasma levels of free anticoagulant protein S. *Blood* **84**, 815–822.
143. Malm, J., Laurell, M., and Dahlback, B. (1988). Changes in the plasma levels of vitamin K-dependent proteins C and S and of C4b Binding Protein during pregnancy and oral contraception. *Br. J. Haematol*. **68**, 437–443.
144. Hesselvik, J. F., Malm, J., Dahlback, B., and Blomback, M. (1991). Protein C, protein S and C4b-binding protein in severe infection and septic shock. *Thromb. Haemost*. **65**, 126–129.
145. D'Angelo, A., Vigano-D'Angelo, S., Esmon, C. T., and Comp, P. C. (1988). Acquired deficiencies of protein S. Protein S activity during oral anticoagulation, in liver disease, and in disseminated intravascular coagulation. *J. Clin. Invest*. **81**, 1445–1449.
146. Kemkes-Matthes, B. (1992). Acquired protein S deficiency. *Clin. Invest*. **70**, 529–534.
147. Carr, M. E., Jr., Steingold, K. A., and Zekert, S. L. (1993). Protein S levels during the normal menstrual cycle and during estrogen therapy for premature ovarian failure. *Am. J. Med. Sci*. **306**, 212–217.
148. Weir, P. E. (1981). Immunofluorescent studies of the uteroplacental arteries in normal pregnancy. *Br. J. Obstet Gynecol*. **88**, 301–307.
149. Kitzmiller, M. D. (1983). Placental pathology of the antiphospholipid syndrome. *Am. J. Obstet. Gynecol*. **115**, 248–251.
150. Hustin, J., Foidant, J. M., and Lambotte, R. (1983). Maternal vascular lesions in preeclampsia and intrauterine growth retardation: Light microscopy and immunofluorescence. *Placenta* **4**, 489–498.
151. Wells, M., Bennett, J., and Bulmer, J. N. (1987). Complement component deposition in uteroplacental (spiral) arteries in normal human pregnancy. *J. Reprod. Immunol.* **12**, 125–135.
152. Malm, J., Laurell, M., and Dahlback, B. (1988). Plasma concentrations of C4b-binding protein and vitamin K-dependent protein S in term and preterm infants: Low levels of protein S–C4b-binding protein complexes. *Br. J. Haematol*. **68**, 437–443.
153. Comp, P. C., Thurnau, G. R., Welsh, J., and Esmon, C. T. (1986). Functional and immunologic protein S levels are decreased during pregnancy. *Blood* **68**, 881–885.
154. Dahlback, B., Frohm, B., and Nelsestuen, G. (1990). High affinity interaction between C4b Binding protein and vitamin K-dependent protein S in the presence of calcium. Suggestion of a third component in blood regulating the interaction. *J. Biol. Chem*. **256**, 16082–16087.
155. Hatanaka, K., Li, X. A., Guo, L., Sakata, T., Gillissen, J. A., Yoshioka, A., and Yamamoto, A. (1994). A case report of deficiency in an inhibitor of calcium-dependent association of protein S with C4b-Binding Protein suggested by a modified crossed immunoelectrophoresis. *Thromb. Res*. **74**, 643–654.
156. Kristensen, T., D'Eustachio, P., Ogata, R. T., Chung, L. P., Reid, K. B., and Tack, B. F. (1987). The superfamily of C3b/C4b-binding proteins. *Fed. Proc.* **46**, 2463–2469.
157. Matsuda, J., Gohchi, K., Kawasugi, K., Gotoh, M., Saitoh, N., and Tsukamoto, M. (1995). Inhibitory activity of anti-beta 2-glycoprotein I antibody on factor Va degradation

by activated-protein C and its cofactor protein S. *Am. J. Hematol*. **49**, 89–91.
158. Camps Garcia, M. T., Guil, M., Sanchez-Lora, J., Grana, M. I., Martinez, J., and de Ramon, E. (1996). Fibrinolytic treatment in primary antiphospholipid syndrome. *Lupus* **5**, 627–629.
159. Merrill, J. T., Shen, C., Butman, B., Jeffries, E., Lahita, R. G., and Myones, B. L. (1999). beta$_2$ glycoprotein-1, a major target antigen of antiphospholipid antibodies, interferes with the binding of protein S by C4b Binding Protein and enhances the anticoagulant function of protein S. *Thromb. Haemost*. **81**,748–757.
160. Griffin, J. H., Gruber, A., and Fernandez, J. A. (1992). Reevaluation of total, free and bound protein S and C4b-binding protein levels in plasma anticoagulated with citrate or hirudin. *Blood* **9**, 3200–3211.
161. Schwalbe, R., Dahlback, B., Hillarp, A., and Nelsestuen, G. (1990). Assembly of protein S and C4b Binding Protein on membranes. *J. Biol. Chem*. **265**, 16074–16081.
162. Guermazi, S., Regnault, V., Gorgi, Y., Ayed, K., Lecompte, T., and Dellagi, K. (2000). Further evidence for the presence of anti-protein S autoantibodies in patients with systemic lupus erythematosus. *Blood Coag. Fibrinol*. **11**, 491–498.
163. Song, K. S., Park, Y. S., and Kim, H. K. (2000) Prevalence of anti-protein S antibodies in patients with systemic lupus erythematosus. *Arthritis Rheum.* **43**, 557–560.
164. Erkan, D., Zhang, H. W., Shriky, R. C., and Merrill, J. T. (2002). Dual antibody reactivity to β_2-glycoprotein I and protein S: Increased association with thrombotic events in the antiphospholipid syndrome lupus., accepted, in press.
165. Levine, S. R., Brey, R. L., Sawaya, K. L., Salowich-Palm, L., Kokkinos, J., Kostrzema, B., Perry, M., Havstad, S., and Carey, J. (1995). Recurrent stroke and thrombo-occlusive events in the antiphospholipid syndrome. *Ann. Neurol.* **38**, 119–124.
166. Silver, R. M., Porter, T. F., van Leeuween, I., Jeng, G., Scott, J. R., and Branch, D. W. (1996). Anticardiolipin antibodies: Clinical consequences of "low titers." *Obstet. Gynecol.* **87**, 494–500.
167. Ghirardello, A., Doria, A., Ruffatti, A., Rigoli, A. M., Vesco, P., Calligaro, A., and Gambari, P. F. (1994). Antiphospholipid antibodies (aPL) in systemic lupus erythematosus. Are they specific tools for the diagnosis of aPL syndrome? *Ann. Rheum. Dis*. **53**, 140–142.
168. Laroche, P., Berard, M., Rouquette, A. M., Desgruelle, C., and Boffa, M. C. (1996). Advantage of using both anionic and zwitterionic phospholipid antigens for the detection of antiphospholipid antibodies. *Am. J. Clin. Pathol*. **106**, 549–554.
169. Gilman-Sachs, A., Lubinski, J., Beer, A. E., Brend, S., and Beaman, K. D. (1991). Patterns of anti-phospholipid specificities. *J. Clin. Lab. Immunol.* **35**, 83–88.
170. Silver, R. M., Pierangeli, S. S., Edwin, S. S., Umar, F., Harris, E. N., Scott, J. R., and Branch, D. W. (1997). Pathogenic antibodies in women with obstetric features of antiphospholipid syndrome who have negative test results for lupus anticoagulant and anticardiolipin antibodies. *Am. J. Obstet. Gynecol*. **176**, 628–633.
171. Caruso, A., De Carolis, S., Ferrazzani, S., Valesini, G., Caforio, L., and Mancuso, S. (1993). Pregnancy outcome in relation to uterine artery flow velocity waveforms and clinical characteristics in women with antiphospholipid syndrome. *Obstet. Gynecol*. **82**, 970–977.
172. Eschwege, V., Laude, I., Toti, F., Pasquali, J. L., and Freyssinet, J. M. (1996). Detection of bilayer phospholipid-binding antibodies using flow cytometry. *Clin. Exp. Immunol*. **103**, 171–175.
173. Roubey, R. A. S. (1996). Antigenic specificities of antiphospholipid antibodies: Implications for clinical laboratory testing and diagnosis of the antiphospholipid syndrome. *Lupus* **5**, 425–430.
174. Forastiero, R. R., Martinuzzo, M. E., Kordich, L. C., and Carreras, L. O. (1996). Reactivity to beta 2 glycoprotein I clearly differentiates anticardiolipin antibodies from antiphospholipid syndrome and syphilis. *Thromb. Haemost.* **75**, 717–720.
175. Levy, R. A., Gharavi, A. E., Sammaritano, L. R., Habina, L., Qamar, T., and Lockshin, M. D. (1990). Characteristics of IgG antiphospholipid antibodies in patients with systemic lupus erythematosus and syphilis. *J. Rheumatol*. **17**, 1036–1041.
176. Loizou, S., Cazabon, J. K., Walport, M. J., Tait, D., and So, A. K. (1997). Similarities of specificity and cofactor dependence in serum antiphospholipid antibodies from patients with human parvovirus B19 infection and from those with systemic lupus erythematosus. *Arthritis Rheum*. **40**, 103–108.
177. Drenkard, C., Villa, A. R., Alarcon-Segovia, D., and Perez-Vazquez, M. E. (1994). Influence of the antiphospholipid syndrome in the survival of patients with systemic lupus erythematosus. *J. Rheumatol*. **21**, 1067–1072.
178. Finazzi, G., Brancaccio, V., Moia, M., Ciaverella, N., Mazzucconi, M. G., Schinco, P. C., Ruggeri, M., Pogliani, E. M., Gamba, G., Rossi, E., Baudo, F., Manotti, C., D'Angelo, A., Palareti, G., De Stefano, V., Berrettini, M., and Barbui, T. (1996). Natural history and risk factors for thrombosis in 360 patients with antiphospholipid antibodies: A four year prospective study from the Italian Registry. *Am. J. Med*. **100**, 530–536.
179. Oshiro, B. T., Silver, R. M., Scott, J. R., Yu, H., and Branch, D. W. (1996). Antiphospholipid antibodies and fetal death. *Obstet. Gynecol*. **87**, 489–493.
180. Cowchock, S., and Reece, E. A. (1997). Do low-risk women with antiphospholipid antibodies need to be treated? Organizing Group of the Antiphospholipid Antibody Treatment Trial. *Am. J. Obstet. Gynecol*. **176**, 1099–1100.
181. Nayer, R., and Lage, J. M. (1996). Placental changes in a first trimester missed abortion in maternal systemic lupus erythematosus with antiphospholipid syndrome; a case report and review of the literature. *Hum. Pathol*. **27**, 201–206.
182. Chamley, L. W., Pattison, N. S., and McKay, E. J. (1993). Elution of anticardiolipin antibodies and their cofactor

beta 2-glycoprotein 1 from the placentae of patients with a poor obstetric history. *J. Reprod. Immunol*. **25**, 2–7.
183. Salafia, C. M., and Parke, A. M. (1997). Placental pathology in systemic lupus erythematosus and phospholipid antibody syndrome. *Rheum. Dis. Clin. North Am*. **23**, 85–97.
184. Weingarten, K., Filippi, C., Barbut, D., and Zimmerman, R. D. (1997). The neuroimaging features of the cardiolipin antibody syndrome. *Clin. Imaging* **21**, 6–12.
185. Kato, T., Morita, A., and Matsumoto, Y. (1997). Hypoperfusion of brain single photon emission computerized tomography in patients wih antiphospholipid antibodies. *J. Dermatol. Sci.* **14**, 20–28.
186. Elubaeva, V. I., Mach, E. S., Reshetniak, T. M., and Alekberova, Z. S. (1994). The cerebral blood circulation in patients with systemic lupus erythematosus and the antiphospholipid syndrome *Terapevticheskii Arkhiv* **66**, 16–18.
187. Barbut, D., Borer, J. S., Wallerson, D., Ameisen, O., and Lockshin, M. (1991). Anticardiolipin antibody and stroke: Possibe relation of valvular heart disease and embolic events. *Cardiology* **79**, 99–109.
188. Provenzale, J. M., and Loganbill, H. A. (1994). Dural sinus thrombosis and venous infarction associated with antiphospholipid antibodies: MR findings. *J. Comput. Assist. Tomogr.* **18**, 719–723.
189. Piette, J. C., Cacoub, P., and Wechsler, B. (1994). Renal manifestations of the antiphospholipid syndrome. *Semin. Arthritis. Rheum*. **23**, 357–366.
190. Merrill, J. T., Shen, C., Lahita, R. G., and Mongee, A.-B. (1997). High prevalence of antiphospholipid antibodies in patients taking procainamide. *J. Rheumatol*. **24**, 1083–1088.
191. Gharavi, A. E., Sammaritano, L. R., Wen, J., Miyawaki, N., Morse, J. H., Zarrabi, M. H., and Lockshin, M. D. (1994). Characteristics of human immunodeficiency virus and chlorpromazine induced antiphospholipid antibodies: Effect of beta 2 glycoprotein I on binding to phospholipid. *J. Rheumatol*. **21**, 94–99.
192. Vlachoyiannopoulos, P. G., Tsiakou, E., Chalevelakis, G., Raptis, S. A., and Moutsopuolos, H. M. (1994). Antiphospholipid syndrome: Clinical and therapeutic aspects. *Lupus* **3**, 91–96.
193. Lima, F., Khamashta, M. A., Buchanan, N. M., Kerslake, S., Hunt, B. J., and Hughes, G. R. (1996). A study of sixty pregnancies in the antiphospholipid syndrome. *Clin. Exp. Rheumatol*. **14**, 131–136.

44

PATHOPHYSIOLOGY OF DRUG-INDUCED LUPUS

Raymond L. Yung
Bruce C. Richardson

PATHOPHYSIOLOGY OF DRUG-INDUCED LUPUS

Since the initial reports of iatrogenic autoimmunity in the 1940s [1], over one hundred drugs have been implicated in the induction of lupus and lupus-like illnesses [2, 3]. Drug-induced lupus (DIL) is perhaps one of the clearest examples of an environmental agent directly causing a systemic autoimmune disease. Since the inciting factors of most autoimmune diseases remain unknown, the observation that specific drugs can induce autoimmunity in susceptible individuals provides a unique opportunity to study early steps in the pathogenesis of autoimmunity. The significant overlap in signs, symptoms, and serologic findings between idiopathic systemic lupus erythematosus (SLE) and DIL suggests that DIL is also a valid model for the study of idiopathic lupus. Thus understanding the mechanisms underlying DIL will likely have important implications for understanding the mechanisms causing idiopathic SLE.

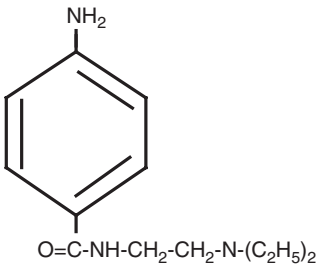
Most investigations into the mechanisms of DIL have focused on procainamide and hydralazine, since these drugs are most frequently implicated in DIL. However, different drugs can cause diverse clinical manifestations with different autoantibody specificities, suggesting that more than one mechanism may be involved. Furthermore, a drug may trigger autoimmunity by more than one mechanism. Advances, including the development of murine models of DIL, have helped clarify the pathophysiology of this disease and its relationship to idiopathic lupus and autoimmunity in general. This chapter will review proposed mechanisms of DIL and summarize the current theories for the pathogenesis of DIL, with an emphasis on *in vivo* models.

STRUCTURES OF DRUGS CAUSING DRUG-INDUCED LUPUS

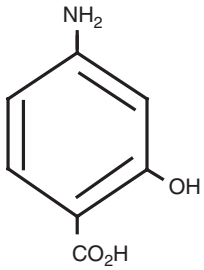
The drugs implicated in DIL include antihypertensives, antiarrhythmics, antibiotics, anticonvulsants, psychotropic agents, and anti-inflammatory medications. Although drugs implicated in DIL have diverse pharmacologic properties, it is tempting to postulate that they, or their metabolites, may share a common pathogenic epitope, or pharmacophore, responsible for eliciting autoimmunity in affected patients. The drugs most commonly associated with DIL contain aromatic amines, hydrazines, sulfhydryl groups, or a phenol ring [4] (Fig. 1). However, the considerable overlap between clinical features of DIL caused by drugs with these diverse chemical structures suggests that no single configuration is responsible for DIL.

ANTI-DRUG ANTIBODIES

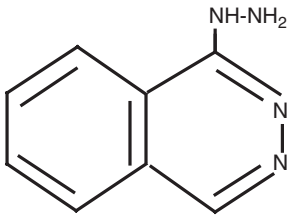
Lupus-inducing drugs are generally poor immunogens, and antibodies raised against them have not been shown to be pathogenic in humans or experimental systems. In support of this, only 6% of patients with



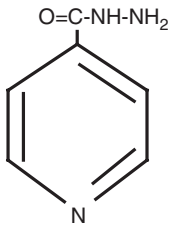
Procainamide



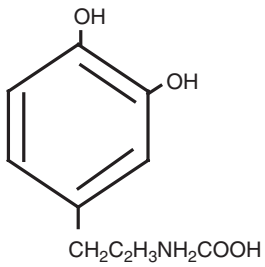
***p*-Aminosalicyclic Acid**



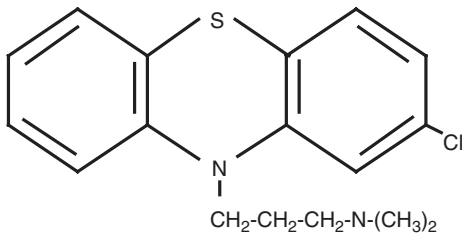
Hydralazine



Isoniazid



Methyl Dopa



Chlorpromazine

FIGURE 1 Structure of selected drugs implicated in DIL. Procainamide is an example of aromatic amines, while hydralazine and isoniazid are example of hydrazines.

procainamide-induced lupus have antibodies against procainamide, compared to 50% of all patients receiving the drug [5]. Interestingly, "natural" antibodies to procainamide have been found in sera of 19–35% patients with idiopathic lupus and rheumatoid arthritis, and in up to 30% of healthy adults never exposed to the drug. These data suggest that development of clinical DIL from procainamide does not depend on the presence of anti-drug antibodies, and that these antibodies are unlikely to cross-react with autoantigens *in vivo* to cause DIL. However, antibodies to a drug–macromolecule complex may cross-react with unrelated autoantigens to cause clinical disease. For example, in one report, all patients with active hydralazine-induced lupus demonstrated anti-hydralazine antibodies [6]. In a second prospective study, anti-hydralazine antibodies were detected in 16 of 21 patients who had received the drug for 1 year [7]. The high incidence of anti-hydralazine antibodies, together with the structural similarities between hydralazine and the nucleotide adenosine, led to the suggestion of possible molecular mimicry and antibody cross-reactivity as a pathogenic mechanism for hydralazine-induced lupus. In support of this, Yamauchi *et al*. [8] demonstrated that rabbits hyperimmunized with hydralazine conjugated to human serum albumin can produce anti-hydralazine antibodies that cross-react with DNA.

Treatment with anti-TNF (tumor-necrosis factor) therapies has been associated with the appearance of anti-nuclear antibodies (ANA), anti-double-stranded (ds)DNA and occasionally clinical lupus [9]. Approximately 13% of patients exposed to infliximab develop infliximab-specific antibodies. Antibody development is lower in recipients on concurrent immunosuppressants such as methotrexate or azathioprine. The presence of antibodies to infliximab increases the likelihood of an infusion reaction but does not predict the development of autoantibodies or DIL. Nonneutralizing antibodies

to the TNF receptor portion or other protein components of etanercept are present in up to 16% of rheumatoid arthritis patient receiving etanercept. There does not appear to be any correlation between antibody development and clinical response or autoimmunity.

DRUG–DNA INTERACTIONS

A number of mechanisms have been proposed to explain how procainamide and hydralazine cause DIL, based on the observation that both procainamide and hydralazine bind polynucleotides *in vitro*. The resulting drug–DNA structures may potentially be more immunogenic than the native DNA molecule. In addition, the amino and hydrazine groups of procainamide and hydralazine are reactive with nucleoproteins [10–15] which may augment their immunogenicity. This raises the possibility that DNA may be modified by these agents in a fashion analogous to haptens, making the DNA more antigenic. These two drugs, and to a lesser extent, isoniazid and D-penicillamine, have also been shown to induce a stable transition from the B (right-handed) to Z (left-handed) configuration of synthetic DNA [16]. DNA in the Z configuration is more immunogenic [17, 18] and the drug–DNA complex may cause the formation of some of the autoantibodies found in DIL. Several studies have demonstrated anti-Z-DNA antibodies in hydralazine-treated patients [19–21] with one reporting that 82% of sera from hypertensive patients receiving hydralazine had evidence of anti-Z-DNA antibodies [20]. In four patients where sera were drawn sequentially, the investigators were able to show that the antibodies developed after the initiation of hydralazine. In this context it is significant that increased levels of natural polyamines have been demonstrated in sera from patients with active idiopathic lupus [22] and some of these polyamines are capable of inducing Z-DNA conformation, possibly contributing to the anti-Z-DNA antibodies demonstrated in idiopathic lupus [23–25]. Hydralazine may also stabilize another unusual and potentially immunogenic form of DNA, known as triplex DNA [26]. Up to 72% of sera from hydralazine-treated patients may have antibodies against triplex DNA. At present, the clinical relevance of these observations is unclear.

DRUG–HISTONE INTERACTIONS AND ANTI-HISTONE ANTIBODIES

Histones are highly conserved DNA-binding proteins that are rich in basic amino acids. There are five major histone subclasses: Hl, H2A, H2B, H3, and H4. (see Chapter 12.) Antibodies against histone subunits are commonly detected in patients with DIL. However, the precise role of these and other autoantibodies in the pathogenesis of DIL is unclear. There are considerable discrepancies among published studies on the fine specificity of anti-histone antibodies in patients with DIL. Part of this may be due to the purity of the substrates used and the different assay conditions employed [27]. In addition, the specificity of anti-histone antibodies appears to differ from those found in idiopathic SLE [28, 29]. Hydralazine has been shown to interact with soluble DNA–histone complexes [30]. The resulting change in structure makes the histone more resistant to proteolytic digestion. The altered structure may also be more immunogenic, suggesting an explanation for the high incidence of anti-histone antibodies in these patients. Different drugs also induce anti-histone antibodies with different fine specificity. It is worth noting that anti-histone antibodies can have higher affinity to native than purified histones. Burlingame *et al*. [31] examined the reactivity of anti-histone antibodies in lupus patients, and demonstrated preferential binding to chromatin and selected histone–DNA subnucleosome complexes compared to purified DNA. Consistent with this, Mohan *et al*. [32] studied autoreactive T-cell clones from lupus-prone mice, and showed that 50% of the clones had specificity against nucleosome antigens and not free DNA or histones. These observations raise the possibility that the immunogenic form of DNA may be the nucleosome, possibly modified by drugs. A pathogenic role of histone or anti-histone antibodies has not been demonstrated. However, one study suggested a statistical correlation between the presence of anti-(H2A-H2B), anti-(H2A-H2B)-DNA, anti-chromatin antibodies, and proteinuria in idiopathic lupus [31].

ABNORMAL IMMUNE REGULATION

Inconsistent results have been reported concerning the *in vitro* effects of lupus-inducing drugs on cells of the immune system. Depending on the concentrations tested, procainamide either promotes or inhibits the mitogenic response of peripheral blood lymphocytes [33–36]. Low concentrations of procainamide hydroxylamine (PAHA), a reactive oxidized metabolite of procainamide, have been shown to enhance lymphocyte mitogenic responses and promote B-cell immunoglobulin (Ig) secretion. Opposite effects, however, have been reported when higher concentrations of the drug were tested [35, 36]. Another report found that sera from patients receiving procainamide will inhibit the Concanavalin-A mitogenic response of peripheral

blood mononuclear cells from normal and procainamide-treated patients [37]. Similarly, T cells from patients with procainamide-induced lupus demonstrate decreased proliferative responses [38]. Most, but not all, researchers report B-lymphocyte activation and increased Ig production in procainamide-treated patients [39–41]. Patients with active procainamide-induced lupus have circulating B cells that spontaneously secrete Ig, and B-cell Ig production in response to pokeweed mitogen is enhanced [42, 43] similar to patients with idiopathic lupus. In one *in vitro* study, researchers showed that procainamide may inhibit T-cell suppressor activity, resulting in excessive B-cell activation. Yet another report found increased Ig production in procainamide-treated patients without concomitant change in T-cell suppressor function [43]. The reason for the inconsistent reporting is not clear, but may in part be related to conditions under which the assays were performed.

The effects of other lupus-inducing drugs on cellular immune responses are less well studied. Peripheral blood lymphocytes of patients with hydralazine-induced lupus have been shown to have a higher transformation index compared with lymphocytes from untreated patients [44]. Litwin *et al*. [45, 46] examined hydralazine-treated patients, and found normal delayed hypersensitivity responses as measured by skin testing. The peripheral blood lymphocytes from the same group of patients had normal mitogenic responses, but had increased proliferation after incubation with hydralazine–albumin conjugates. Decreased suppressor T-cell function, with enhanced B-cell Ig production, has been reported in patients taking methyldopa, and in normal T-cells treated with methyldopa *in vitro*. Others, however, found depressed peripheral blood mononuclear cell mitogenic responses after methyldopa treatment [47]. Diphenylhydantoin was found to inhibit lymphocyte proliferation [48], but had no effect on T-cell suppressor function [49].

A number of cytokine/anticytokine therapies have more recently been reported to cause autoimmunity in humans. Between 0.15 and 0.7% of interferon-α (IFN-α) treated patients develop a lupus-like illness [50, 51]. Interestingly, IFN-α levels are increased in idiopathic lupus and may correlate with anti-DNA antibody titers [52]. It has been postulated that IFN-α may modulate autoimmunity through its effects on apoptosis. However, this is uncertain because IFN-α has been shown to upregulate the expression of both the pro-apoptotic Fas ligand [53] and the antiapoptotic Bcl-2 [54] genes on lymphoid cells. Because IFN-α induces IFN-γ expression, it is also possible that IFN-α-induced lupus occurs in part through IFN-γ-dependant mechanisms. There are additional data showing that the cytokine may activate autoreactive T cells by stimulating viral superantigen production [55]. Finally, another report suggests that IFN-α may drive the autoimmune response in lupus by inducing monocyte differentiation into antigen-presenting dendritic cells [56].

The mechanism behind anti-TNF therapy-associated lupus is unclear. Serum levels of TNF and soluble TNF receptors are increased in patients with idiopathic lupus, and may correlate with lupus disease activity [57–59]. TNF and interleukin-1 (IL-1) β expression are also increased in the kidneys of MRL/lpr mice with autoimmune lupus nephritis [60]. In contrast, NZB/NZW F1 mice have low serum TNF levels. TNF supplementation prior to the onset of nephritis in these mice delays proteinuria and improves survival, but does not affect anti-DNA antibody production [61]. One postulated mechanism is that anti-TNF antibodies may bind to TNF-α-bearing cells, inducing apoptosis and the release of potentially immunogenic nuclear antigens into the circulation [9]. Interestingly, newly described members of the TNF/TNF receptor family of proteins, including B-cell-activating factor (BAFF, also known as BlyS, zTNF4) and APRIL, and their receptors TACI, BCMA, and BAFF-R, have been implicated in the pathogenesis of lupus [62]. However, the relationship of these molecules and TNF antagonist-induced autoimmunity is unclear.

GENETIC FACTORS

Human Leukocyte Antigen and Complement

Extensive studies have documented the association between specific major histocompatibility complex (MHC) alleles and the development of idiopathic lupus [63, 64]. These include human leukocyte antigen (HLA)-DR2 and HLA-DR3, and class III C4A and C4B null alleles of complement (reviewed in Chapters 4 and 5 of this book). In contrast, there are only limited and sometimes conflicting reports of HLA associations in DIL. Initial studies from England by Batchelor *et al*. [65] showed that 79% of patients with hydralazine-induced lupus expressed the HLA-DR4 allele, compared with 25% of asymptomatic patients receiving hydralazine. Twenty-five of the 26 patients with hydralazine-induced disease were also slow acetylators. No association between the presence of DR4 and acetylator phenotype was found. In 1987, a second English study [66] also reported an association between the HLA-DR4 allele and hydralazine-induced lupus. The researchers found that 70% of their 20 patients studied were HLA-DR4 positive, compared with 33% of the

general population. Of note, 6 of the 20 patients were also included in the initial study by Batchelor *et al*. [65]. In contrast, a third study from Australia failed to find an increased incidence of HLA-DR4 in 18 patients with hydralazine-induced disease when compared to untreated controls [67]. It now appears that the putative HLA association in the initial English study may be the result of linkage disequilibrium with the complement C4 null allele. C4 is encoded at two loci, C4A and C4B, located between the class I MHC and HLA-DR genes. Multiple alleles, including null or nonfunctioning alleles, are present within each of these loci [68]. When the researchers from the English study reexamined their patients, they found a much higher incidence of the C4 null allele in the study group (16 of 21 patients, or 76%) compared with controls (35 of 82 patients, or 43%) [69]. The observed HLA-DR4 association is possibly the result of linkage disequilibrium between the C4-null-DR4 haplotype and the C4 null trait. Of note, partial deficiencies of C4, especially the C4A isotype, are risk factors for idiopathic lupus. The occasional patients with total C4 deficiency have a high incidence of the disease [70].

Another potential pathogenic mechanism in DIL also involves the complement system. The classic complement pathway plays an important role in removing circulating immune complexes [68]. Some of the drugs implicated in DIL, including hydralazine, penicillamine, isoniazid, and metabolic products of procainamide, have been shown to inhibit the covalent binding of C4 to C2 [68, 71, 72]. This may inhibit the activation of C3 by the classic pathway, resulting in defective immune-complex clearance. Increased amounts of circulating immune complexes have been reported, but not consistently, in patients with DIL [68, 73].

Very few studies have examined possible HLA associations in other forms of DIL. One report suggested an association between HLA-DR6Y and procainamide-induced lupus [74]. Fifty-three percent of the clinically affected patients were HLA-DR6Y positive, compared with 25% of procainamide-treated patients who were asymptomatic but ANA positive, and 17% of controls. However, this has not been confirmed by other investigators [75]. Another report suggested an association between HLA-Bw44 and chlorpromazine-related autoimmunity [76]. Forty-six percent of patients with chlorpromazine-induced autoantibodies were HLA-Bw44 positive, compared with 19% of the normal population. HLA-DR4 was linked with penicillamine-induced lupus in one study [77]. Another study, however, failed to confirm the association, but found increased frequencies of HLA-All and B15 in a group of penicillamine-treated rheumatoid arthritis patients [78]. In summary, there has not been any consistent report of an HLA association in DIL. Genetic factors predisposing patients to DIL are likely to lie outside the MHC region.

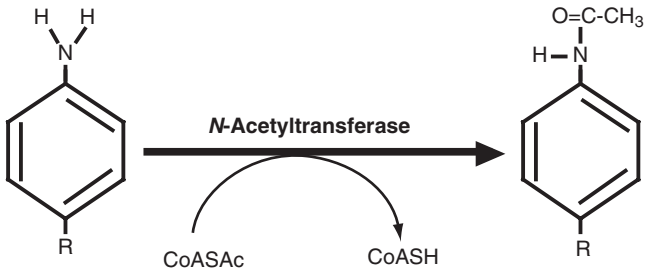


FIGURE 2 *N*-acetyltransferase catalyzes the acetylation of aromatic amines by acetyl-coenzyme A (CoASAc).

Acetylator Phenotype and DIL

Acetylation is the dominant pathway for the metabolism of aromatic amines and hydrazines. The enzyme responsible for this chemical conversion is *N*-acetyltransferase (Fig. 2). The enzyme(s) is found primarily in the liver and gut, although acetylation activity has also been detected in human erythrocytes and peripheral blood mononuclear cells [79]. The rate of acetylation of a drug is influenced by genetic factors. Individuals who are phenotypically slow acetylators are homozygous for the recessive allele, and fast acetylators are either heterozygous or homozygous for the dominant allele encoding the hepatic acetyltransferase gene. Humans with the slow acetylator phenotype have decreased or absent *N*-acetyltransferase activities in the liver [80]. Drugs known to be metabolized by this pathway include procainamide, hydralazine, isoniazid, sulfapyridine, sulfadimidine, dapsone, an amine metabolite of nitrazepam, and some carcinogenic aromatic amines [81]. Nongenetic factors may also affect the rate of acetylation. For example, acetylation rate is decreased in patients with renal insufficiency [82] and increased with ethanol ingestion [83].

There are approximately equal numbers of people with the slow and fast acetylator phenotypes in North America [84]. Although DIL can occur in patients with either phenotype, the majority of patients who develop procainamide or hydralazine-induced lupus have the slow acetylator phenotype [65, 85–87]. Individuals who are slow acetylators develop autoantibodies after a shorter exposure to hydralazine than do fast acetylators (Fig. 3), and are also more prone to developing the clinical manifestations of hydralazine-induced lupus [65, 85, 86, 88, 89]. Perry *et al*. [85] were the first to document the relationship between hepatic acetyltransferase activity, hydralazine, and the development of ANA and

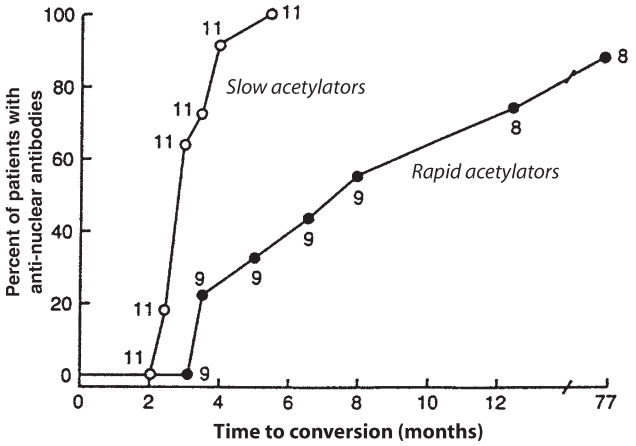


FIGURE 3 Rate of development of procainamide-induced ANA in slow acetylators (open circles) and rapid acetylators (closed circles) with time. (From Woolsey *et al*. (1987). *N. Engl. J. Med.* **298**, 1157, with permission.)

DIL. Of the 57 patients receiving hydralazine in their study, 33 were slow acetylators and 24 were fast acetylators. Twelve patients developed hydralazine-induced lupus, and all were slow acetylators. The cumulative dose and the steady-state concentration of the drugs are important factors in determining the onset of DIL. The incidence of DIL is about the same in patients with the slow and fast acetylator phenotypes, if the serum concentration of procainamide is maintained at the same level [90]. *N*-Acetylprocainamide (NAPA), a metabolite of procainamide, does not have the same lupus-inducing potential as the parent drug [91], suggesting that the aromatic amino group in procainamide may be important in inducing this disease. This suggests that the primary amino or hydrazine group on the parent drugs, rather then their metabolites, may be important elements in the pathogenesis of procainamide and hydralazine-induced lupus.

Only a small number of drugs implicated in DIL utilize the acetylation metabolic pathway. The contribution of a patient's acetylation phenotype in determining the development of DIL appears to vary with different drugs. Although acetylation is the primary determinant of the serum half-life of isoniazid, patients who are slow acetylators and receive the drug have been reported to be both equally likely [92] or more likely [93, 94] to develop ANAs and clinical disease, compared with fast acetylators.

Acetylator Phenotype and Idiopathic SLE

The association between slow acetylator phenotype and some forms of DIL suggests that acetylation inactivates the inciting drug, and it thus provides protection. Unless other environmental aromatic amines play a role in the etiology of idiopathic lupus, there should be no relationship between acetylation phenotype and idiopathic lupus. Initial studies by Reidenberg and Martin [95] showed that 71% of patients with idiopathic SLE may be slow acetylators, compared with the 52% expected incidence in the general population. Subsequent studies, however, showed positive associations, statistically insignificant associations, or no association between acetylator status and idiopathic lupus [96–106]. The association, if present, appears to be small. It is highly doubtful that genetic slow acetylation plays a significant role in the pathogenesis of idiopathic lupus. The varying results in different studies may be in part due to the use of historical controls, or variation of other unidentified geographic factors important in idiopathic lupus [96].

NEUTROPHIL-MEDIATED OXIDATIVE METABOLISM

Because lupus-inducing drugs have diverse chemical structures and pharmacological actions it has been postulated that these agents may cause clinical autoimmunity via common reactive oxidative metabolites. This model postulates that reactive oxygen species released by neutrophils modify xenobiotics, resulting in toxic metabolites. Immune cells including monocytes and Langerhans cells possess more limited amounts of enzymes such as myeloperoxidase and prostaglandin H synthase but may have some activity. Hepatic metabolism involving the microsomal mono-oxygenase system and esterases also produces some potentially toxic compounds. These highly reactive agents are very labile and generally do not circulate in the body in the active forms. A role for these reactive metabolites in immune responses therefore must involve contact with immunocytes in the liver, or locally within areas of inflammation (Fig. 4). Peripheral blood neutrophils can oxidize procainamide to the toxic metabolite PAHA [107]. Mixing experiments and cell-free studies suggest that myeloperoxidase and reactive oxygen radicals are released by activated neutrophils during this chemical transformation [107–109]. Therapeutic levels of six different lupus-inducing drugs, including hydralazine, were found to be cytotoxic to EL4 cells when cultured in the presence of activated neutrophils [109]. It is possible that PAHA, oxygen radicals, and other metabolic byproducts contribute to the cytotoxicity [109, 110]. Kinetics of inhibition assays suggest that the observed cytotoxicity may be related to the ability of these drugs to serve as substrates for the myeloperoxidase enzyme. However, these highly reactive drug metabolites are

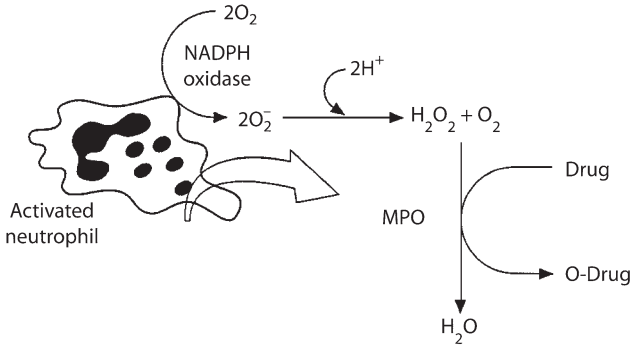


FIGURE 4 Mechanism for biotransformation of drugs by activated neutrophils. (From Rubin, R. L. (1994). *Lupus* **3**, 479, with permission.)

unstable and it has been difficult to detect their presence *in vivo*. Of interest but uncertain significance are the observations that PAHA and its nitroso derivatives have also been shown to bind covalently to histones [111], and autoantibodies to myeloperoxidase have been found in the serum of patients with DIL [112, 113]. These observations are consistent with the notion that myeloperoxidase-mediated metabolism may be involved in the pathogenesis of drug-induced autoimmunity.

Kretz-Rommel and Rubin examined the possibility that local production of PAHA may affect processes involved in T-cell tolerance to self-antigens [114]. Partial activation of T cells through T-cell receptor (TCR) engagement in the absence of costimulating signals can induce anergy to peptide antigens. Interference with this process may potentially affect autoimmune tolerance, resulting in the production of autoreactive T cells. Kretz-Rommel and Rubin [114] showed that treatment with PAHA, but not procainamide, will block the induction of anergy in cloned T cells by anti-CD3 antibody. Although PAHA is unstable in solution, its effects on T-cell anergy may be up to 8 h long. This model suggests that colocalization of potentially autoreactive T cells, activated neutrophils, and persistent inflammation is required to maintain the tolerant state. However, it is uncertain whether the concentrations of PAHA achieved *in vivo* approach the 1–5 μ*M* concentrations necessary to modify tolerance *in vitro*.

EPIGENETIC MECHANISMS

Evidence suggests that epigenetic mechanisms may also play a role in the pathogenesis of DIL and idiopathic lupus. In particular, biochemical modification of chromatin can affect gene expression and may be important in the induction of autoimmune diseases. These posttranslational modifications include methylation of DNA [115–118] and the phosphorylation, acetylation, and adenosine diphosphate (ADP)-ribosylation of histones [119].

ADP-Ribosylation and DIL

Limited studies have been performed examining the effects of lupus-inducing agents on ADP-ribosylation of histones. Poly-(ADP-ribose)-polymerase (PADPRP) is a chromatin-bound enzyme that catalyzes the transfer of ADP-ribose from nicotinamide to histones, including HI and H2B [120]. Autoantibodies reactive to HI and H2B, and to poly-(ADP-ribose) (PADPR) and PADPRP, have been demonstrated in patients with DIL and idiopathic lupus [121–123]. Interestingly ultraviolet light (UV), which has been implicated in the activation of lupus, also activates PADPR [124]. These observations have prompted further studies. Ayer *et al*. [125] examined the effect of procainamide and hydralazine on PADPRP activity in human T- and B-cell lines. These investigators found that therapeutic concentrations of procainamide increased PADPRP activity by up to 2.5-fold. Hydralazine and NAPA also increased the enzyme's activity, though to a smaller degree. It is possible that ADP-ribosylation of intracellular molecules, including histone proteins, may alter the structure and increase the immunogenicity of the self-antigens.

T-Cell DNA Hypomethylation and DIL

Evidence indicates an association between abnormal methylation of T-lymphocyte DNA and some forms of DIL. DNA methylation refers to the postsynthetic methylation of cytosine at the 5 position [115, 116, 126–129] and is one of the mechanisms regulating gene expression. For genes regulated by DNA methylation, hypomethylation of regulatory sequences correlates with gene expression, whereas methylation results in transcriptional suppression. DNA methylation can affect gene expression by several mechanisms. The methylation of cytosine residues can prevent the binding of some transcription factors if located in their recognition sequences. In addition, a family of specific nuclear proteins recognize and bind the methylated sequences, preventing the binding of transcription factors. Finally, it is now clear that the process of DNA methylation acts in concert with a related epigenetic process, that of histone deacetylation, in chromatin remodeling and suppression of gene transcription. Some methylcytosine binding proteins like MeCP2 attract a chromatin inactivation complex containing histone deacetylases, which condenses chromatin into an inactive configuration, inhibiting interactions

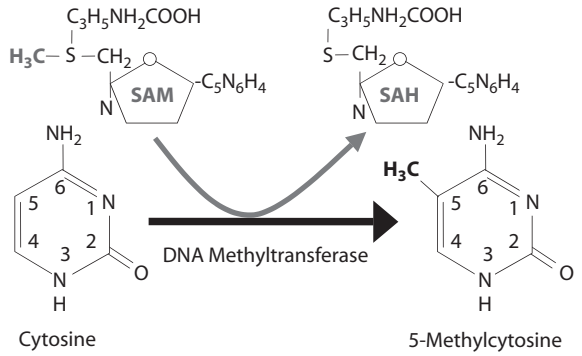


FIGURE 5 Formation of 5-methylcytosine. DNA methyltransferase enzyme catalyzes the transfer of the methyl group (CH_3) from *S*-adenosylmethionine (SAM) to (deoxy) cytosin producing 5-(deoxy)methylcytosine and *S*-adenosylhomocystine (SAH). (From Attwood *et al*. (2002). *Cell Mol. Life Sci.* **59**, 241, with permission.)

with transcription factors required for gene expression [130].

DNA methylation patterns are replicated during mitosis by the enzyme DNA (cytosine-5-)-methyltransferase 1 (Dnmt1) [115]. During mitosis, Dnmt1 recognizes hemimethylated CpG dinucleotides in the parent and daughter DNA strands, and catalyzes the transfer of the methyl group from *S*-adenosylmethionine to the cytosine residues in the unmethylated daughter DNA strand, producing symmetrically methylated sites and maintaining methylation patterns [131–134] (Fig. 5). The importance of DNA methylation is evidenced by its role in X-chromosome inactivation [135], and by its role in genomic imprinting [136]. Disruption of the *Dnmt1* gene in embryonal stem cells results in abnormal development and embryonic death [137], suggesting that DNA methylation is important in ontogeny. Additional DNA methyltransferases have been identified in the past few years, including Dnmt3a and Dnmt3b. In contrast to Dnmt1, these enzymes will methylate both unmethylated and hemimethylated DNA equally well, and may function as *de novo* methyltransferases. Homozygous deletion of these enzymes also causes death during embryonic development or in the early neonatal period [130].

Certain drugs inhibit the DNA methyltransferases, modifying methylation patterns with sometimes striking effects. For example, 5-azacytidine (5-azaC), an irreversible DNA methyltransferase inhibitor, causes 10T1/2 cells to differentiate into myocytes, adipocytes, and chondrocytes by altering expression of genes determining cellular differentiation [116, 138]. This approach has been used to clone the *MyoD* gene, which regulates myocytic differentiation [139, 140]. The observation that classic DNA hypomethylating agents like 5-azaC can affect the activity of histone deacetylases [141] suggests another potential mechanism whereby lupus-inducing drugs may alter gene expression and promote autoimmunity. However, the role of histone deacetylation has not been examined in DIL.

The role of DNA methylation in regulating T-cell function and gene expression has more recently been examined. 5-azaC treatment of cloned, antigen-specific $CD4^+$ cells caused them to lose their requirement for nominal antigen and respond to autologous antigen-presenting cells (APC) alone [142–145], thus becoming autoreactive. This was confirmed using a panel of $CD4^+$ antigen-specific human cloned cells, PHA activated polyclonal $CD4^+$ human T cells, cloned murine antigen-specific $CD4^+$ T cells, alloreactive murine $CD4^+$ T cells, and Concanavalin-A-activated $CD4^+$ murine T cells. This autoreactive response is specific for autologous or syngeneic APC, and requires specific MHC class II and T-cell antigen–MHC receptor interactions, indicating that the autoreactive response uses the TCR to transmit one of the activation signals.

The autoreactivity correlates with an approximately 10-fold overexpression of leukocyte function antigen-1 (LFA-1) (CDlla-CD18) on the surface of the treated cells [142–145], and transfecting human or murine T-cell clones with LFA-I causes an identical autoreactivity [146, 147]. In either 5-azaC-treated or LFA-1-transfected cells, small amounts of antibody to CD11a or intercellular adhesion molecule I (ICAM-1) (CD54), an LFA-1 ligand [144, 146, 147], completely inhibits the autoreactive response to APC without antigen, while only minimally inhibiting the response to the same APC presenting specific antigen. This suggests that LFA-I overexpression might contribute to the autoreactivity, and inhibiting the effects of the additional LFA-1 molecules reverses the effect. LFA-1 is a member of the β2 integrin family of adhesion molecules, and serves to stabilize the interaction of the TCR with class II MHC molecules on APC [148, 149]. Others have also shown that LFA-1–ICAM interactions are particularly important when the affinity of the TCR–class II interaction is low [150]. LFA-1 overexpression may cause autoreactivity by overstabilizing the normally low-affinity interaction between the TCR and class II MHC molecules, allowing T-cell activation by the class II determinants alone or with other peptides than those normally recognized by the T cell.

The observation that normal, antigen-specific T cells can be made autoreactive through chemical exposure prompted studies examining the relevance of this phenomenon to autoimmunity. A similar situation, in which $CD4^+$ T cells respond to host MHC determinants, occurs in the murine chronic graft-vs-host (GVH) disease model. In this system, adoptive transfer of $CD4^+$ T cells from a parent strain into the F1 hybrid of certain his-

toincompatible parental strains results in the development of an autoimmune disease with features of human lupus [151, 152]. The recipients develop an immune–complex glomerulonephritis, splenomegaly, and arthritis, as well as an autoimmune liver disease. Serologic features include hypergammaglobulinemia, ANA, and anti-double-stranded DNA (dsDNA) antibodies. Subsequent studies demonstrated that therapeutic concentrations of procainamide or hydralazine inhibit DNA methylation in human and murine T cells [153–155]. Procainamide was subsequently shown to reversibly inhibit human T-cell DNA MTase activity in a dose-dependent fashion [153, 154]. Activated normal human and murine $CD4^+$ T-cell clones treated with therapeutic concentrations of procainamide or hydralazine also became autoreactive, similar to 5-azaC-treated cells [142–145]. This autoreactivity also correlates with increased LFA-1 expression. Conversely, NAPA, a metabolite of procainamide that does not cause lupus, is about 100-fold less potent in its ability to induce T-cell autoreactivity *in vitro*. More recently, it was shown that UV light, implicated in activating lupus, also inhibits T-cell DNA methylation, increases LFA-I expression, and induces autoreactivity at relatively modest doses [146]. It is likely that DNA demethylating drugs also affect other methylation sensitive genes that may contribute to autoimmunity. Our group examined the effects of 5-azaC on the expression of approximately 7000 T-cell genes using a microarray genome scanning approach [156]. One hundred eighteen genes were identified that have greater than two-fold increases in expression following the drug treatment, including IFN-γ and CD70 as B-cell costimulating molecules, and perforin as a molecule participating in cytotoxic responses.

Defects in signal transduction have been identified in lupus T cells that may account for the aberrant cellular and humoral immune responses in lupus patients [157]. Evidence has shown that Dnmt1 is upregulated following T-cell stimulation in part by signals transmitted through the MAPKK-catalyzed ERK phosphorylation pathway, and that decreased signaling in the pathway may result in T-cell DNA hypomethylation in lupus patients [158]. Interestingly, although hydralazine has no direct effect on Dnmt1 activity, it may indirectly decrease Dnmt1 expression and function via inhibition of the ERK signaling pathway [159, 160]. Together, these studies suggest that certain environmental agents may also induce T-cell autoreactivity by inhibiting T-cell DNA methylation, thereby altering T-cell gene expression.

T cells made autoreactive by treatment with DNA methylation inhibitors interact with other cells of the immune system in a way that could contribute to the development of autoimmunity. For example, coculture of B lymphocytes with $CD4^+$ T cells treated with DNA methylation inhibitors, including 5-azaC, procainamide, and hydralazine, induces differentiation of the B cells into IgG-secreting cells, without the addition of antigen or mitogen [161]. An identical amount of antibody secretion was induced with supernatant from activated treated or untreated cells, suggesting that the autoreactive T cells were activated by MHC determinants on autologous B cells, and the T cells then secreted B-cell differentiation factors, such as IFN-γ, interleukin-4 (IL-4) and/or IL-6, activating the B cells. However, a role for cell–cell communication through surface molecules such as CD40 and the CD40 ligand [162] has not been excluded. This mechanism could contribute to the polyclonal B-cell activation observed in patients with procainamide-induced lupus [39–41]. In addition, the autoreactive cells spontaneously induce apoptosis in autologous or syngeneic macrophages [144, 145] through pathways including Fas, TWEAK, and TRAIL [163]. These studies have led to the proposal that a similar T-cell modification occurring *in vivo* could result in the release of intracellular and nuclear antigens. If the autoreactive T cells traffic to lymphatic tissue such as the lymph nodes or spleen, macrophage lysis in the immediate proximity of B cells and other T cells, together with the release of cytokines like IL-4 and IL-6, might contribute to the generation of autoantibodies. Macrophage lysis in the liver or spleen may also contribute to impaired immune-complex clearance.

T-Cell DNA Methylation in Idiopathic Lupus

The DNA hypomethylation model suggests that certain chemical agents can inhibit T-cell DNA methylation, modify T-cell LFA-1 expression, and induce autoreactivity. Furthermore, the autoreactive T cells can interact with MHC-identical B cells and macrophages, inducing macrophage apoptosis and B-cell activation, and *in vivo* the same cells can cause a disease with many manifestations of human lupus (see the following section Animal Models). This model thus predicts that patients with active lupus might have hypomethylated T-cell DNA, T cells that overexpress LFA-1 and lyse autologous macrophages, and evidence for B-cell activation due to IL-4 or IL-6 production. Evidence for these predictions has been sought in patients with active lupus. Using reverse-phase high-pressure liquid chromatography (HPLC) to quantitate total genomic deoxycytosine (dC) content, patients with active, but not inactive, lupus were shown to have significantly less d^mC content than age-matched controls [164]. A small decrease was also found in T cells from some patients

with rheumatoid arthritis, but not in T cells from patients with chronic infections, osteoarthritis, gout, or multiple sclerosis. These results have been confirmed by an independent group [165]. The lupus patients also had approximately one-half the amount of T-cell DNA MTase enzymatic activity as healthy controls and rheumatoid arthritis patients. This decrease is similar to that achieved *in vitro* with procainamide [164] and suggests that impaired DNA MTase activity may be common to T cells from lupus patients and the *in vitro* model. More recent evidence suggests that this may be due to a failure to upregulate Dnmt1 following T-cell stimulation, possibly due to a defect in the ras signaling pathway [166]. Evidence for T-cell LFA-1 overexpression has also been sought. A subset of peripheral blood T cells were found to express high levels of LFA-1 relative to other cell surface markers in 25 of 49 lupus patients compared with no increase in LFA-1 in more than 50 controls, including normal individuals, patients with active infections, and patients with multiple sclerosis. There was also a strong positive correlation between the number of cells expressing high levels of LFA-1 and clinical disease activity [161]. These observations have been confirmed by an independent group [167]. When isolated from other T cells, the population with high LFA-1 expression was found to be autoreactive, in that the cells spontaneously lysed autologous, but not allogeneic, macrophages, again similar to T cells treated with DNA hypomethylating agents. Patients with active lupus were found to have circulating apoptotic monocytes, suggesting that macrophage killing was occurring *in vivo* [168]. Finally, IL-6 overexpression by T and B cells also occurs in lupus patients [169–171], and anti-IL-6 will inhibit the spontaneous B-cell Ig secretion characteristic of lupus. Together, these observations are consistent with the predictions generated by the DNA hypomethylation model, and suggest that a similar mechanism may contribute to idiopathic lupus. However, whether similar changes in T-cell DNA methylation, LFA-1 expression, and T-cell autoreactivity occur in DIL remains to be established.

ANIMAL MODELS

Early Animal Models

Since the initial description of DIL, investigators have tried to reproduce the disease in animals. Most of these reports involved administering large (and often nonpharmacological) amounts of test drugs to laboratory animals. Unfortunately, the majority of studies have yielded conflicting results, or results with uncertain clinical significance. As early as 1956, Comens reported induction of a lupus-like disease in dogs given large doses of oral hydralazine over a few weeks to months [172]. Disease manifestations included anemia, hypergammaglobulinemia, lupus erythematosus (LE) cells, renal disease (fibrinoid degeneration and "wire-loop" lesions), weight loss, weakness, convulsions, and sometimes death. Other researchers were able to reproduce the acute, severe, and prolonged illness induced in dogs given toxic doses of hydralazine, but were unable to confirm the more specific lupus-like features such as the presence of LE cells or renal disease [173, 174].

A few researchers investigated the effects of lupus-inducing drugs in guinea pigs, in part because of a report that this species produces LE cells when given serum from human lupus patients [175]. Oral hydralazine was found to induce LE cells in 7 out of 15 guinea pigs [176]. A few of these animals also developed anemia, leukopenia, elevated α2 globulins, anorexia, weight loss, and eventually death within 4 months. However, histologic abnormalities were not documented. Braverman and Lerner [177] fed hydralazine to guinea pigs (10–100 mg/kg) for up to 3 months, and demonstrated that 17% of the animals developed LE cells and/or ANAs in their blood or sera. Minimal thickening of the renal glomerular basement membrane was seen in 17% of the animals, but otherwise no histologic abnormality was found. In a similar experiment, Leovey *et al*. [178] found circulating anti-hydralazine antibodies in up to 71% of guinea pigs receiving oral or intramuscular hydralazine. Twenty-three percent and 27% of these animals also developed LE cells and ANA activity, respectively. Increased α2 globulins, anemia, leukopenia, interstitial myocarditis, thickening of the glomerular basal membrane, iridocyclitis, chronic hepatitis, and amyloid-like substance in the liver and spleen were also occasionally observed. Ellman *et al*. [179] immunized guinea pigs with hydralazine in Freund's complete adjuvant, and showed strain-specific differences in anti-hydralazine antibody production and delayed hypersensitivity to hydralazine. ANA and anti-DNA antibodies were not detected, and strain differences in hepatic acetylation rates were not confirmed.

Cannat and Seligmann [180] administered hydralazine and isoniazid orally to two inbred strains of mice, BALB/c and C57BL/6, for 3–12 months. They demonstrated that mice treated with either drug developed a positive ANA test after 6 months of treatment, in titers significantly greater than age-matched controls. Ten Veen and Feltkamp [181] examined the development of ANA and anti-native DNA antibodies in three inbred strains of mice (BALB/c, C57BL/6, and A/Jackson) receiving hydralazine, isoniazid, procainamide, α-methyldopa, and chlorthalidone in their drinking water. They were able to detect increased ANA

production in all treated mice compared with age-matched controls. Anti-native DNA antibodies were detected in A/JAX mice without drug treatments, confirming a previous report [182]; otherwise no increased titers of anti-native DNA antibody were found. IgG and IgM deposition was found in the kidneys of treated and untreated mice. These investigators also described increased glomerulosclerosis and immune-complex glomerulonephritis in drug-treated mice, beyond expected age-related changes [183]. The ability of different drugs to induce autoantibody production or clinical disease in other animals has also been tested. Yamauchi *et al*. [184] immunized rabbits with hydralazine–human serum albumin conjugates over 12–17 weeks, and found rising titers of anti-hydralazine, anti-single-stranded DNA (ssDNA), and anti-native DNA antibodies. Histologic changes were not observed in the immunized rabbits. Administration of hydralazine subcutaneously or orally failed to induce DIL in rats [185] or swine [186].

In summary, earlier attempts to establish animal models of DIL have yielded at best mixed results. In general, these studies have not gained wide acceptance, often because of failure of other investigators to reproduce the findings. In many instances, the doses of drugs administered to the animals were much higher than therapeutic doses that caused DIL in humans. Finally, most studies only reported induction of LE cells or the presence of a positive ANA test with no histologic evidence for disease.

Recent Animal Models

In Vivo Effects of PAHA

PA14A, a reactive metabolite of procainamide discussed previously, has more recently been shown to induce autoantibodies in mice, possibly by interfering with central T-cell tolerance [187]. Anti-chromatin antibodies were detected in C57BL/6 × DBA/2 Fl mice following two intrathymic injections with PAHA. Interestingly, the PAHA-induced antibodies showed specificity to the (H2A–H2B)–DNA complex, similar to that found in the sera of many patients with procainamide-induced lupus. The immune response appears to be clonally restricted, as there was no evidence of polyclonal B-cell activation. Similar to earlier reports of DIL in animal models, however, no evidence for histologic changes was presented. Thus, this model suggests a mechanism by which procainamide can induce autoantibodies, although their pathologic significance is uncertain. Limiting this model, though, is that high concentrations of PAHA (20 µl of a 4 m*M* solution injected into each thymic lobe) are required. How such high concentrations of this highly reactive metabolite would be generated in the thymus, and whether a similar result would be found following thymic involution and analogous to the elderly patients usually receiving procainamide, is uncertain.

In Vivo Effects of T-Cell DNA Hypomethylation

The effects of T cells modified by treatment with DNA methylation inhibitors have been examined *in vivo*. In the first published report, DBA/2 mice were used, in part because this strain does not develop autoimmunity, and in part because others have used this strain successfully in the chronic GVH model [151, 152, 188]. Alloreactive and mitogen-stimulated $CD4^+$ T-cell lines, established from DBA/2 mice, were treated with 5-azaC or procainamide, and shown to overexpress LFA-1 and respond to syngeneic APC without antigen, similar to human cells [144]. The treated T cells secreted IL-4, IL-6, and IFN-γ, suggesting that both Th1 and Th2, or ThO, cells were present. The autoreactive cells also killed syngeneic macrophages without antigen, similar to the human system [143]. The autoreactive cells were then injected intravenously into normal unirradiated female DBA/2 mice. The recipients developed hematuria, proteinuria, and red-cell casts beginning 7–21 days after each injection and lasting 7–14 days. Histologic analysis demonstrated a proliferative glomerulonephritis with IgG, IgM, and IgA immune-complex deposition, as well as IgG deposition at the dermal–epidermal junction resembling the lupus band test. Antibodies to histones and ssDNA in titers comparable to those found in New Zealand Black/New Zealand White (NZB/NZW) mice were also found in the mice receiving drug-treated cells. Controls included mice receiving untreated cells, heat-killed 5-azaC-treated cells, or unstimulated procainamide-treated $CD4^+$ T cells. The controls did not develop pathologic or serologic findings.

These results were confirmed using a cloned conalbumin-reactive murine Th2-cell line (D10.G4. 1, $H\text{-}2^k$, derived from AKR mice) [145]. Procainamide and 5-azaC treatment again induced LFA-1 overexpression and autoreactivity. Drug-treated cells, as well as untreated cells, produced IL-6 and relatively smaller amounts of IL-4, but no detectable IL-2 or IFN-γ. Syngeneic macrophages were again found to die by apoptosis following coculture with either drug-treated cells alone or with untreated cells plus conalbumin. Chromium and fluorescein labeling studies demonstrated that autoreactive as well as untreated cells homed to the spleen, as predicted by the model and by earlier trafficking studies [189, 190]. Adoptive transfer of the autoreactive D10 cells into AKR mice produced

a more severe immune-complex glomerulonephritis, pulmonary alveolitis, and central nervous system lesions resembling human lupus with vascular fibrinoid necrosis, cerebral infarcts, and sterile meningitis. Other pathologic findings included liver lesions with periportal inflammatory infiltration and bile duct proliferation resembling primary biliary cirrhosis. The mice also developed significant titers of anti-ssDNA, anti-dsDNA, and anti-histone antibodies. The reason for the difference in disease severity between the DBA/2 and AKR models is not known. Possible explanations include the use of a cloned Th2 line in the AKR model, with less inhibition by Thl cells, or differences in the genetic makeup of the hosts. However, a more recent study showed that cloned Th1 cells overexpress LFA-1 and become autoreactive following 5-azaC treatment, similar to Th2 cells [191]. Adoptive transfer of the autoreactive Th1 cells also induces similar anti-DNA antibodies. Comparison of effector mechanisms suggests that the T-cell cytokine repertoire is probably not crucial in this system. In contrast, macrophage/monocyte killing is common to all three models and may be an important early step providing antigenic nucleosomes and decreasing clearance of apoptotic materials.

The D10 model has also been used to compare relative potency of procainamide, NAPA, hydralazine, and phthalazine. NAPA does not cause DIL, whereas phthalazine is the parent compound of hydralazine but lacks the hydrazine side chain, which has been implicated in autoimmunity. Procainamide and hydralazine are also more potent DNA methylation inhibitors than NAPA [155]. Mice receiving hydralazine and procainamide developed a similar disease. Phthalazine was less potent than hydralazine, and NAPA had no effect in this system. These results lend further credibility to the DNA hypomethylation model, and suggest a reason for the differing potencies of these drugs for inducing DIL.

Gender predisposition to lupus has also been examined in the DNA hypomethylation model. Lupus is predominantly a female disease, and sex hormones have been shown to play an important role in modulating immune responses in humans and murine models of lupus [192–194]. Consistent with data from humans and autoimmune prone mice, normal female mice receiving procainamide-treated T cells develop a more severe disease, and have higher titers of autoantibodies than age-matched male recipients [195]. Oophorectomized female mice develop a much milder disease, with anti-DNA titers similar to orchiectomized and normal male mice receiving drug-treated cells.

Further studies demonstrated gender-specific effects of T-cell trafficking *in vivo*, with up to seven times as many T cells homing to the female spleens compared to male spleens, and this female-specific increase in splenic homing also decreased following oophorectomy. Splenectomized females did not develop autoantibodies or autoimmune pathology, supporting an important pathogenic role for the spleen in this model. These studies also indicated that relatively few cells accumulating in the spleen (a total of approximately 360,000) are sufficient to induce autoimmunity in mice [195]. These observations suggest that female-specific increases in T-cell trafficking to the spleen may cause increased disease severity in this model, presumably due to effects of female sex hormones on T-cell trafficking patterns. Estrogens have been reported to upregulate endothelial cell adhesion molecule expression [196], providing a plausible explanation for the gender-specific differences in T-cell trafficking observed. However, the effect of gender appears to be less prominent in DIL [2]. Since DIL affects primarily older individuals, this may be due to the decline in sex-hormone production with aging. Alternately, gender-specific factors may play a less important role in DIL.

In summary, two well-documented *in vivo* models have been described that suggest mechanisms that could contribute to the pathogenesis of DIL. The PAHA model generates autoantibodies resembling those seen in procainamide-induced DIL. Limitations of the model are described earlier. The DNA methylation model provides a common mechanism for procainamide and hydralazine, but the pathologic lesions produced more closely resemble idiopathic lupus than DIL, which characteristically spares the kidney and central nervous system [2]. Furthermore, both systems are somewhat artificial, and evidence that either is operant in human DIL remains indirect. Further studies will be required to determine whether the PAHA model, the DNA methylation model, neither, or both contribute to human DIL. However, it is reasonable to propose that both mechanisms contribute to human DIL. The diseases induced by procainamide and hydralazine have distinct differences, including clinical manifestations and autoantibody specificity [2]. It is possible that the influence of PAHA could modify disease manifestations induced by DNA hypomethylation resulting from procainamide treatment, accounting for these differences. It is anticipated that both models will be useful for understanding DIL and possibly idiopathic lupus as well.

XENOBIOTICS

Environmental exposure to certain toxins, known as xenobiotics, has been linked to the development of lupus-like illnesses and autoimmunity in humans and in

several animal models. Selected environmental factors include heavy metals, occupational and industrial contaminants, foods, and dietary supplements. This is a broad field, and it is difficult to discuss every environmental agent linked to autoimmunity. Therefore, only selected agents, including those with more recent associations, will be discussed. Interested readers are referred to some excellent reviews on this subject [197–202].

Foods and Dietary Supplements

L-Canavanine

A lupus-like syndrome associated with the ingestion of the nonessential amino acid L-canavanine has been described in humans and animals. Dietary supplements containing alfalfa were initially implicated in triggering disease exacerbations in lupus patients [203]. In 1981, Malinow *et al*. [204] reported that ingestion of alfalfa seeds was the cause of a reversible autoimmune hemolytic anemia and pancytopenia in one patient. L-Canavanine was subsequently shown to be the principal ingredient in alfalfa seeds responsible for this disease. Primates fed alfalfa seeds, alfalfa sprouts, or L-canavanine developed a similar disease, with hemolytic anemia, high-titer ANA, and anti-dsDNA antibodies. Withdrawal of alfalfa seeds from the diet results in resolution of the clinical condition, although autoantibodies remain detectable for up to 2 years [205, 206].

L-Canavanine is an amino acid structurally similar to L-arginine, and can compete with L-arginine for the charging of transfer RNA (tRNA) Arg. The isoelectric point of canavanine is lower than arginine. The substitution may change the structure and function of the resulting proteins, including arginine-rich histone proteins, altering their immunogenicity [207, 208]. L-Arginine also affects the immune system, including the arginine–nitrous oxide pathway. L-Canavanine may therefore influence the immune system by several mechanisms. At high doses, this nonessential amino acid inhibits the proliferative response of human mononuclear cells to mitogens through its effect on DNA synthesis. L-Canavanine may also affect B-cell function. Under *in vitro* conditions, L-canavanine can alter the surface charge of B cells, but not T cells, from NZB/NZW Fl mice [209]. It is possible that the activated B cells cause increased incorporation of canavanine into surface proteins, modifying the surface charge [210]. Interestingly, L-canavanine can also stabilize DNA in the more antigenic Z configuration [198]. The enhanced antigenicity may contribute to the development of anti-DNA antibodies.

Anilines and L-Tryptophan

Two more recent epidemics of environmentally induced autoimmunity highlight the pathologic potential of chemicals the human body may be exposed to and their clinical sequellae. Ingestion of rapeseed oil denatured with aniline was responsible for reports of toxic oil syndrome (TOS) in Spain in 1981 [211]. Affected patients developed acute pneumonitis, followed by a chronic illness characterized by a scleroderma-like skin disease, neuromyopathy, and sicca syndrome. Eosinophilia, elevated IgE, and occasionally the presence of ANAs and other autoantibodies were also reported. A second outbreak of a similar disease, the eosinophilic myalgia syndrome (EMS), was reported in the late 1980s [211, 212]. Epidemiologic studies identified the dietary supplement L-tryptophan, an essential amino acid, as the causative agent in the outbreak. Although not conclusive, it appears that contaminants or by-products introduced during the manufacturing process by a single manufacturer may be the culprit [212]. The precise mechanism underlying this disease has not been worked out, and only a small percentage of people who ingested the offending agents were affected. Human and animal studies suggest that immunogenetic and metabolic phenotypes are important factors in the expression of both diseases.

Increased frequencies of HLA-DR3 and HLA-DR4 [198], enhanced T-suppressor cell activity, and antibodies to T and B cells have all been reported in patients with TOS. Interestingly, patients with TOS may also have abnormal tryptophan metabolism, including abnormal IFN-γ activation of indoleamine-2,3-dioxygenase, an enzyme involved in tryptophan breakdown metabolism. A number of studies have been performed using a variety of animal systems. Unfortunately, no animal model of TOS that truly resembles the human disease has been described. Mice fed aniline develop high serum IgE titers and increased IL-1β, IL-6, and IFN-γ [213]. It was suggested that a breakdown in tolerance and polyclonal B-cell activation may be important in this disease. Only one study reported lung toxicity in rats [214], with other researchers reporting uniformly negative lung pathology in a number of animal systems [215–217]. In one long-term study, rats were fed for 200 days with "case oil" obtained from the National Center for Food and Nutrition in Majadahonda [218]. These rat developed a large increase in dermal collagen deposition. Electron microscopic analysis showed increased collagen fibers of uniform diameter arranged in thick bundles. A pathogenic mechanism for TOS akin to GVH has also been proposed [219–221]. Both diseases display a biphasic clinic course. 1-phenyl-5-vinyl-2-imidazolidinethione (IZT), a possible constituent of

unrefined rapeseed oils and a possible reaction product of aniline, shares structural similarities with anticonvulsants (such as phenytoin) and antithyroid drugs known to be capable of inducing GVH or lupus-like illnesses in humans. However, only traces of IZT could be found in the case oils. The role of IZT in TOS is currently unclear [222, 223].

Eosinophilic myalgia syndrome has been linked to HLA-Aw33, HLA-B44, and HLA-DR6 [224]. Abnormal tryptophan metabolism, enhanced kynurenine production, and roles for IFN-γ and transforming growth factor β (TGF-β) have all been proposed for this disease. Kynurenine is a metabolic product of L-tryptophan. Of interest, increased urinary excretion of kynurenine and plasma kynurenine levels has been reported in patients with scleroderma, CREST syndrome, and eosinophilic fasciitis [225, 226]. Female Lewis rats fed with L-tryptophan have enhanced tryptophan metabolism with increased plasma L-kynurenine levels [227, 228]. Other features of this model include suppression of the hypothalamic–pituitary–adrenal (HPA) axis and pathologic findings including fasciitis and perimyositis. However, several features of the human disease are absent in this model, including pulmonary and skin involvement and peripheral or tissue eosinophilia. The role of eosinophils in this and other eosinophilic rheumatic diseases is unclear. Cytokine-mediated activation of eosinophils, with resulting degranulation and release of toxic products (e.g., major basic protein, eosinophil-derived neurotoxin), may contribute to tissue injury [212]. Other alterations of cellular immunity have also been described, including phenotypic changes in peripheral blood mononuclear cells and activation of peripheral T lymphocytes. A contaminant, termed peak E, was isolated from contaminated oil [229, 230]. The compound was determined to be 1,1′-ethylidenebis(tryptophan), composed of two L-tryptophan molecules joined by an ethylidene bridge. A definitive pathogenic role of this novel amino acid in EMS has not been established. Despite clinical similarities with TOS, a convincing link between EMS and TOS has not been shown [231].

Heavy Metals

Gold, mercury, cadmium, and to a lesser extend lithium, chromium, and zinc have all been associated with autoimmune diseases. Lupus-like illnesses have been reported with exposure to gold, chromium, and lithium. How various heavy metals can induce autoimmunity is unclear. One proposed mechanism is that they may modify nuclear antigens by binding to carboxyl, nitryl, sulfhydryl, and phosphoryl groups, rendering them immunogenic.

Gold

Gold salts have been used in the treatment of human diseases for centuries, and are still an important therapy for rheumatoid arthritis. Between 1 and 3% of rheumatoid arthritis patients receiving gold salts develop a reversible autoimmune thrombocytopenia [232]. This is associated with the development of antibodies that bind to platelets in the presence of gold or its metabolites. The thrombocytopenia occurs following weeks to months of treatment, and is usually reversible within a week after cessation of therapy. Six to 17% of gold-treated patients also develop nephropathy with proteinuria [233, 234]. Biopsy studies suggest that most cases of renal disease are due to membranous glomerulonephritis. Other changes, including minimal change glomerulonephritis and interstitial nephritis, have also been reported.

The pathogenic mechanisms underlying gold-induced renal disease are unclear. HLA-DR3 and HLA-B8 have been shown to be a risk factor for gold-related renal disease in rheumatoid arthritis patients, and may be present in up to half of these patients [235]. Immune-complex deposition is commonly found in the glomeruli of affected patients, and is important in membranous glomerulonephritis. Mononuclear phagocytes can metabolize Au (I) to Au (III), a reactive metabolite, *in vitro* [236, 237]. It is possible that subsequent oxidation of nuclear proteins may allow sensitization of T cells to the modified antigen. To help understand this disease, an animal model of gold-induced autoimmunity was developed. Brown Norway (BN) rats injected with gold salts develop an autoimmune disease, with polyclonal B-cell activation, lymphoproliferation, IgE hypergammaglobulinemia, and the production of autoantibodies [238]. These animals also develop an immune-complex glomerulonephritis. Anti-glomerular basement membrane antibodies, anti-tubular basement membrane antibodies, and anti-brush border antibodies have all been demonstrated. There may be strain specificity in this model, since Lewis rats receiving gold do not develop glomerulopathy, although evidence of interstitial nephritis and polyclonal B-cell activation can still be found. However, the substantial differences between this model and gold-induced human disease make the relevance of the model to the human disease uncertain, and the mechanisms involved remain unknown.

Mercury

Mercury was once widely used as a component of laxatives, diuretics, and teething powders. The heavy metal can still be found in fish, dental amalgams, electrical

products, pharmaceuticals, and paper and pulp mills. Renal complications, including proteinuria, nephrotic syndrome, and immune-mediated glomerulonephritis have all been documented following mercury exposure [239, 240]. A role for mercury in the generation of anti-mitochondrial antibodies and in human primary biliary cirrhosis has been proposed. Mercuric chloride may interact with disulfide- or thiol-groups in bile duct cells, altering the structure of mitochondrial enzymes and increase their immunogenicity [241, 242]. Similarities between clinical features of Kawasaki's disease and mercury intoxication, including fever, erythematosus mucosa, lymphadenopathy, and increased IgE levels, have been noticed by different authors. However, no definite association has yet been found [243–245].

A number of well-characterized animal models of mercury-induced autoimmunity have been described [246–250]. In most cases, the animals develop autoantibodies and kidney disease following mercury treatment. Features of mercury-induced autoimmune disease in BN rats include IgE hypergammaglobulinemia, autoantibodies to ssDNA and glomerular basement membrane, lymphoproliferation, mucositis, a Sjögren's-like syndrome, and an immune-complex glomerulonephritis. The renal disease in mercury-treated rats is characterized by a two-stage process, with initial linear then granular deposition of IgG anti-laminin antibody in the glomerular and tubular basement membrane.

Studies of animal models suggest that susceptibility to mercury-induced autoimmunity may be genetically controlled, with considerable variation in the autoimmune features among the strains and species studied [251, 252]. Granular deposits of IgG, IgM, and C3 have been detected in the kidneys of mercury-treated mice, and eluates from immune deposits of SJL mice contain anti-nucleolar antibodies. B10.S mice developed anti-nuclear (e.g., anti-chromatin and/or anti-histone) and anti-nucleolar (e.g., anti-fibrillin, or U3 ribonucleoprotein (RNP)) antibodies, with IgG deposition in vessel walls and glomerular basement membranes. Development of anti-fibrillarin ANAs in mercury-treated mice has been linked to the I and S regions of the H2 complex. In SJL mice, susceptibility to anti-nucleolar antibody production resides in the $H2^a$ locus, is inherited in a codominant fashion in crosses with strains carrying the $H2^b$ and $H2^d$ haplotypes, and is strongly modulated by non-H2 genes. Rats that carry the RT1 haplotype (Lewis, Fischer 344) do not develop autoimmunity, in contrast to the Rtn haplotype, which are susceptible to anti-glomerular basement membrane and laminin antibodies. Rats with other haplotypes show intermediate susceptibility.

Mercury-induced autoimmunity is a T-cell dependent disease, and shares some similarity with the murine chronic GVH autoimmunity model. Normal BN rats develop the disease following adoptive transfer of $CD4^+$ T cells from mercury-treated rats [253, 254]. Host $CD8^+$ cells are important, as treatment with anti-CD8 antibodies exacerbates the disease [255]. T-cell depleted rats (BN rnu/rnu and BN "B" rats) do not develop mercury-related autoimmunity, unless their T cells are reconstituted [256]. Finally, low-dose cyclosporin-A can ameliorate this disease in rats, supporting a role for T cells [257]. Autoreactive T-cell clones have been isolated from mercury-treated animals, and a T-cell response to modified Ia antigens may produce autoimmunity by mechanisms similar to chronic GVH disease [258]. It has also been proposed that T cells recognizing mercury–nuclear protein (e.g., fibrillarin) complexes may result in cross-reactivity with the native self-antigen, possibly by epitope spreading.

A role for anti-idiotype antibodies in mercury-induced autoimmunity has also been proposed. B cells producing antibodies recognizing a V κ-associated idiotype on anti-glomerular-basement-membrane antibodies have been isolated from BN rats [259]. Corresponding auto-anti-idiotypic antibodies can be identified in BN mice with the disease. However, a pathogenic role for these anti-idiotypic antibodies has not been confirmed. Other mechanisms implicated in mercury-related autoimmunity include a possible role for cytokines. Depressed IL-12 production, as well as preferential expansion of Th2 T cells and upregulation of IL-4, have both been reported [260, 261].

Cadmium

Exposure to cadmium in the workplace has been linked to the development of renal disease, including proteinuria and interstitial and glomerular disease [197]. Rodents may be more prone to develop cadmium-induced autoimmune disease than humans. Susceptibility to cadmium toxicity varies with different animal strains, underscoring the importance of genetic factors. Sprague Dawley rats treated with this heavy metal have been reported to develop proliferative glomerulonephritis with immune-complex deposition and anti-laminin antibodies [262]. On the other hand, BN rats are resistant to the disease. Low levels of exposure are sufficient to cause ICR mice to develop ANAs. Finally, BALB/c mice will develop autoantibodies after treatment with high-dose cadmium, but C57BL/6 mice are resistant. Again, the mechanism is unknown.

Vinyl Chloride and Industrial Solvents

Exposure to vinyl chloride has been linked to a number of different autoimmune diseases in humans.

These include a scleroderma-like illness, Raynaud's phenomenon, mixed connective tissue disease, arthritis, neuropathy, and vasculitis [263]. Ingestion of trichloroethylene and other chemicals in well water may also be responsible for an outbreak of a lupus-like syndrome in Arizona [264]. Similar to most environmental exposures, the underlying pathogenic mechanisms are poorly understood. Vinyl chloride appears to be toxic to endothelial cells [265]. The resin can bind to nucleotides, and may induce production of antibodies against nuclear antigens [266]. The oxidized metabolites of vinyl chloride are highly reactive [267]. They can bind to both sulfhydryl and amino groups, causing oxidative cell damage with increased immunogenicity of altered self-antigens. Rats exposed to vinyl chloride for prolonged periods of time have increased collagen deposition in the skin.

Silica, Silicon, and Silicones

Silicon is the second most abundant element on the earth. In nature, it is found in combination with oxygen to yield various forms of silicas, silicates, glasses, and sand. Silicone is a synthetic polymer containing a repeating Si—O backbone, and the organic groups attached to the silicon atom via silicon—carbon bonds determine the class of silicone. Exposure to silica dust has been linked to the development of autoimmune diseases, including scleroderma, and to a lesser extent rheumatoid arthritis. A more recent Spanish report examined the incidence of systemic autoimmune diseases in a group of workers at a scouring-powder factory [268]. The scouring powder is composed of 90% silica. Seventy-two percent of the patients were found to be ANA positive, and 64% of the 50 studied workers were found to have a systemic autoimmune illness. These included 6 patients with Sjögren's syndrome, 5 with scleroderma, 3 with SLE, 5 with a lupus-scleroderma overlap, and 19 with an undefined collagen disease. The incidence of lupus and other autoimmune diseases in this group is much higher than would be expected in the general population. Although interesting, further studies will be needed to determine if the association truly exists.

Silica particles have been shown to induce IL-1 production by macrophages [269]. Animal studies also suggest a potential role for TNF-α. This proinflammatory cytokine may potentially play a role in inflammatory and fibrotic reactions. Workers exposed to silica may have decreased numbers of circulating T cells, and increased numbers of circulating B cells [270]. Hilar lymph nodes in some patients also showed reversal of normal T-helper cells: suppressor-cell ratios, with increased T-helper and decreased T-suppressor cells.

Considerable controversy exists regarding the role of silicone breast implants in the induction of autoimmune diseases, including scleroderma and SLE [271–274]. The pathologic and immunologic effects of silicone have been studied in detail [275, 276]. However, the clinical significance of these observations is unclear. Local inflammatory changes are common. Pathologic studies often show granulomatous, macrophage-rich chronic inflammatory reactions. Silicone has been found to exhibit adjuvant activity in a rat model, and monocytes and macrophages produce IL-1β when exposed to silicone rubber in culture. Fibroblasts have twice as much rough endoplasmic reticulum when they are grown on silicone surfaces compared with polystyrene, and mice exposed to silicone gel have increased numbers of granulocyte-macrophage colony-forming units in the bone marrow, increased hepatic macrophage uptake of radio-labeled sheep erythrocytes, and reduced responsiveness of natural killer cells against YAC target cells. However, despite anecdotal case reports of an association between silicone breast implants and autoimmunity, large numbers of epidemiologic studies including meta-analysis of up to 24 studies have failed to show a causal relationship with any specific autoimmune disease including lupus [277–281].

Hair Dye and SLE

Hair dye has been proposed as a possible etiologic agent in SLE. Hydrazine, an aromatic amine implicated in DIL (see previous discussion), is a common component of hair dyes, and the compound can be absorbed through the scalp [282, 283]. One case-control study suggested an association between permanent hair dye use and connective tissue disease [284]. However, other researchers were unable to confirm this finding [285, 286]. At present, it seems unlikely that this exposure is a significant risk for the development of SLE.

CONCLUSIONS

Many mechanisms have been proposed for how various drugs may cause DIL. Of these, the evidence is best for procainamide and hydralazine, in part because these have been the most extensively studied. The more recent development of animal models for autoimmunity induced by these two drugs is likely to greatly accelerate our understanding of the underlying mechanisms involved.

References

1. Hoffman, B. J. (1945). Sensitivity of sulfadiazine resembling acute disseminated lupus erythematosus. *Arch. Dermatol. Syph.* **51**, 190.
2. Yung, R. L., and Richardson, B. C. (1945). Drug-induced lupus. *Rheum. Dis. Clin. North Am.* **20**, 61.
3. Yung, R. L., and Richardson, B. C. (2003). Drug-induced lupus. In "Rheumatology" (M. Hochberg, A. Silman, J. Smolen, M. Weinblatt, and M. Weisman, Eds.), 3rd ed., in press. Harcourt Health Sciences, London.
4. Adams, L. E., and Hess, E. V. (1991). Drug related lupus. Incidence, mechanisms and clinical implications. *Drug Safety* **6**, 431.
5. Russell, A. S., and Ziff, M. (1968). Natural antibodies to procainamide. *Clin. Exp. Immunol.* **3**, 901.
6. Hahn, B. H., Sharp, G. C., Irvin, W. S., *et al*. (1972). Immune response to hydralazine and nuclear antigens in hydralazine-induced lupus erythematosus. *Ann. Intern. Med.* **76**, 365.
7. Carpenter, J. R., McDuffie, F. C., Sheps, S. G., *et al*. (1980). Prospective study of immune response to hydralazine and development of antideoxyribonucleoprotein in patients receiving hydralazine. *Am. J. Med.* **69**, 395.
8. Yamauchi, Y., Litwin, A., Adams, L. E., Zimmer, H., and Hess, E. V. (1975). Induction of antibodies to nuclear antigens in rabbits by immunization with hydralazine-human serum albumin conjugates. *J. Clin. Invest.* **56**, 958.
9. Charles, P. J., Smeenk, R. J. T., De Jong, J., Feldmann, M., and Maini, R. N. (2000). Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor α. *Arthritis Rheum.* **43**, 2383.
10. Bluestein, H. G., Redelman, D., and Zvaifler, N. J. (1981). Procainamide-lymphocyte reactions. A possible explanation for drug-induced autoimmunity. *Arthritis Rheum.* **24**, 1019.
11. Dubroff, L. M., Reid, R., Jr., and Papalian, M. (1981). Molecular models for hydralazine-related systemic lupus erythematosus. *Arthritis Rheum.* **24**, 1082.
12. Dubroff, L. M., and Reid, R., Jr. (1980). Hydralazine–pyrimidine interactions may explain hydralazine-induced lupus erythematosus. *Science* **208**, 404.
13. Eldredge, N. T., van Robertson, V. V. B., and Jiller, J. J. (1974). The interaction of lupus-inducing drugs with deoxyribonucleic acid. *Clin. Immunol. Immunopathol.* **3**, 262.
14. Tomura, T., and van Lancker, J. L. (1988). Procainamide–DNA interaction. *J. Rheumatol.* **15**, 59.
15. Sinha, B. K., and Patterson, M. A. (1983). Free radical metabolism of hydralazine. Binding and degradation of nucleic acids. *Biochem. Pharmacol.* **32**, 3279.
16. Thomas, T. J., and Messner, R. P. (1986). Effects of lupus-inducing drugs on the B to Z transition of synthetic DNA. *Arthritis Rheum.* **29**, 638.
17. Lafer, E. M., Moller, A., Nordheim, A., Stoller, B. D., and Rich, A. (1981). Antibodies specific for left-handed Z-DNA. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3546.
18. Gunnia, U. B., Thomas, T., and Thomas, T. J. (1991). The effects of polyamines on the immunogenicity of polynucleotides. *Immunol. Invest.* **20**, 337.
19. Rubin, R. L., Bell, S. A., and Burlingame, R. W. (1992). Autoantibodies associated with lupus induced by diverse drugs target a similar epitope in the (H2A–H2B)–DNA complex. *J. Clin. Invest.* **90**, 165.
20. Thomas, T. J., Seibold, J. R., Adams, L. E., and Hess, E. V. (1993). Hydralazine induces Z-DNA conformation in a polynucleotide and elicits anti (Z-DNA) antibodies in treated patients. *Biochem. J.* **294**, 419.
21. Burlingame, R. W., and Rubin, R. L. (1991). Drug-induced anti-histone autoantibodies display two patterns of reactivity with substructures of chromatin. *J. Clin. Invest.* **88**, 680.
22. Thomas, T. J., Gunnia, U. B., Seibold, J. R., and Thomas, T. (1995). Defective signal-transduction pathways in T-cells from autoimmune MRL-lpr/lpr mice are associated with increased polyamine concentrations. *Biochem. J.* **311**, 175.
23. Lafer, E. M., Vae, R.-P., Moller, A., *et al*. (1983). Z-DNA-specific antibodies in human systemic lupus erythematosus. *J. Clin. Invest.* **71**, 314.
24. Sano, H., and Morirnoto, C. (1982). DNA isolated from DNA/anti-DNA antibody immune complexes in systemic lupus erythematosus is rich in guanine-cytosine content. *J. Immunol.* **128**, 1341.
25. Sano, H., and Moirimoto, C. (1981). Isolation of DNA from DNA/anti-DNA antibody immune complexes in systemic lupus erythematosus. *J. Immunol.* **126**, 538.
26. Thomas, T. J., Seibold, J. R., Adams, L. E., and Hess, E. V. (1995). Triplex-DNA stabilization by hydralazine and the presence of anti-(triplex DNA) antibodies in patients treated with hydralazine. *Biochem. J.* **311**, 183.
27. Monestier, M., Fasy, T. M., Debbas, T. M., and Patel, K. (1990). Deoxyribonuclease I treatment of histones for the detection of anti-histone antibodies in solid-phase immunoassays. Effect of protease contamination in commercial deoxyribonuclease I preparations. *J. Immunol. Methods* **127**, 289.
28. Bernstein, R. M., Hobbs, R. N., Lea, D. J., Ward, D. J., and Hughes, G. R. V. (1985). Patterns of antihistone antibody specificity in systemic rheumatic disease 1. Systemic lupus erythematosus, mixed connective tissue disease, primary sicca syndrome, and rheumatoid arthritis with vasculitis. *Arthritis Rheum.* **28**, 285.
29. Gohill, J., Cary, P. D., Couppez, M., and Fritzier, M. J. (1985). Antibodies from patients with drug-induced and idiopathic lupus erythematosus react with epitopes restricted to the arnino and carboxyl termini of histone. *J. Immunol.* **135**, 3116.
30. Portanova, J. P., Arndt, R. E., Tan, E. M., and Kotzin, B. L. (1987). Anti-histone antibodies in idiopathic and drug-induced lupus recognize distinct intrahistone regions. *J. Immunol.* **138**, 446.

31. Burlingame, R. W., Boey, M. L., Starkebaum, G., and Rubin, R. L. (1994). The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. *J. Clin. Invest.* **94**, 184.
32. Mohan, C., Adams, S., Stanik, V., and Datta, S. K. (1993). Nucleosome: A major immunogen for pathogenic autoantibody-inducing T cells of lupus. *J. Exp. Med.* **17**, 1367.
33. Bluestein, H. G., Zvaifler, N. J., Weisman, M. H., and Shapiro, R. F. (1979). Lymphocyte alteration by procainamide: Relation to drug-induced lupus erythematosus syndrome. *Lancet* **2**, 816.
34. de Boccardo, G., Drayer, D., Rubin, A. L., *et al*. (1985). Inhibition of pokeweed mitogen-induced B cell differentiation by compounds containing primary amine or hydralazine groups. *Clin. Exp. Immunol.* **59**, 69.
35. Ochi, T., Goldings, E. A., Lipsky, P. E., and Ziff, M. (1983). Immunomodulatory effect of procainamide in man. Inhibition of human suppressor T-cell activity *in vitro*. *J. Clin. Invest.* **71**, 36.
36. Adams, L. E., Sanders, C. E., Jr., Budinsky, R. A., *et al*. (1989). Immunomodulatory effects of procainamide metabolites: Their implications in drug-related lupus. *J. Lab. Clin. Med.* **113**, 482.
37. Tannen, R. H., and Cunningham-Rundles, S. (1982). Inhibition of Con A mitogenesis by serum from procainamide-treated patients and patients with systemic lupus erythematosus. *Immunol. Commun.* **11**, 33.
38. Yu, C. L., and Ziff, M. (1985). Effects of long-term procainamide therapy on immunoglobulin synthesis. *Arthritis Rheum.* **28**, 276.
39. Forrester, J., Golbus, J., Brede, D., Hudson, J., and Richardson, B. (1988). B cell activation in patients with active procainamide induced lupus. *J. Rhumatol.* **15**, 1384.
40. Klajman, A., Camin-Belsky, N., Kimchi, A., and Ben-Efraim, S. (1970). Occurrence, immunoglobulin pattern and specificity of antinuclear antibodies in sera of procainamide treated patients. *Clin. Exp. Immunol.* **7**, 641.
41. Yu, C. L., and Ziff, M. (1985). Effects of long-term procainamide therapy on immunoglobulin synthesis. *Arthritis Rheum.* **28**, 276.
42. Green, B. J., Wyse, D. G., Duff, H. J., Mitchell, L. B., and Matheson, D. S. (1988). Procainamide in vivo modulates suppressor T cell activity. *Clin. Invest. Med.* **11**, 425.
43. Miller, K. B., and Salem, D. (1982). Immune regulatory abnormalities produced by procainamide *in vitro* suppressor cell function of IgG secretion. *Am. J. Med.* **73**, 487.
44. Hahn, B. H., Sharp, G. C., Irvin, W. S., *et al*. (1972). Immune responses to hydralazine and nuclear antigens in hydralazine-induced lupus crythematosus. *Ann. Intern. Med.* **76**, 365.
45. Litwin, A., Adams, L. E., Zimmer, H., *et al*. (1981). Prospective study of immunologic effects of hydralazine in hypertensive patients. *Clin. Phannacol. Ther.* **29**, 447.
46. Litwin, A., Adams, L. E., Zimmer, H., and Hess, E. V. (1981). Immunologic effects of hydralazine in hypertensive patients. *Arthritis Rheum.* **24**, 1074.
47. Kirtland, H. H., Mohler, D. N., and Horwitz, D. A. (1980). Methyldopa inhibition of suppressor-lymphocyte function: A proposed cause of autoimmune hemolytic anemia. *N. Engl. J. Med.* **302**, 825.
48. MacKinney, A. A., and Booker, H. E. (1972). Diphenylhydantoin effects on human lymphocytes *in vitro* and in vivo. A hypothesis to explain some drug interactions. *Arch. Intern. Med.* **129**, 988.
49. Alarcon-Segovia, D., and Palacios, R. (1981). Differences in immunoregulatory T cell circuits between diphenylhydantoin-related and spontaneously occurring systemic lupus erythematosus. *Arthritis Rheum.* **24**, 1086.
50. Okanoue, T., Sakamoto, S., Itoh, Y., Minami, M., Yasui, K., Sakamoto, M., *et al*. (1996). Side effects of high-dose interferon therapy for chronic hepatitis C. *J. Hepatol.* **25**, 283.
51. Ronnblom, L. E., Alm, G. V., and Oberg, K. E. (1991). Autoimmunity after alpha-interferon therapy for malignant carcinoid tumors. *Ann. Intern. Med.* **324**, 509.
52. Kim, T., Kanayama, Y., Negoro, N., Okamura, M., Takeda, T., and Inoue, T. (1987). Serum levels of inteferons in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **70**, 562.
53. Kirou, K. A., Vakkalanka, R. K., Butler, M. J., and Crow, M. K. (2000). Induction of Fas ligand-mediated apoptosis by interferon-alpha. *Clin. Immunol.* **95**, 218.
54. Jewell, A. P., Worman, C. P., Lydyard, P. M., Yong, K. L., Giles, F. J., and Goldstone, A. H. (1994). Interferon-alpha up-regulates bcl-2 expression and protects B-CLL cells from apoptosis *in vitro* and in vivo. *Br. J. Haematol.* **88**, 268.
55. Stauffer, Y., Marguerat, S., Meylan, F., Ucla, C., Sutkowski, N., Huber, B., Pelet, T., and Conrad, B. (2001). Interferon-α-induced endogenous superantigen: A model linking environment and autoimmunity. *Immunity* **15**, 591.
56. Blanco, P., Palucka, A. K., Gill, M., Pascual, V., and Banchereau, J. (2001). Induction of dendritic cell differentiation by IFN-alpha in systemic lupus erythematosus. *Science* **294**, 1540.
57. Heilig, B., Fiehn, C., Brockhaus, M., *et al*. (1996). Evaluation of soluble tumor necrosis factor alpha and its soluble receptors parallel clinical disease and autoimmune activity in systemic lupus erythematosus. *Br. J. Rheumatol.* **35**, 1067.
58. Aderka, D., Wysenbeek, A., Engelmann, H., *et al*. (1993). Correlation between serum levels of soluble TNF-α and disease activity in SLE. *Arthritis Rheum.* **36**, 1111.
59. Maury, C. P., and Teppo, A. M. (1989). Tumor necrosis factor in the serum of patients with systemic lupus erythematosus. *Arthritis Rheum.* **32**, 146.
60. Boswell, J. M., Yui, M. A., Burt, D. W., and Kelley, V. E. (1988). Increased tumor necrosis factor and IL-1 beta gene expression in the kidneys of mice with lupus nephritis. *J. Immunol.* **141**, 3050.
61. Jacob, C. O., and McDevitt, H. O. (1988). Tumor necrosis factor-α in murine autoimmune "lupus" nephritis. *Nature* **331**, 356.

62. Dorner, T., and Putterman, C. (2001). B cells, BAFF/zTNF4, TACI, and sytemic lupus erythematosus. *Arthritis Res.* **3**, 197.
63. Goldstein, R., and Arnett, F. C. (1987). The genetics of rheumatic disease in man. *Rheum. Dis. Clin. North Am.* **13**, 487.
64. Shoenfeld, Y., and Schwartz, R. S. (1984). Immunologic and genetic factors in autoimmune diseases. *N. Engl. J. Med.* **311**, 1019.
65. Batchelor, J. R., Welsh, K. I., Tinoco, R. M., *et al*. (1980). Hydralazine-induced systemic lupus erythematosus: Influence of HLA-DR and sex on susceptibility. *Lancet* **1**, 107.
66. Russell, G. I., Bing, R. F., Jones, J. A., Thurston, H., and Swales, J. D. (1987). Hydralazine sensitivity: Clinical features, autoantibody changes and HLA-DR phenotype. *Q. J. Med.* **65**, 45.
67. Brand, C., Davidson, A., Littlejohn, G., and Ryan, P. (1984). Hydralazine-induced lupus: No association with HLA-DR4. *Lancet* **1**, 462.
68. Sim, E. (1991). Drug-induced immune complex disease. *Biochem. Soc. Trans.* **19**, 164.
69. Speirs, C., Fielder, A. H., Chapel, U., Davey, N. J., and Batchelor, J. R. (1989). Complement system protein C4 and susceptibility to hydralazine-induced systemic lupus erythematosus. *Lancet* **1**, 922.
70. Arnett, F. C. (1992). Genetic aspects of human lupus. *Clin. Immunol. Immunopathol.* **63**, 4.
71. Sim, E., Dodds, A. W., and Goldin, A. (1989). Inhibition of the covalent binding reaction of complement component C4 by penicillamine, an anti-rheumatic agent. *Biochem. J.* **259**, 415.
72. Sim, E., Gill, E. W., and Sim, R. B. (1984). Drugs that induce systemic lupus erythematosus inhibit complement component C4. *Lancet* **2**, 422.
73. Mitchell, J. A., Batchelor, J. R., Chapel, H., *et al*. (1987). Erythrocyte complement receptor type I (CRI) expression and circulating immune complex (CIC) levels in hydralazine-induced SLE. *Clin. Exp. Immunol.* **68**, 446.
74. Whiteside, T., Mulhern, L., Buckingham, R., and Luksick, J. (1982). Procainamide-induced lupus (PIL) is associated with an increased frequency of HLA-DR 6Y (abstract). *Arthritis Rheum.* **25**, S41.
75. Totoritis, M. C., Tan, E. M., McNally, E. M., and Rubin, R. L. (1988). Association of antibody to histone complex H2A–H2B with symptomatic procainamide-induced lupus. *N. Engl. J. Med.* **318**, 1431.
76. Canoso, R. T., Lewis, M. E., and Yunis, E. J. (1982). Association of HLA-Bw44 with chlorpromazine-induced autoantibodies. *Clin. Immunol. Immunopathol.* **25**, 278.
77. Chin, G. L., Kong, N. C., Lee, B. C., and Rose, I. M. (1991). Penicillamine induced lupus-like syndrome in a patient with classical rheumatoid arthritis (letter). *J. Rheumatol.* **18**, 947.
78. Chalmers, A., Thompson, D., Stein, H. E., Reid, G., and Patterson, A. C. (1982). Systemic lupus erythematosus during penicillamine therapy for rheumatoid arthritis. *Ann. Intern. Med.* **97**, 659.
79. Hutabarat, R. W., Smith, A. L., and Unadkat, J. D. (1994). Disposition of drugs in cystic fibrosis. VII. Acetylation of sulfamethoxazole in blood cells. *In vitro*–in vivo correlation and characterization of its kinetics of acetylation in lymphocytes. *Clin. Pharmacol. Ther.* **259**, 427.
80. Grant, D. M., Morike, K., Eichelbaum, M., and Meyer, U. A. (1990). Acetylation pharmacogenetics. The slow acetylator phenotype is caused by decreased or absent arylamine *N*-acetyltransferase in human liver. *J. Clin. Invest.* **85**, 968.
81. Weber, W. W. (1987). Acetylation of drugs. In "The Acetylator Genes and Drug Response," pp. 50–84. Oxford Univ. Press, New York.
82. Reidenberg, M. M., Kostenbauder, H., and Adams, W. P. (1969). Rate of drug metabolism in obese volunteers before and during starvation and in azotemic patients. *Metabolism* **18**, 209.
83. Olsen, H., and Morland, J. (1978). Ethanol-induced increases in drug acetylation in man and isolated rat liver cells. *Br. Med. J.* **2**, 1260.
84. Evans, D. A. (1989). *N*-acetyltransferase. *Pharmacol. Ther.* **42**, 157.
85. Perry, H. M., Jr., Tan, E. M., Cordody, S., and Sahamato, A. (1970). Relationship of acetyl transferase activity to antinuclear antibodies and toxic symptoms in hypertensive patients treated with hydralazine. *J. Lab. Clin. Med.* **76**, 114.
86. Strandberg, I., Boman, G., Hassler, L., and Sjoqvist, F. (1976). Acetylator phenotype in patients with hydralazine-induced lupoid syndrome. *Acta. Med. Scand.* **200**, 367.
87. Woosley, R. L., Drayer, D. E., Reidenberg, M. M., *et al*. (1978). Effect of acetylator phenotype on the rate at which procainamide induces antinuclear antibodies and the lupus syndrome. *N. Engl. J. Med.* **298**, 1157.
88. Litwin, A., Adams, L. E., Zimmer, H., and Hess, E. V. (1981). Immunological effects of hydralazine in hypertensive patients. *Arthritis Rheum.* **24**, 1074.
89. Mansilla-Tinoco, R., Harland, S. J., Ryan, P. J., *et al*. (1982) Hydralazine, antinuclear antibodies, and the lupus syndrome. *Br. Med. J.* **284**, 936.
90. Sonnhag, C., Karlsson, E., and Hed, J. (1979). Procainamide-induced lupus erythematosus-like syndrome in relation to acetylator phenotype and plasma levels of procainamide. *Acta. Med. Scand.* **206**, 245.
91. Roden, D. N. A., Reele, S. B., Higgins, S. B., *et al*. (1980). Antiarrhythmic efficacy, pharmacokinetics and safety of *N*-acetylprocainamide in human subjects: Comparison with procainamide. *Am. J. Cardiol.* **46**, 463.
92. Lee, S. L., Rivero, I., and Siegel, M. (1975). Activation of systemic lupus erythematosus: A critical review. *Semin. Arthritis Rheum.* **5**, 83.
93. Alarcon-Segovia, D., Fishbein, E., and Alcala, H. (1971). Isoniazid acetylation rate and development of antinuclear antibodies upon isoniazid treatment. *Arthritis Rheum.* **14**, 748.
94. Evans, D. A., Bullen, M. F., Houston, J., Hopkins, C. A., and Vetters, J. M. (1972). Antinuclear factor in rapid and

slow acetylator patients treated with isoniazid. *J. Med. Genet.* **9**, 53.
95. Reidenberg, N. A. M., and Martin, J. H. (1974). The acetylator phenotype of patients with systemic lupus erythematosus. *Drug Metab. Dispos.* **2**, 71.
96. Uetrecht, J. P., and Woosley, R. L. (1981). Acetylator phenotype and lupus erythematosus. *Clin. Pharmacokinet.* **6**, 118.
97. Johansson, E., Mustakallio, K. K., and Mattila, M. J. (1981). Polymorphic acetylator phenotype and systemic lupus erythematosus. *Acta. Med. Scand.* **210**, 193.
98. Ishizaki, T., Horai, Y., Koya, G., Matsuyama, K., and Iguchi, S. (1981). Acetylator phenotype and metabolic disposition of isoniazid in Japanese patients with systemic lupus erythematosus. *Arthritis Rheum.* **24**, 1245.
99. Hess, E. V. (1987). Acetylator phenotype in patients with systemic lupus erythematosus (letter). *Arthritis Rheum.* **30**, 1074.
100. Emery, P. (1987). Further evidence for the lack of association between acetylator phenotype and systemic lupus erythematosus (letter). *Arthritis Rheum.* **30**, 357.
101. Sardas, S., Karakaya, A. E., and Sardas, O. S. (1986). Acetylator phenotype in patients with systemic lupus erythematosus (letter). *Arthritis Rheum.* **29**, 1412.
102. Baer, A. N., Woosley, R. L., and Pincus, T. (1986). Further evidence for the lack of association between acetylator phenotype and systemic lupus erythematosus. *Arthritis Rheum.* **29**, 508.
103. Reidenberg, M. M., Drayer, D. E., Lorenzo, B., *et al*. (1993). Acetylation phenotypes and environmental chemical exposure of people with idiopathic systemic lupus erythematosus. *Arthritis Rheum.* **36**, 971.
104. Ong, M. L., Mant, T. G., Veerapen, K., *et al*. (1990). The lack of relationship between acetylator phenotype and idiopathic systemic lupus erythematosus in a Southeast Asian population: A study of Indians, Malays and Malaysian Chinese. *Br. J. Rheumatol.* **29**, 462.
105. Marsden, J. R., Mason, G. G., Coburn, P. R., Rawlins, M. D., and Shuster, S. (1985). Drug acetylation and expression of lupus erythematosus. *Eur. J. Clin. Pharmacol.* **28**, 387.
106. Weber, W. W., Hein, D. W., Litwin, A., and Lower, G. M., Jr. (1983). Relationship of acetylator status to isoniazid toxicity, lupus erythematosus, and bladder cancer. *Fed. Proc.* **42**, 3086.
107. Rubin, R. L., and Curnutte, J. T. (1989). Metabolism of procainamide to the cytotoxic hydroxylamine by neutrophils activated *in vitro*. *J. Clin. Invest.* **83**, 1336.
108. Rubin, R. L. (1992). Autoantibody specificity in drug-induced lupus and neutrophil-mediated metabolism of lupus-inducing drugs. *Clin. Biochem.* **25**, 223.
109. Jiang, X., Khursigara, G., and Rubin, R. L. (1994). Transformation of lupus-inducing drugs to cytotoxic products by activated neutrophils. *Science* **266**, 810.
110. Hofstra, A. H., Matassa, L. C., and Uetrecht, J. P. (1991). Metabolism of hydralazine by activated leukocytes: Implications for hydralazine induced lupus. *J. Rheumatol*. **18**, 1673.
111. Uetrecht, J. P. (1985). Ractivity and possible significance of hydroxylamine and nitroso metabolites of procainamide. *J. Pharmacol. Exp. Ther.* **232**, 420.
112. Nassberger, L., Jonsson, H., Sjoholm, A. G., Stuefelt, G., and Akesson, A. (1990). Autoantibodies against neutrophil cytoplasmic components in systemic lupus erythematosus and in hydralazine-induced lupus. *Clin. Exp. Immunol.* **81**, 380.
113. Cambridge, G., Wallace, U., Bernstein, R. M., and Leaker, B. (1994). Autoantibodies to myeloperoxidase in idiopathic and drug-induced systemic lupus erythematosus and vasculitis. *Br. J. Rheumatol.* **33**, 109.
114. Kretz-Rommel, A., and Rubin, R. L. (1997). A metabolite of the lupus-inducing drug procainamide prevents anergy induction in T cell clones. *J. Immunol.* **158**, 4465.
115. Holliday, R., and Pugh, J. E. (1975). DNA modification mechanisms and gene activity during development. *Science* **187**, 226.
116. Jones, P. A., and Taylor, S. M. (1980). Cellular differentiation, cytidine analogs and DNA methylation. *Cell* **20**, 85.
117. Bird, A. (1992). The essentials of DNA methylation. *Cell* **70**, 5.
118. Riggs, A. D. (1975). X-inactivation, differentiation and DNA methylation. *Cytogenet. Cell. Genet.* **14**, 9.
119. Levy-Wilson, B., Gjerset, R. A., and McCarthy, B. J. (1977). Acetylation and phosphorylation of *Drosophila* histones. Distribution of acetate and phosphate groups in fractionated chromatin. *Biochim. Biophys. Acta.* **475**, 168.
120. Althaus, F. R. (1992). Poly ADP-ribosylation: A histone shuttle mechanism in DNA excision repair. *J. Cell Sci.* **102**, 663.
121. Kanai, Y., Kawaminami, Y., Miwa, M., Matsushima, T., and Sugimura, T. (1977). Naturally-occurring antibodies to poly(ADP-ribose) in patients with systemic lupus erythematosus. *Nature* **265**, 175.
122. Kanai, Y., and Sugirnura. T. (1981). Comparative studies on antibodies to poly(ADP-ribose) in rabbits and patients with systemic lupus erythematosus. *Immunology* **43**, 101.
123. Hobbs, R. N., Clayton, A. L., and Bernstein, R. M. (1987). Antibodies to the five histones and poly (adenosine diphosphate-ribose) in drug induced lupus: Implications for pathogenesis. *Ann. Rheum. Dis.* **46**, 408.
124. Berger, N. A., Sikorski, G. W., Petzold, S. J., and Kurohara, K. K. (1979). Association of poly(adenosine diphosphoribose) synthesis with DNA damage and repair in normal human lymphocytes. *J. Clin. Invest.* **63**, 1164.
125. Ayer, L. M., Edworthy, S. M., and Fritzler, M. J. (1993). Effects of procainamide and hydralazine on poly (ADP-ribosylation) in cell lines. *Lupus* **2**, 167.
126. Razin, A., and Szyf, M. (1984). DNA methylation patterns formation and function. *Biochim. Biophys. Acta.* **782**, 331.
127. Adams, R. L. P., and Burdon, R. H. (1985). DNA methylation in the cell. In "Molecular Biology of DNA Methylation" (A. Rich, Ed.), pp. 9–18. Springer-Verlag, New York.
128. Bird, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature* **321**, 209.

129. Zacharias, W. (1993). Methylation of cytosine influences in DNA structure. In "DNA Methylation: Molecular Biology and Biological Significance" (J. P. Jost and H. P. Saluz, Eds.), pp. 27–38. Birkhaeuser, Basel.
130. Attwood, J. T., Yung, R. L., and Richardson, B. C. (2002). DNA methylation and the regulation of gene transcription. *Cell. Mol. Life Sci.* **59**, 241.
131. Bestor, T., Laudano, A., Mattaliano, R., and Ingram, V. (1988). Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. *J. Mol. Biol.* **203**, 971.
132. Adams, R. L. P., and Burdon, R. H. (1985). *S*-Adenosyl-L-methionine-donor of methyl groups. In "Molecular Biology of DNA Methylation" (A. Razin, H. Cedar, and A. D. Riggs, Eds.), pp. 31–41. Springer-Verlag, New York.
133. Bestor, T. H., and Ingram, V. M. (1985). Growth-dependent expression of multiple species of DNA methyltransferase in murine erythroleukemia cells. *Proc. Natl. Acad. Sci. U.S.A.* **82**, 2674.
134. Gruenbaum, Y., Cedar, H., and Razin, A. (1982). Substrate and sequence specificity of a eukaryotic DNA methylase. *Nature* **95**, 620.
135. Riggs, A. D. (1985). X-inactivation, DNA methylation, and differentiation revered. In "DNA Methylation: Biochemistry and Biological Significance" (A. Razin, H. Cedar, and A. D. Riggs, Eds.), pp. 269–278. Springer-Verlag, New York.
136. Stoger, R., Kubicka, P., Liu, C.-G., *et al*. (1993). Maternal specific methylation of the imprinted mouse Igf2r locus identified the expressed locus as carrying the imprinting signal. *Cell* **73**, 61.
137. Li, E., Bestor, T. H., and Jaenisch, R. (1992). Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* **69**, 915.
138. Taylor, S. M., and Jones, P. A. (1979). Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. *Cell* **17**, 771.
139. Davis, R. L., Weintraub, H., and Lassar, A. B. (1987). Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51**, 987.
140. Pinney, D. F., Pearson-White, S. H., Konieczny, S. F., Latham, K. E., and Emerson, C. P., Jr. (1988). Myogenic lineage determination and differentiation: Evidence for a regulatory gene pathway. *Cell* **53**, 781.
141. Patra, S. K., Patra, A., and Dahiya, R. (2001). Histone deacetylase and DNA methyltransferase in human prostate cancer. *Biochem. Biophy. Res. Commun.* **287**, 705.
142. Richardson, B., Kahn, L., Lovett, E. J., and Hudson, J. (1986). Effect of an inhibitor of DNA methylation on T cells. I. 5-Azacytidine induces T4 expression on $T8^+$ T cells. *J. Immunol.* **137**, 35.
143. Richardson, B. (1986). Effect of an inhibitor of DNA methylation on T cells. II. 5-Azacytidine induces self-reactivity in antigen-specific $T4^+$ cells. *Hum. Immunol.* **17**, 456.
144. Quddus, J., Johnson, K. J., Gavalchin, J., *et al*. (1993). Treating activated $CD4^+$ T cells with either of two distinct DNA methyltransferase inhibitors, 5-Azacytidine or procainamide, is sufficient to cause a lupus-like disease in syngeneic mice. *J. Clin. Invest.* **92**, 38.
145. Yung, R. L., Quddus, J., Chrisp, C. E., Johnson, K. J., and Richardson, B. C. (1995). Mechanisms of drug induced lupus. I. Cloned Th2 cells modified with DNA methylation inhibitors in vitro cause autoimmunity in vivo. *J. Immunol.* **154**, 3025.
146. Richardson, B. C., Powers, D., Hooper, F., Yung, R. L., and O'Rourke, K. (1994). Lymphocyte function-associated antigen I overexpression and T cell autoreactivity. *Arthritis Rheum.* **37**, 1363.
147. Yung, R. L., Powers, D., Johnson, K., *et al*. (1996). Mechanisms of drug-induced lupus. II. T cells overexpressing LFA-1 cause a lupus-like disease in syngeneic mice. *J. Clin. Invest.* **97**, 2866.
148. Springer, T. A. (1990). Adhesion receptors of the immune system. *Nature* **346**, 425.
149. Hynes, R. O. (1992). Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11.
150. Altmann, D. M., Hogg, N., Trowsdale, J., and Wilkinson, D. (1989). Cotransfection of ICAM-1 and HLA-DR reconstitutes human antigen-presenting cell function in mouse L cells. *Nature* **338**, 512.
151. Rolink, A. G., and Gleichmann, E. (1983). Allosuppressor- and allohelper-T cells in acute and chronic graft-vs-host (GvH) disease. 111. Different Lyt subsets of donor T cells induce different pathological syndromes. *J. Exp. Med.* **158**, 546.
152. Gleichmann, E., van Elven, E. H., and van der Veen, J. P. W. (1982). A systemic lupus erythematosus (SLE)-like disease in mice induced by abnormal T–B cell cooperation. Preferential formation of autoantibodies characteristic of SLE. *Eur. J. Immunol.* **12**, 139.
153. Scheinbart, L. S., Johnson, M. A., Gross, L. A., Edelstein, S. R., and Richardson, B. C. (1991). Procainamide inhibits DNA methyltransferase in a human T cell line. *J. Rheumatol.* **18**, 530.
154. Cornacchia, E., Golbus, J., Maybaum, J., *et al*. (1988). Hydralazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. *J. Immunol.* **140**, 2197.
155. Yung, R., Chang, S., Hemati, N., Johnson, K., and Richardson, B. (1997). Mechanisms of drug-induced lupus IV. Comparison of procainamide and hydralazine with analogs *in vitro* and in vivo. *Arthritis Rheum.* **40**, 1436.
156. Richardson, B. C., Attwood, J. T., Ray, D., Richardson, D. K., and Deng, C. (2001). Identification of methylation sensitive T cell genes capable of participating in autoimmunity. *Arthritis Rheum.* **44**, S201.
157. Kammer, G. M., Perl, A., Richardson, B. C., and Tsokos, G. C. (2002). Abnormal T cell signal transduction in sytemic lupus erythematosus. *Arthritis Rheum.* **46**, 1139.
158. Deng, C., kaplan, M. J., Yang, J., Ray, D., Zhang, Z., McCune, W. J., Hanash, S. M., and Richardson, B. C. (2001). Decreased Ras-mitogen-activated protein kinase signaling may cause DNA hypomethylation in T lymphocytes from lupus patients. *Arthritis Rheum.* **44**, 397.

159. Deng, C., Zhang, Z. Y., Rao, T., Attwood, J., and Richardson, B. C. (2003). Hydralazine inhibits ERK pathway signaling in human T cells. *Arthritis Rheum*. **48**, 746.
160. Deng, C., Yang, J., Scott, J., Hanash, S., and Richardson, B. C. (1998). Role of the ras–MAPK signaling pathway in the DNA methyltransferase response to DNA hypomethylation. *Biochemistry* **379**, 1113.
161. Richardson, B. C., Liebling, M. R., and Hudson, J. L. (1990). $CD4^+$ cells treated with DNA methylation inhibitors induce autologous B cell differentiation. *Clin. Immunol. Immunopathol.* **55**, 368.
162. Reth, M. (1992). Antigen receptors on B lymphocytes. *Annu. Rev. Immunol.* **10**, 97.
163. Kaplan, M. J., Ray, D., Mo, R. R., Yung, R. L., and Richardson, B. C. (2000). TRAIL (APO2 ligand) and TWEAK (Apo3 ligand) mediate $CD4^+$ T cell killing of antigen-presenting macrophages. *J. Immunol.* **164**, 2897.
164. Richardson, B. C., Strahler, J. R., Pivirotto, S., *et al*. (1992). Phenotypic and functional similarities between 5-azacytidine-treated cells and a T cell subset in patients with active systemic lupus erythematosus. *Arthritis Rheum.* **35**, 647.
165. Corvetta, A., Della Bitta, R., Luchetti, M. M., and Pomponio, G. (1991). 5-Methylcytosine content of DNA in blood, synovial mononuclear cells and synovial tissue from patients affected by autoimmune rheumatic diseases. *J. Chromatogr.* **566**, 481.
166. Yang, J., Deng, C., Hemati, N., Hanash, S. M., and Richardson, B. C. (1997). Effect of mitogenic stimulation and DNA methylation on human T cell DNA methyltransferase expression and activity. *J. Immunol.* **159**, 1303.
167. Takeuchi, T., Amano, K., Sekine, H., Koide, J., and Abe, T. (1993). Upregulated expression and function of integrin adhesive receptors in systemic lupus erythematosus patients with vasculitis. *J. Clin. Invest.* **92**, 3008.
168. Richardson, B. C., Yung, R. L., Johnson, K. J., Rowse, P. E., and Lalwani, N. D. (1996). Monocyte apoptosis in patients with active lupus. *Arthritis Rheum.* **39**, 1432.
169. Linker-Israeli, M., Deans, R. J., Wallace, D. J., *et al*. (1991). Elevated levels of endogenous IL-6 in systemic lupus erythematosus. A putative role in pathogenesis. *J. Immunol.* **147**, 117.
170. Klashman, D. J., Martin, R. A., Martinez-Maza, O., and Stevens, R. H. (1991). *In vitro* regulation of B cell differentiation by interleukin-6 and soluble CD23 in systemic lupus erythematosus B cell subpopulations and antigen-induced normal B cells. *Arthritis Rheum.* **34**, 276.
171. Kitani, A., Hara, M., Hirose, T., *et al*. (1989). Heterogeneity of B cell responsiveness to interleukin 4, interleukin 6 and low molecular weight B cell growth factor in discrete stages of B cell activation in patients with systemic lupus erythematosus. *Clin. Exp. Immunol.* **77**, 31.
172. Comens, P. (1956). Experimental hydralazine disease and its similarity to disseminated lupus erythematosus. *J. Lab. Clin. Med.* **47**, 444.
173. Dubois, E. L., Katz, Y. J., Freeman, V., and Garbak, F. (1957). Chronic toxicity studies of apresoline in dogs with particular reference to the production of the "hydralazine syndrome." *J. Lab. Clin. Med.* **50**, 119.
174. Gardner, D. L. (1957). Response of the dog to oral L-hydrazinopthalazine (hydralazine). *Br. J. Exp. Pathol.* **38**, 227.
175. Carrera, A. E., Reid, M. V., and Kurnick, N. B. (1954). Differences in susceptibility of polymorphonuclear leucocytes from several species to alteration by S.L.E. serum: Application to a more sensitive L.E. phenomenon. *Test Blood* **9**, 1165.
176. Siguier, F., Betourne, C. L., and Bonnet De La Tour, J. (1958). Le lupus erythemateux hydralazinique. *Semin. Hop. Paris* **34**, 773.
177. Braverman, I. M., and Lerner, A. B. (1967). Hydralazine disease in the guinea-pig as an experimental model for lupus erythematosus. *J. Invest. Dermatol.* **39**, 317.
178. Leovey, A., Szegedi, G., Bobory, J., and Devenyi, I. (1967). Experimental "hydralazine erythematodes" of the guinea-pig. *Acta. Rheum. Scand.* **13**, 119.
179. Ellman, L., Inman, J., and Green, I. (1971). Strain difference in the immune response to hydralazine in inbred guinea-pigs. *Clin. Exp. Immunol.* **9**, 927.
180. Cannat, A., and Seligmann, M. (1968). Induction by isoniazid and hydralazine of antinuclear factors in mice. *Clin. Exp. Immunol.* **3**, 99.
181. Ten Veen, J. H., and Feltkamp, T. E. W. (1972). Studies on drug induced lupus erythematosus in mice. I. Drug induced antinuclear antibodies (ANA). *Clin. Exp. Immunol.* **11**, 265.
182. Thoburn, R., Koffler, D., and Kunkel, U. G. (1971). Distribution of antibodies to native DNA, single-stranded DNA, and double-stranded RNA in mouse serums. *Proc. Soc. Exp. Biol. Med.* **136**, 711.
183. Ten Veen, J. H., and Feltkamp-Vroom, T. M. (1973). Studies on drug induced lupus erythematosus in mice. 111. Renal lesions and splenomegaly in drug-induced lupus erythematosus. *Clin. Exp. Immunol.* **15**, 591.
184. Yamauchi, Y., Litwin, A., Adams, L., Zimmer, H., and Hess, E. V. (1975). Induction of antibodies to nuclear antigens in rabbits by immunization with hydralazine-human serum albumin conjugates. *J. Clin. Invest.* **56**, 958
185. Mollerberg, H. (1958). Attempts to produce the "hydralazine syndrome" in the albino rat. *Acta. Med. Scand.* **161**, 443.
186. McCoy, F. W., and Leach, W. J. (1959). Experimental attempt to produce the L.E. syndrome (arthritis) in swine with hydralazine. *Proc. Soc. Exp. Biol. Med.* **101**, 183.
187. Kretz-Rommel, A., Duncan, S. R., and Rubin, R. L. (1997). Autoimmunity caused by disruption of central T cell tolerance. A murine model of drug-induced lupus. *J. Clin. Invest.* **99**, 1888.
188. De Wit, D., Van Mechelen, M., Zanin, C., *et al*. (1993). Preferential activation of Th2 cells in chronic graft-versus-host reaction. *J. Immunol.* **150**, 361.

189. Lepault, F., Gagnerault, M. C., Faveeuw, C., and Boitard, C. (1994). Recirculation, phenotype and functions of lymphocytes in mice treated with monoclonal antibody MEL-14. *Eur. J. Immunol.* **24**, 3106.
190. Jung, L. K. L., Good, R. A., and Fernandes, G. A. (1986). Studies on lymphocyte homing in autoimmune prone NZB mice. *Immunol. Invest.* **15**, 11.
191. Yung, R., Kaplan, M., Ray, D., Schneider, K., Mo, R. R., Johnson, K., and Richardson, B. (2001). Autoreactive murine Th1 and Th2 cells kill syngeneic macrophages and induce autoantibodies. *Lupus* **10**, 539.
192. Grossman, C. J. (1985). Interactions between the gonadol steroids and the immune system. *Science* **227**, 257.
193. Cutolo, M., Sulli, A., Seriolo, B., Accardo, S., and Masi, A. T. (1995). Estrogens, the immune response and autoimmunity. *Clin. Exp. Rheumatol.* **13**, 217.
194. Roubinian, J. R., Talal, N., Greenspan, J. S., Goodman, J. R., and Siteri, P. K. (1978). Effect of castration and sex hormone treatment on survival, antinucleic acid antibodies, and glomerulonephritis in NZB/NZW Fl mice. *J. Exp. Med.* **147**, 1568.
195. Yung, R., Williams, R., Johnson, K., *et al*. (1997). Mechanisms of drug-induced lupus III. Sex-specific differences in T cell homing may explain increased disease severity in female mice. *Arthritis Rheum.* **140**, 1334.
196. Cid, C., Kleinman, H. K., Grant, D. S., *et al*. (1994). Estradiol enhances leukocyte binding to tumor necrosis factor (TNF)-stimulated endothelial cells via an increase in TNF-induced adhesion molecules E-selectin, intercellular adhesion molecule type I and vascular cell adhesion molecule type I. *J. Clin. Invest.* **93**, 17.
197. Bigazzi, P. E. (1994). Autoimmunity and heavy metals. *Lupus* **3**, 449.
198. Yoshida, S., and Gershwin, M. E. (1993). Autoimmunity and selected environmental factors of disease induction. *Semin. Arthritis Rheum.* **22**, 399.
199. Bigazzi, P. E. (1988). Autoimmunity induced by chemicals. *J. Toxicol. Clin. Toxicol.* **26**, 125.
200. Gleichmann, E., Kimber, I., and Purchase, I. F. H. (1989). Immunotoxicology: Suppressive and stimulators effects of drugs and environmental chemicals on the immune system. *Arch. Toxicol.* **63**, 257.
201. Kaplan, M., and Richardson, B. C. (1999). Mechanisms of autoimunity in environmentally-induced connective tissue diseases. In "Rheumatic Diseases and the Environment" (J. Varga and L. Kauffman, Eds.), pp. 19–31. McGraw-Hill, New York.
202. Montanaro, A., and Bardana, E. J., Jr. (1991). Dietary amino-acid-induced systemic lupus erythematosus. *Rheum. Dis. Clin. North Am.* **17**, 323.
203. Roberts, J. L., and Hayashi, J. A. (1983). Exacerbation of SLE associated with alfalfa ingestion. *N. Engl. J. Med.* **380**, 1361.
204. Malinow, M. R., Bardana, E. J., Jr., and Goodnight, S. H., Jr. (1981). Pancytopenia during ingestion of alfalfa seeds. *Lancet* **1**, 615.
205. Bardana, E. J., Malinow, M. R., Houghton, D. C., *et al*. (1982). Diet-induced systemic lupus erythematosus (SLE) in primates. *Am. J. Kidney Dis.* **1**, 345.
206. Malinow, M. R., Bardana, E. J., Jr., Pirofsky, B., Craig, S., and McLaughlin, P. (1982). Systemic lupus erythematosus-like syndrome in monkeys fed alfalfa sprouts: Role of nonprotein amino acid. *Science* **216**, 415.
207. Rosenthal, G. A. (1977). The biological effects and mode of action of L-canavanine, a structural analogue of L-arginine. *Q. Rev. Biol.* **52**, 155.
208. Alcocer-Varela, J., Iglesias, A., Llorente, L., and Alarcon-Segovia, D. (1985). Effects of L-canavanine on T cells may explain the induction of systemic lupus erythematosus by alfalfa. *Arthritis Rheum.* **28**, 52.
209. Prete, P. E. (1985). Effects of L-canavanine on immune function in normal and autoimmune mice: Disordered B cell function by a dietary amino acid in the irnmuno-regulation of autoimmune disease. *Can. J. Physiol. Pharmacol.* **63**, 843.
210. Prete, P. E. (1986). Membrane surface properties of lymphocytes of normal DBA/2 and autoimmune NZB/NZW Fl hybrid mice: Effects of L-canavanine and a proposed mechanism for diet-induced autoimmune disease. *Can. J. Physiol. Pharmacol.* **64**, 1189.
211. Philen, R. M., and Posada, M. (1993). Toxic oil syndrome and eosinophilia–myalgia syndrome. 8–10 May 1991, World Health Organization meeting report. *Semin. Arthritis Rheum.* **23**, 104.
212. Varga, J., Jimenez, S. A., and Uitto, J. (1993). L-Tryptophan and the eosinophilia–myalgia syndrome: Current understanding of the etiology and pathogenesis. *J. Invest. Dermatol.* **100**, 97S.
213. Bell, S. A., Hobbs, M. V., and Rubin, R. L. (1992). Isotype-restricted hyperimmunity in a murine model of the toxic oil syndrome. *J. lmmunol.* **148**, 3369.
214. Tena, G. (1982). Fatty acid anilides and the toxic oil syndrome. *Lancet* **1**, 98.
215. Aldridge, W. N., and Connors, T. A. (1982). Toxic oil syndrome in Spain. *Food Chem. Toxicol.* **20**, 989.
216. Casals, C., Garcia-Barreno, P., and Municio, A. M. (1983). Lipogenesis in liver, lung and adipose tissue of rats fed with oleoylanilide. *Biochem. J.* **212**, 339.
217. Suarez, A., Viloria, M. D., Garcia-Barreno, P., and Municio, A. M. (1985). Toxic oil syndrome, Spain: Effect of oleoylanilide on the release of polysaturated fatty acids and lipid peroxidation in rats. *Arch. Environ. Contam. Toxicol.* **14**, 131.
218. Aldridge, W. N. (1992). Experimental studies. In "Toxic Oil Syndrome Current Knowledge and Future Perspectives" *WHO Regional Publications, European Series, No. 42*, pp. 67–97.
219. Kammuller, M. E., Bloksma, N., and Seinen, W. (1988). Chemical-induced autoimmune reactions and Spanish toxic oil syndrome. Focus on hydantoins and related compounds. *J. Toxicol. Clin. Toxicol.* **26**, 157.
220. Kammuller, M. E., Penninks, A. H., and Seinen, W. (1984). Spanish toxic oil syndrome and chemically

induced graft-versus-host-like reactions (letter). *Lancet* **2**, 805.
221. Gleichmann, H., and Gleichmann, E. (1984). GVHD, a model for Spanish toxic oil syndrome? *Lancet* **1**, 1474.
222. Kammuller, M. E., Verhaar, H. J., Versluis, C., *et al*. (1988). 1-Phenyl-5-vinyl-2-imidazolidinethione, a proposed causative agent of Spanish toxic oil syndrome: Synthesis, and identification in one of a group of case-associated oil samples. *Food Chem. Toxicol.* **26**, 119.
223. Verhaar, H. J., Kammuller, M. E., Terlouw, J. K., Brandsma, L., and Seinen, W. (1989). Spanish toxic oil syndrome: An isothiocyanate-derived compound cannot be substantiated as a causative agent (letter). *Food Chem. Toxicol.* **27**, 205.
224. Mizutani, T., Mizutani, H., Hashimoto, K., *et al*. (1991). Simultaneous development of two cases of eosinophilia–myalgia syndrome with the same lot of L-tryptophan in Japan. *J. Am. Acad. Dermatol.* **25**, 512.
225. Silver, R. M., Sutherland, S. E., Carreira, P., and Heyes, M. P. (1992). Alterations in tryptophan metabolism in the toxic oil syndrome and in the eosinophilia–myalgia syndrome. *J. Rheumatol.* **19**, 69.
226. Pestana, A., and Munoz, E. (1982). Anilides and the Spanish toxic oil syndrome. *Nature* **298**, 608.
227. Love, L. A., Rader, J. I., Crofford, L. J., *et al*. (1993). Pathological and immunological effects of ingesting L-tryptophan and 1,1′-ethyl-idenebis (L-tryptophan) in Lewis rats. *J. Clin. Invest.* **91**, 804.
228. Crofford, L. J., Rader, J. I., Dalakas, M. C., *et al*. (1990). L-Tryptophan implicated in human eosinophilia–myalgia syndrome causes fasciitis and perimyositis in the Lewis rat. *J. Clin. Invest.* **86**, 1757.
229. Belongia, E. A., Hedberg, C. W., Gleich, G. J., *et al*. (1990). An investigation of the cause of the eosinophilia–myalgia syndrome associated with tryptophan use. *N. Engl. J. Med.* **323**, 357.
230. Mayeno, A. N., Lin, F., Foote, C. S., *et al*. (1990). Characterization of "peak E," a novel amino acid associated with eosinophilia–myalgia syndrome. *Science* **250**, 1707.
231. Varga, J., Griffin, R., Newman, J. U., and Jimenez, S. A. (1991). Eosinophilic fasciitis is clinically distinguishable from the eosinophilia–myalgia syndrome and is not associated with L-tryptophan use. *J. Rheumatol.* **18**, 259.
232. Bigazzi, P. E. (1994). Autoimmunity and heavy metals. *Lupus* **3**, 449.
233. Lockie, L. M., and Smith, D. M. (1985). Forty-seven years experience with gold therapy in 1,019 rheumatoid arthritis patients. *Semin. Arthritis. Rheum.* **14**, 238.
234. Romagnoli, P., Spinas, G. A., and Sinigaglia, F. (1992). Gold-specific T cells in rheumatoid arthritis patients treated with gold. *J. Clin. Invest.* **89**, 254.
235. Hall, C. L. (1989). The natural course of gold and penicillamine nephropathy: A long term study of 54 patients. *Adv. Exp. Med. Biol.* **252**, 247.
236. Goebel, C., Kubicka-Muranyi, M., Tonn, T., Gonzalez, J., and Gleichmann, E. (1995). Phagocytes render chemicals immunogenic. Oxidation of gold (I) to the T-cell sensitizing gold (III) metabolite generated by mononuclear phagoeytes. *Arch. Toxicol.* **69**, 450.
237. Schuhmann, D., Kubicka-Muranyi, M., Mirtcheva, J., *et al*. (1990). Adverse immune reactions to gold: Chronic treatment with an Au (I) drug sensitizes mouse T cells not to Au (I), but to Au (III) and induces autoantibody formation. *J. Immunol.* **145**, 2132.
238. Tournade, H., Guery, J. C., Pasquier, R., *et al*. (1991). Experimental gold-induced autoimmunity. *Nephrol. Dial. Transplant.* **6**, 621.
239. Roger, J., Zilikens, D., Burg, G., and Gleichmann, E. (1992). Systemic autoimmune disease in a patient with long standing exposure to mercury. *Eur. J. Dermatol.* **2**, 168.
240. Tubbs, R. R., Gephardt, G. N., McMahon, J. T., *et al*. (1982). Membraneous glomerulonephritis associated with industrial mercury exposure. *Am. J. Clin. Pathol.* **77**, 409.
241. Gershwin, M. E., and Mackay, I. R. (1991). Primary biliary cirrhosis: Paradigm or paradox for autoimmunity. *Gastroenterology* **100**, 822.
242. Gregus, Z., Stein, A. F., Varga, F., *et al*. (1992). Effect of lipoic acid on biliary excretion of gluthathione and metals. *Toxicol. Appl. Pharmacol.* **114**, 88.
243. Aschner, M., and Aschner, J. L. (1989). Mucocutaneous lymph node syndrome: Is there a relation to mercury exposure? *Am. J. Dis. Child.* **143**, 1133.
244. Adler, R., Boxstein, D., Schoff, P., and Kerly, D. (1982). Metallic mercury vapour poisoning simulating mucocutaneous lymph node syndrome. *J. Pediatr.* **14**, 967.
245. Orlowki, J. P., and Mercen, R. D. (1980). Urine mercury levels in Kawasaki disease. *Pediatrics* **66**, 633.
246. Hultman, P., and Enestrom, S. (1988). Mercury-induced antinuclear antibodies in mice: Characterization and correlation with renal immune complex deposits. *Clin. Exp. Immunol.* **71**, 269.
247. Bariety, J., Druet, P., Laliberte, F., and Sapin, C. (1971). Glomerulonephritis with gamma and beta 1C globulin deposits induced in rats by mercuric chloride. *Am. J. Pathol.* **65**, 293.
248. Aten, J., Veninga, A., Brujin, J. A., *et al*. (1992). Antigenic specificities of glomerular-bound autoantibodies in membraneous glomerulopathy induced by mercuric chloride. *Clin. Immunol. Immunopathol.* **63**, 89.
249. Hultman, P., Johansson, U., Turley, S. J., *et al*. (1996). Adverse immunological effects and autoimmunity induced by dental amalgam and alloy in mice. *FASEB J.* **8**, 1183.
250. Bowman, C., Mason, D. W., Pusey, C. D., and Lockwood, C. M. (1984). Autoregulation of autoantibody synthesis in mercuric chloride nephritis in the Brown Norway rat. I. A role for T suppressor cells. *Eur. J. Immunol.* **14**, 464.
251. Hultman, P., Bell, L. J., Enestrom, S., and Pollard, K. M. (1993). Murine susceptibility to mercury. 11. Autoantibody profiles and renal immune deposits in hybrid, backcross, and H-2 congenic mice. *Clin. Immunol. Immunopathol.* **68**, 9.

252. Sapin, C., Mandet, C., Druct, E., Gunther, E., and Druet, P. (1982). Immune complex type disease induced by HgCl2 in Bown Norway rats: Genetic control of susceptibility. *Clin. Exp. Immunol.* **48**, 700.
253. Kubicka-Muranyi, M., Behmer, O., Uhrberg, M., *et al*. (1993). Murine systemic autoimmune disease induced by mercuric chloride (HgCl2): Hg-specific helper T-cells react to antigen stored in macrophages. *Int. J. Immunopharmacol.* **15**, 151.
254. Kubicka-Muranyi, M., Kremer, J., Rottman, N., *et al*. (1996). Murine systemic autoimmune disease induced by mercuric chloride: T helper cells reacting to self proteins. *Int. Arch. Allergy Immunol.* **109**, 11.
255. Pelletier, L., Rossert, J., Pasquier, R., Vial, M. C., and Druct, P. (1990). Role of $CD8^+$ T cells in mercury-induced autoimmunity or immunosuppression in the rat. *Scand. J. Immunol.* **31**, 65.
256. Pelletier, L., Pasquier, R., Rossert, J., *et al*. (1988). Autoreactive T cells in mercury-induced autoimmunity. Ability to induce the autoimmune disease. *J. Immunol.* **140**, 750.
257. Baran, D., Vendeville, B., Vial, M. C., Bascou, C., Teychenne, P., and Druet, P. (1986). Effect of cyclosporin A on mercury-induced autoimmune glomerulonephritis in the Brown-Norway rat. *Clin. Nephrol.* **25**(Suppl.), 175.
258. Rossert, J., Pelletier, L., Pasquier, R., and Duet, P. (1988). Autoreactive T cells in mercury-induced autoimmunity. Demonstration by limiting dilution analysis. *Eur. J. Immunol.* **18**, 1761.
259. Guery, J. C., and Druet, P. (1990). A spontaneous hybridoma producing auto-anti-idiotypic antibodies that recognize a V kappa-associated idiotype in mercury induced autoimmunity. *Eur. J. Immunol.* **20**, 1027.
260. Mathieson, P. W. (1995). Mercury: God of Th2 cells? *Clin. Exp. Immunol.* **102**, 229.
261. Gillespie, K. M., Qasim, F. J., Tibbats, L. M., *et al*. (1995). Interleukin-4 gene expression in mercury-induced autoimmunity. *Scand. J. Immunol.* **41**, 268.
262. Sudo, J., Hayashi, T., Kimura, S., *et al*. (1996). Mechanism of nephrotoxicity induced by repeated administration of cadmium chloride in rats. *J. Toxicol. Environ. Health* **48**, 333.
263. Kahn, M. F., Bourgeois, P., Aeschlimann, A., and de Truchis, P. (1989). Mixed connective tissue disease after exposure to polyvinyl chloride. *J. Rheumatol.* **16**, 533.
264. Kilburn, K. H., and Warshaw, R. H. (1992). Prevalence of symptoms of systemic lupus erythematosus (SLE) and of fluorescent antinuclear antibodies associated with chronic exposure to trichloroethylene and other chemicals in well water. *Environ. Res.* **57**, 1.
265. Storck, J., Del Razek, A., and Zimmermann, E. R. (1996). Effect of polyvinyl chloride plastic on the growth and physiology of human umbilical vein endothelial cells. *Biomaterials* **17**, 1791.
266. Bergman, K. (1982). Reactions of vinyl chloride with RNA and DNA of various mouse tissues in vivo. *Arch. Toxicol.* **49**, 117.
267. Ward, A. M., Udnoon, S., Watkins, J., Walker, A. E., and Darke, C. S. (1976). Immunological mechanisms in the pathogenesis of vinyl chloride disease. *Br. Med. J.* **1**, 936.
268. Sanchez-Roman, J., Wichmann, I., Salaberri, J., Varela, J. M., and Nunez-Roldan, A. (1993). Multiple clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. *Ann. Rheum. Dis.* **52**, 534.
269. Allison, A. C. (1996). Fibrogenic and other biological effects of silica. *Curr. Top. Microbiol. Immunol.* **210**, 147.
270. Scheule, R. K., and Holian, A. (1991). Immunologic aspects of pneumoconiosis. *Exp. Lung. Res.* **17**, 661.
271. Smith, H. R. (1995). Do silicone breast implants cause autoimmune rheumatic diseases? *J. Biomater. Sci. Polym. Ed.* **7**, 115.
272. Rohrich, R. J., Clark III, C. P., and Clark, C. P. (1993). Controversy over the silicone gel breast implant: Current status and clinical implications. *Tex. Med.* **89**, 52.
273. Bridges, A. J., Conley, C., Wang, G., Burns, D. E., and Vasey, F. B. (1993). A clinical and immunologic evaluation of women with silicone breast implants and symptoms of rheumatic disease. *Ann. Intern. Med.* **118**, 929.
274. Laing, T. J., Schottenfeld, D., Lacey, J. V., Jr., Gillespie, B. W., Garabrant, D. H., Cooper, B. C., Heeringa, S. G., Alcser, K. H., and Mayes, M. D. (2001). Potential risk factors for undifferentiated connective tissue disease among women: Implanted medical devices. *Am. J. Epidemiol.* **154**, 610.
275. Kossovsky, N., and Freiman, C. J. (1994). Silicon breast implant pathology. Clinical data and immunologic consequences. *Arch. Pathol. Lab. Med.* **118**, 686.
276. Naim, J. O., and van Oss, C. J. (1995). Silicone gels as adjuvants. Effects on humoral and cell-mediated immune responses. *Adv. Exp. Med. Biol.* **383**, 1.
277. Sanchez-Guerrero, J., Colditz, G. A., Karlson, E. W., *et al*. (1995). Silicone breast implants and the risk of connective-tissue diseases and symptoms. *N. Engl. J. Med.* **332**, 1666.
278. Kurland, L. T., and Homburger, H. A. (1966). Epidemiology of autoimmune and immunological diseases in association with silicone implants: Is there an excess of clinical disease or antibody response in population-based or other "controlled" studies? *Curr. Top. Microbiol. Immunol.* **210**, 427.
279. Hennekens, C. H., Lee, I. M., Cook, N. R., *et al*. (1966). Self-reported breast implants and connective-tissue diseases in female health professionals. A retrospective cohort study. *JAMA* **275**, 616.
280. Janowsky, E. C., Kupper, L. L., and Hulka, B. S. (2000). Meta-analysis of the relationship between silicone breast implants and the risk of connective-tissue disease. *N. Engl. J. Med.* **342**, 781.
281. Tugwell, P., Wells, G., Peterson, J., Welch, V., Page, J., Davison, C., McGowan, J., Ramroth, D., and Shea, B. (2001). Do silicon breast implants cause rheumatic disorders? A systemic review for a court-appointed national science panel. *Arthritis Rheum.* **44**, 2477.

282. Reidenberg, M. M. (1983). Aromatic amines and the pathogenesis of lupus erythematosus. *Am. J. Med.* **75**, 1037.
283. Ames, B. N., Kammen, H. O., and Yamasaki, E. (1975). Hair dyes are mutagenic: Identification of a variety of mutagenic ingredients. *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2423.
284. Freni-Titulaer, L. W., Kelley, D. B., Grow, A. G., *et al*. (1989). Connective tissue disease in southeastern Georgia: A case-control. Study of etiologic factors. *Am. J. Epidemiol.* **130**, 404.
285. Sanchez-Guerrero, J., Karlson, E. W., Colditz, G. A., *et al*. (1996). Hair dye use and the risk of developing systemic lupus erythematosus. *Arthritis Rheum.* **39**, 657.
286. Petti, M., and Allbritton, J. (1992). Hair product use in systemic lupus erythematosus. A case-control study. *Arthritis Rheum.* **35**, 625.

45

DRUG AND ENVIRONMENTAL LUPUS: CLINICAL MANIFESTATIONS AND DIFFERENCES

Anne-Barbara Mongey
Evelyn V. Hess

INTRODUCTION

There are increasing numbers of reports and case series of patients who develop lupus-like syndromes following exposure to certain medications and environmental agents. There have been at least 70 pharmacological and other agents implicated in the development of drug-related lupus with more recent reports implicating minocycline, the statin agents, and tumer necrosis factor (TNF) α inhibitors. The most commonly reported clinical features include constitutional symptoms, arthralgias/arthritis, serositis, and the development of autoantibodies. Typically there is resolution of clinical features on discontinuation of the medication, although autoantibodies may persist for prolonged periods of time. There is evidence to suggest that genetic factors play a role in determining which patients develop lupus-like syndrome. Physicians need to be aware of possible associations between the development of autoimmune-like features and exposure to medications and other therapies.

Systemic lupus erythematosus (SLE) is an autoimmune disease for which the etiology(ies) remain undetermined despite intensive research. There is evidence to suggest that genetic factors play a major role in the pathogenesis of this disease. However, results from monozygotic twin studies [1] would indicate that other, most likely environmental, factors are required for the induction of the disease. There have been many reports in the literature of the development of autoimmune phenomenon, such as autoantibodies and autoimmune-like diseases, following exposure to certain environmental agents including chemicals and drugs. The term drug-related lupus (DRL) refers to the development of a lupus-like syndrome following exposure to a certain drug. Typically there is a rapid resolution of the lupus-like clinical features following removal of the offending agent, although autoantibodies may persist for some time. With the introduction of new pharmacological agents, including biological agents, there has been an increase in the number of reports of drug-related lupus syndromes. Unfortunately, many of these reports are in the form of case reports that provide weak evidence for an association between the ingestion of a particular agent and the development of a lupus-like syndrome. In the absence of good controlled prospective studies on large numbers of patients, it can be difficult to confirm a definitive association between the ingestion of an agent and the development of an autoimmune-like disease. In some cases, exposure to the so-called offending agent may either have been coincidental with or even possibly have resulted in the induction of idiopathic SLE. It is important to try to make the distinction between this and the development of drug-related lupus since, in the case of the latter, removal of the offending agent should result in resolution of the

lupus-like syndrome. Ideally, one would like to confirm the association by rechallenge with the offending agent; however, this is seldom done in clinical practice. With the ever increasing availability of new medications, biological therapies, and herbal and other alternative remedies, physicians need to be aware of possible associations between the development of autoimmune-like diseases and the ingestion of these agents.

HISTORY

The first report of an association between ingestion of a drug and a lupus-like syndrome was made in 1945 by Hoffman [2] in which he described the development of fever, rash, myalgia, and nephritis in a 19-year-old man receiving sulfadiazine. Further reports implicating sulfonamides and penicillin followed in the 1950s [3–6] but since then, there has been no further evidence to support a definite association. It is now thought that an association with either of these drugs is unlikely and that previous reports may represent coincidental development of idiopathic SLE. It is also possible that the infection, for which the antibiotic was prescribed, may have played a role in the development of SLE as suggested by the current increasing speculation on a relationship between viral and bacterial infections and SLE. Hydralazine was first incriminated in 1953 by Morrow *et al.* [7]. Since that time there have been numerous other reports confirming this association [8, 9]. Anticonvulsants were first incriminated in 1957 when Lindqvist reported the development of a lupus-like syndrome in two patients administered mesantoin [10]. Procainamide, which is the most common cause of DRL, was first implicated by Ladd in 1962 [11]. A report from France in 1966 suggested an association with isoniazid which was subsequently supported by others [12, 13]. In 1971, Fabius and Gaulhofer [14] implicated the psychotrophic agents. Since the 1970s at least 70 pharmacological and other agents have been reported to be associated with DRL (Table 1). These include a variety of antiarrhythmic, antihypertensive, antipsychotic, antithyroid, antibiotic, antirheumatic, and various biological agents. With increasing physician awareness and introduction of new medications it is likely that this number will continue to increase. It is more common to find reports of an association with serological changes alone than with the development of DRL.

DEFINITION

There are as yet no established criteria for the diagnosis of DRL. The American College of Rheumatology requires 4 of 11 specified criteria to be present in order

TABLE 1 Drugs Definitively Associated with Drug-Related Lupus

Chlorpromazine
Hydralazine
Isoniazid
Methyldopa
Minocycline
Procainamide
Quinidine

Other drugs associated with drug-related lupus

Acebutolol	Metrizamide
Acecainide	Minoxidil
Allopurinol	Nalidixic acid
Aminoglutethimide	
Amoproxan	Nitrofurantoin
Anthiomaline	Nomifensine[a]
Anti-tumor necrosis factor alpha	Oxyphenisatin
Atenolol	Oxyprenolol
Atorvastatin	*Para*-amino salicyclic acid
Benoxaprofen	Penicillamine
Captopril	Penicillin
Carbamazepine	Perazine
Chlorprothixene	Perphenazine
Chlorthalidone	Phenelzine
Cimetidine	Phenopyrazone[a]
Cinnarazine	Phenylbutazone[a]
Clonidine	Phenylethylacetylurea[a]
Danazol	Phenytoin
Diclofenac	Practalol[a]
1-2-dimethyl-3 hydroxy-pyride-4-1	Prazosin
Diphenylhydantoin	Primidone
Disopyriamide	Prindolol
Enalapril	Promethazine
Estrogens	Propafenone
Ethosuximide	Prophythiouracil
Ethylphenacemide	Propranolol
Gold salts	Psoralen[a]
Griseofulvin	Pyrathiazine
Guanoxan	Pyrithoxine
Ibuprofen	Quinine
Interferon alpha	Reserpine
Interferon gamma	Simvastatin
Interleukin 2	Spironolactone
Labetalol	Streptomycin
Leuprolide acetate	Sulindac
Levadopa	Sulfadimethoxine
Levomeprazone	Sulfamethoxypyridazine
Lithium carbonate	Sulfasalazine
Lovastatin	Tetracyclines
Mephenytoin	Tetrazine
Mesalazine	Thionamide[a]
Methimazole	Thioridazide
Methlysergide[a]	Timolol eyedrops
Methylthiouracil[a]	Tolazamide
Metoprolol	Tolmetin
	Trimethadione

[a] No longer available.

to diagnose idiopathic SLE [15]. However, patients with DRL frequently present with less than 4 of these features. A temporal association between the ingestion of an agent and the development of the lupus-like features is required to make the diagnosis. Unlike drug hypersensitivity, it generally requires weeks to months of exposure to an agent before DRL develops. There should be remission of the clinical features with a decrease in the autoantibody titers following withdrawal of the offending agent. Recurrence of the syndrome following reintroduction of the agent would provide confirmatory evidence for an association but this is seldom done in clinical practice given the large number of alternative medications available. The development of anti-nuclear antibodies (ANAs) alone is not sufficient to diagnose DRL. It is now known that many patients will develop anti-nuclear antibodies while taking certain medications but the majority do not appear to develop the features of a lupus-like syndrome. Hence, the development of a positive ANA in the absence of other clinical features is not sufficient reason to discontinue the medication.

PREVALENCE

The real prevalence of DRL is unknown. It has been estimated that approximately one million people in the United States have idiopathic SLE and that up to 5% of these may be related to the ingestion of a pharmacological agent. The prevalence of DRL among certain populations is influenced by the pattern of drug prescription within these groups. With the increasing availability of alternative drugs and therapies, it is likely that the incidence of procainamide- and hydralazine-related lupus will decrease. However, physicians need to be alert to the possibility of new associations, including possible associations with biological agents, herbs, and other such compounds which are being used more frequently.

DEMOGRAPHIC FEATURES

The demographic features of DRL largely reflect those of the diseases for which the incriminated drugs are being prescribed. Hence, DRL occurs more frequently in the elderly population because of their higher incidence of diseases for which the drugs are prescribed. Exceptions include the anticonvulsant drugs and minocycline. Anticonvulsants are more commonly prescribed for children and, hence, most of the reports of anticonvulsant-related lupus have been described in younger age groups [16, 17]. Minocycline is generally used to treat acne in young females and, therefore, minocycline-related lupus has been most frequently reported in this population. In contrast to idiopathic SLE which has a male to female ratio of 1:9, DRL occurs only slightly more frequently in females [16, 18]. This reflects the demographic features of the underlying diseases for which the drugs are prescribed. Drug-related lupus in the United States occurs more frequently in Caucasians than in African-Americans, unlike idiopathic SLE.

CLINICAL FEATURES

Specific clinical features for the diagnosis of DRL have not as yet been defined (Table 2). Hence, the ARA revised criteria for the classification of SLE are often used as a guideline. However, as noted most patients with DRL have fewer than four criteria. A set of ascertainment criteria has been proposed by the

TABLE 2 Frequency of Clinical and Laboratory Features in Idiopathic SLE and DRL

Features	Idiopathic SLE	DRL
Constitutional	40–85	40–50
Arthralgias/arthritis	75–95	80–95
Myalgias	40–80	35–57
Rash	50–70	0–30
Lymphadenopathy	23–67	<15
Pleurisy	42–60	0–52
Pleural effusion	16–20	0–33
Pulmonary infiltrates	0–10	5–40
Pericarditis	20–30	0–18
Hepatomegaly	10–31	0–25
Splenomegaly	9–46	0–20
Renal involvement	50	0–13 (Hydralazine)
Neurological involvement	25–70	0–2
Anemia	30–90	0–53
Leukopenia	35–66	0–33
Thrombocytopenia	20–50	0–10
Positive Coomb's	18–30	0–23
Elevated ESR	50–75	60–93
Anti-nuclear antibodies	>95	100
Antibodies to histones	50–70	>95
Antibodies to dsDNA	50	<5
Anti-Sm antibodies	25	<5
Hypocomplementemia	40–65	0–25
Rheumatoid factor	25	20–40

Environmental Study Section of the American College of Rheumatology [19]. These include a positive ANA.

In the majority of patients, the symptoms are relatively mild compared with idiopathic SLE and acutely ill patients are rarely seen. DRL patients frequently present with constitutional symptoms such as malaise, low grade fever, and myalgia, which may occur acutely or insidiously. Articular features such as arthralgias and arthritis occur in over 80%. Arthralgias occur more commonly than frank arthritis. As seen with idiopathic SLE, there is typically symmetrical polyarticular involvement of mild to moderate intensity. Articular involvement most commonly affects the small joints of the hands followed by wrists, elbows, shoulders, ankles, and knees. Typically the synovial fluid is noninflammatory although inflammatory fluids have also been reported [20]; effusions are an uncommon finding. Pleuropulmonary disease occurs most frequently in procainamide-related lupus. Manifestations include pleurisy, pleural effusion, and pulmonary infiltrates. Pulmonary parenchymal disease has been reported in up to 40% of these patients unlike idiopathic SLE in which it is relatively uncommon [21]. LE cells may be found in pleural fluid of both DRL and idiopathic SLE patients and may be helpful in ruling out other possible causes for effusions, such as pneumonia and pulmonary infarction which can occur in the elderly population. Pericarditis is another manifestation of DRL occurring most frequently in procainamide-related lupus [22]. It may be complicated by the development of a pericardial effusion and even tamponade [23, 24]. LE cells may be detected in the pericardial fluid. Constricted pericarditis has been reported although it is an extremely uncommon manifestation of DRL [25]. Pericarditis has also been associated with mesalazine-induced lupus [26–29].

Other clinical features of idiopathic SLE, in particular dermatological, renal, and neurological features are rare in DRL. There have been reports of discoid lesions occurring with the use of griseofulvin and etanercept [30–32] and two reports of acute neutrophilic dermatosis "Sweet's syndrome" related to the use of hydralazine [33, 34]. The classic malar rash is rarely noted. Elkayam *et al.* [35] reported cutaneous involvment among 5 of 7 patients with minocycline-related lupus, of whom 3 had livedo reticularis and 2 had subcutaneous nodules. None of the 3 patients with livedo had anti-cardiolipin antibodies. Tournigand *et al.* [36] reported the recurrence of a lupus-like syndrome, characterized by arthralgias, fever, and a maculopapular eruption in a 14-year-old girl who had restarted minocycline after previously developing minocycline-related lupus. A few patients have been reported to have hepatosplenomegaly [37] and minocycline has been implicated in the development of an autoimmune hepatitis [38].

Renal involvement has been noted in patients receiving hydralazine [39, 40], although renal biopsy findings are generally minimal and often difficult to distinguish from hypertensive-related changes. However, there have been some reports of patients developing acute nephritis which has resolved on discontinuation of the hydralazine [41]. Reported histopathological findings included focal, segmental, membranous proliferative, and necrotizing glomerulonephritis which are indistinguishable from those seen in idiopathic SLE [39, 42, 43]. While improvement was noted in the majority of patients following discontinuation of the drug, some required treatment with steroids and other immunosuppressant agents while others failed to respond at all. It is unclear whether failure to respond reflected some underlying renal insufficiency secondary to another disease process such as hypertensive-related renal insufficiency. Clinical neurological disease occurs very rarely in DRL although there are a few reports of peripheral and cranial neuropathy occurring in procainamide and hydralazine DRL [44–46]. Vasculitis has also been reported as a manifestation of DRL [42, 47–52]. Lawson *et al.* reported the occurrence of cutaneous vasculitis in 2 of 23 patients with minocycline-induced lupus [51]. Cases of anti-neutrophil cytoplasmic antibodies (ANCA)-positive vasculitis, particularly when associated with anti-myeloperoxidase antibodies, have been associated with exposure to certain medications, such as hydralazine and propylthiouracil [52].

LABORATORY FINDINGS

Laboratory, including hematological, abnormalities occur less commonly in DRL than idiopathic SLE. One needs to keep in mind that many of the patients will exhibit abnormalities as a result of the underlying disease for which the medication has been prescribed. The erythrocyte sedimentation rate (ESR) is frequently elevated and often reaches moderate to high values; it quickly returns to the normal range with resolution of the clinical features of DRL. C-reactive protein is increased in cases of minocycline induced lupus. A mild, generally normochromic, normocytic, anemia may be seen although severe hematological abnormalities are rare [53]. Mild leukopenia and thromobcytopenia can also occur. A positive Coombs test has been reported in patients receiving methyldopa, procainamide, and chlorpromazine [54–59]. Hemolytic anemia has been reported in some patients receiving methyldopa [60].

Increased immunoglobulin levels may occur but less frequently than with idiopathic SLE. Circulating immune complexes have been described in procainamide- and hydralazine-related lupus [61, 62]. There have been a few

reports of decreased C3 and C4 levels in patients with procainamide- and quinidine-related lupus [63, 64]. However, levels tend to return to normal on discontinuation of the drug. Persistently low complement levels should suggest idiopathic SLE. Deposition of immunoglobulins at the dermal–epidermal junction (lupus band test) has been reported in patients with DRL secondary to procainamide and penicillamine [65, 66]. Immunoglobulin and complement deposition has also been noted in the glomeruli of a few patients with hydralazine-related lupus [40,43,67].

It is generally accepted that anti-nuclear antibodies need to be present in order to diagnose DRL, although their presence alone is not sufficient to make the diagnosis. Typically a diffuse/homogeneous staining pattern is seen although other patterns have occasionally been reported. The antigenic specificity of these antibodies are more restrictive compared with idiopathic SLE [68]. In particular, antibodies to the Sm antigen have rarely been described in DRL and their presence should strongly suggest idiopathic SLE. Antibodies to double-stranded (ds)DNA are uncommon findings in drug-related lupus but they have been reported to develop in patients treated with TNF-α inhibitors, sulphasalazine, and minocycline [69–71]. Studies by Charles *et al.* indicated that the incidence of anti-dsDNA antibodies among patients receiving infliximab varied according to the assay used [71]. Their data suggested that high affinity anti-dsDNA antibodies were more predictive of the development of lupus-like syndromes. Antibodies to single-stranded DNA are a relatively common finding but are considered nonspecific. Positive rheumatoid factors have also been reported [57] and considered to be nonspecific most likely representing cross-reactivity with anti-histone antibodies.

The homogeneous/diffuse fluorescent ANA staining pattern represents binding of antibodies to chromatin which consists of DNA and histones. There are five different histone proteins, namely H1, H2A, H2B, H3, and H4 seen in humans. The specificity of the autoantibody for these different histone proteins varies between the different drugs. Anti-histone antibodies seen in patients receiving procainamide commonly react with the H2A–2B dimer, its complex with DNA, and also with chromatin itself [72, 73]. It has been suggested that IgG antibodies to (H2A–2B)–DNA complex and to chromatin are specific for drug-related lupus while IgM antibodies to this complex and antibodies directed against the H2A–2B are most commonly found in asymptomatic patients receiving procainamide [73]. Antibodies against the (H2A–2B)–DNA complex have also been reported in DRL secondary to isoniazid, quinidine, sulfasalazine, penicillamine, and timolol [64, 74–76]. Antibodies from procainamide patients also exhibit reactivity with individual subfractions, most commonly the H1 [77]. Anti-histone antibodies from patients with hydralazine-related lupus recognize a broader array of autoantigenic epitopes including the individual histones H1, H3, and H4 and the H2A–2B and H3–H4 complexes [78–80]. Antibodies from procainamide-related lupus patients have also demonstrated reactivity with the H3–H4 complex but to a lesser degree than that to the H2A–2B complex [79]. Antibodies to the H1 and H2B subfractions have been reported in quinidine-related lupus [81]. It has been suggested that a switch from the IgM to IgG isotype precedes the development of lupus-like syndrome in patients receiving procainamide and this may also be true for other drugs [82]. While anti-histone antibodies are frequently found in DRL they are also found in idiopathic SLE and hence are not considered diagnostic for drug-related lupus [68, 77, 83]. In addition, anti-histone antibodies are not a common feature of minocycline-related lupus. In contrast, antibodies to double-stranded DNA are highly specific for idiopathic SLE and their presence would tend to rule out a diagnosis of drug-related lupus although they have been described in patients with minocycline-related lupus and patients receiving infliximab [35, 71].

Anti-phospholipid antibodies and lupus anticoagulants have been reported with drug-related lupus. They have been described in patients receiving chlorpromazine, perphenazine, procainamide, quinine, quinidine, hydralazine, sulfasalazine, and minocycline [35, 51, 59, 84–101]. These antibodies are most frequently of the IgM subclass and in prospective studies have not been associated with a significant increase in thrombotic events [84, 101] although there have been individual case reports suggesting an association [87, 89, 93].

Anti-neutrophilic cytoplasmic antibodies giving a perinuclear pattern (p-ANCA) on indirect immunofluorescence have been associated with the use of some medications. These include minocycline, sulphasalazine, hydralazine, and propylthiouracil [35, 51, 52]. Anti-neutrophilic cytoplasmic antibodies have been described with hydralazine-related lupus; these antibodies react with myeloperoxidase and elastase [102–104]. Choi *et al.* determined the prevalence of exposure to medications associated with ANCA-positive vasculitis among 30 patients with the highest titers of anti-myeloperoxidase antibodies among 250 patients in whom p-ANCA and anti-myeloperoxidase antibodies had been assayed [52]. Of the 30 patients, 10 (33%) had been exposed to hydralazine, 3 (10%) to propylthiouracil, 2 to penicillamine, one to sulphasalazine, and 2 to allopurinol. All patients had clinical and histological findings typical of ANCA-positive vasculitis. There was a strong association between the presence of anti-elastase and/or anti-lactoferrin antibodies and exposure to these medications. Although not all the 30 patients

had documented histological findings diagnostic of ANCA-positive vasculitis, all had the typical features of this entity. There are also reports associating anti-myeloperoxidase antibodies with exposure to hydralazine and propylthiouracil in the absence of vasculitis (105–107).

It is difficult to estimate the incidence of drug-induced ANCAs because of the lack of prospective studies. Choi *et al.* evaluated sera prospectively collected as part of three separate double-blind controlled trials for ANCA seroconversion [108]. The three prospective studies consisted of a 40-week trial of minocycline and a 37-week trial of sulfasalasine, both for treatment of rheumatoid arthritis, and a 104-week trial of penicillamine for the treatment of early systemic sclerosis. Sera of 12 patients from the combined total of 228 patients contained anti-myeloperoxidase antibodies, but no patients seroconverted to a positive ANCA during the duration of the respective studies. Hence, the exact role of drugs in the development of ANCAs remains unclear.

Lymphocytotoxic antibodies have been reported in patients with drug-related lupus secondary to procainamide, hydralazine, and phenytoin [109–112].

Antibodies to hydralazine and procainamide have been reported in patients receiving these medications [113, 114]. Antibodies against the incriminated drug appear to occur more frequently in patients with hydralazine-related lupus compared to those with procainamide-related lupus. The actual significance of these antibodies has yet to be defined.

DIFFERENTIAL DIAGNOSIS

Since many of the features of drug-related lupus are relatively nonspecific, it can be difficult to make a definitive diagnosis. A variety of inflammatory, infectious, and other autoimmune diseases can present with similar features and patients may not give accurate histories of previous medication usage as noted by Gordon and Porter [70]. In particular it is often difficult to differentiate DRL from idiopathic SLE. Since renal, cutaneous, neurological, and severe hematological involvement is rarely seen in drug-related lupus, the presence of these features would suggest idiopathic SLE. Demographic features may also be helpful in distinguishing between these two diseases since, with the exception of anticonvulsants and minocycline, DRL generally occurs more frequently in elderly Caucasian males in contrast to idiopathic SLE. The presence of antibodies to double-stranded DNA, Smith, and Ro antigens would favor idiopathic SLE. Other differential diagnoses would include infections, in particular viral syndromes which are a recognized cause of arthropathies. However, in most cases of virus-related arthropathy the symptoms resolve spontaneously within a matter of weeks to months. Osteoarthritis is a frequent finding in the elderly population and the articular symptoms of DRL may be mistaken for this especially if the symptoms are of an insidious onset. The diagnosis of DRL related to the use of minocycline and TNF-α inhibitors may be particularly problematic since both of these drugs are used to treat rheumatoid arthritis and it may be difficult to distinguish the development of DRL from a flare of rheumatoid arthtitis. Supervised rechallenge with the suspected medication may be helpful in confirming the diagnosis as suggested by the study by Lawson *et al.* [51].

THERAPY

The definitive treatment is withdrawal of the offending agent. In some cases, this can be difficult since many patients are on multiple medications. Generally, there is significant improvement in the symptoms within days of discontinuation of the medication. Rarely, it may take some weeks for improvement to occur. However, occasionally some patients with persistent symptoms may require further therapy. Nonsteroidal anti-inflammatory agents are useful to treat constitutional symptoms such as low grade fevers, arthralgias, and myalgias and may also be used to treat pleuritis and pericarditis. In patients with more severe involvement, such as large pleural or pericardial effusions, steroids are required. Generally these can be successfully tapered once the clinical features have resolved. Pericardectomy has been used in the treatment of constrictive pericarditis secondary to procainamide. In the rare instance where a patient requires continuation of the offending medication for medical reasons, symptoms have been treated with either a nonsteroidal anti-inflammatory agent or steroids. However, with the greater availability of alternative medications, in particular the antiarrythmic agents, this is rarely if ever necessary.

COMMENTS ON CERTAIN DRUGS ASSOCIATED WITH DRUG-RELATED LUPUS

Many of the incriminated drugs have (1) an arylamine or hydrazine functional group, (2) a sulfhydryl or thiono sulfur group, or (3) are hydantoins possibly metabolized to phenol. Since not all individuals exposed to a certain drug will develop autoimmunity, this suggests that other factors may be very important. Certain

individuals may metabolize drugs and other agents at a faster rate than others. Polymorphisms of genes coding for enzyme systems exist and these may be important in determining exposure time of the host to certain agents or their metabolites. Study of the metabolites of the various compounds is very important to determine if a metabolite(s) is involved in the autoimmune phenomenon. A number of polymorphisms have been reported for the cytochrome P450 enzyme system which is known to play an important role in the metabolism of both endogenous and exogenous agents.

Hydralazine

Hydralazine was introduced as a new antihypertensive agent in 1952 and it is generally accepted to be the first drug definitively associated with DRL [7]. Reports have indicated that between 24 and 54% of patients receiving hydralazine develop anti-nuclear antibodies; however, only 2 to 21% of patients develop lupus-like symptoms [9]. The most common symptoms are fever, malaise, arthralgias, and arthritis. Serositis may also be seen but occurs less commonly than with procainamide-related lupus. Renal involvement has also been reported with this drug although in some cases it is unclear whether the renal manifestations may not in fact be related to the underlying hypertension. The development of hydralazine-related lupus is dose related. Patients receiving greater than 200 mg per day of hydralazine have a significant increase risk of developing DRL compared with those receiving smaller doses [48]. The risk has also been related to the acetylator phenotype which is under genetic control [115]. DRL occurs more commonly in patients who are slow acetylators. This is believed to be due to the slower rate of inactivation of the parent compound through acetylation of its hydrazine group by the hepatic acetyl transferase enzymes.

The development of hydralazine-related lupus has been linked to the HLA Class II and III genes. In 1980, Batchelor *et al.* reported a frequency of 79% for the HLA-DR4 antigen in patients who developed hydralazine-related lupus compared to 25% in asymptomatic hydralazine-treated controls [18]. However, a subsequent study by Brand *et al.* of 18 Australian patients with hydralazine-related lupus failed to show an increased frequency of the HLA-DR4 antigen compared with a control group from Melbourne [116]. A study by Russell *et al.* [117], reported a frequency of HLA-DR4 antigen of 70% among 20 patients with hydralazine-related lupus compared with a frequency of 33% in the general population. Six of these 20 patients had been included in the 1980 study by Batchelor *et al.* A further study by the Batchelor group examining a possible role of polymorphisms of the HLA Class III gene noted a significant increase in the frequency of one or more null alleles of the genes coding for C2, C4A, and C4B among 21 cases with hydralazine DRL compared with 82 normal controls [118]. Of interest was the finding of a significantly higher frequency of the HLA-DR4 antigen among the patients compared with the controls. The genes which code for C2, C4A, and C4B are located on chromosome 6 adjacent to the HLA DR regions and their alleles exhibit linkage disequilibrium with HLA DR alleles. In particular, the C4B null allele is in linkage disequilibrium with HLA-DR4 antigen. It has therefore been postulated that it may be the C4 null allele in linkage disequilibrium with the DR4 antigen that is important in determining susceptibility to the development of DRL in patients receiving hydralazine. If no linkage disequilibrium exists between the HLA-DR4 and the C4 null alleles among the Australian population, this could explain the discrepancy between the results of the previously mentioned studies.

Anti-phospholipid, lymphocytotoxic, anti-neutrophilic cytoplasmic antibodies and antibodies to poly A and Z-DNA have been described in patients receiving hydralazine in addition to anti-nuclear and anti-histone antibodies [99, 102–104, 111, 119, 120]. The pathogenic significance of these autoantibodies have yet to be elucidated. The frequency of hydralazine-related lupus appears to have decreased during the more recent years as the dosages and use of the drug have decreased.

Procainamide

Procainamide is a class I antiarrhythmic agent and is believed to be the commonest cause of DRL in the United States. A positive ANA has been reported to develop in up to 90% of patients [72]. Approximately 30% of these patients will subsequently develop DRL [121]. The duration of therapy prior to the onset of symptoms varies from 1 month to as late as 12 years with an average of 1 year [57]. Common features include constitutional and musculoskeletal symptoms with approximately half of the patients developing pleuropulmonary involvement and/or pericarditis. Although there is a weaker relationship with acetylator status compared to hydralazine, it has been reported that patients with a slow acetylator phenotype will develop ANAs and DRL after a shorter duration of therapy compared to those with a fast acetylator phenotype [121]. However, this difference in phenotypes is less obvious if the dose is titrated such that the plasma levels of the drug are equivalent between the two groups [122]. A more recent study did not find an association between slow acetylator status and the development of procainamide-related lupus although patient numbers

were small [123]. However, this suggests that other factors are involved. A positive association of DQβ1 DQw7 (split of DQw3) with antibodies to histone subfractions (H2A–2B), Z-DNA, and poly A has been reported in asymptomatic patients receiving PA [124].

As with hydralazine, lymphocytotoxic, antiphospholipid antibodies, lupus anticoagulants, and antibodies to poly A and Z-DNA have been described in patients on procainamide [72, 88–94, 109]. Antibodies to procainamide have also been reported [114].

Isoniazid

Anti-nuclear antibodies develop in approximately 20–25% of patients receiving isoniazid; however, less than 1% of patients develop a lupus-like syndrome [125]. Common features of isoniazid-related lupus include fever, arthralgias, and arthritis and less commonly serositis. IgG anti-nuclear antibodies against the nucleohistone complex (H2A–H2B)–DNA have been reported in a patient with isoniazid related lupus [126]. Since other antituberculous agents and tuberculosis itself have been reported to be associated with ANAs and rheumatoid factors, it may be difficult for the clinician to determine if isoniazid has a pathogenic role in the development of new lupus-like symptoms. Although isoniazid is also inactivated through acetylation, no association between acetylator phenotype and the development of ANA and/or DRL has been reported [127, 128].

Quinidine

Quinidine is another class I antiarrhythmic agent associated with the development of DRL. The first report by Kendal and Hawkins was published in 1970 [129]. At least 29 further reports of quinidine-related lupus have appeared in the English literature since then [130]. Quinidine-related lupus occurs primarily in older White patients (mean age of 63 years) and equally in men and women. There has been one case report in an 11-year-old girl. Typically, symptoms develop after 1–18 months of therapy, and resolve within 1–4 weeks. Of interest is the report of development of quinidine-related lupus in two patients who had a previous history of procainamide-related lupus [131]. In both cases, there had been resolution of the symptoms following discontinuation of procainamide and prior to the institution of quinidine.

The most common clinical features are those of arthralgias and arthritis which are characterized by symmetrical involvement of the hands and wrists and less commonly involvement of the shoulders and knees. Hence, it may be mistaken for rheumatoid arthritis. Less frequently reported features include fever, rash, serositis, and hepatic and neurological involvement, primarily manifested by carpal tunnel syndrome and peripheral neuropathy. Thrombocytopenia and leukopenia have been reported in 47 and 24% of patients, respectively. Hypocomplementemia has been reported in a few patients. While the ANA fluorescence pattern associated with DRL is typically homogeneous, both speckled and homogeneous patterns have been seen in patients with quinidine-related lupus. Both IgG and IgM classes of anti-histone antibodies have been reported which may demonstrate reactivity with H1, H2B, and the complexes H2A–2B and H3–H4 [130]. The lupus anticoagulant has also been described.

There have been three cases of development of polyarthropathy alone in patients receiving quinidine which resolved on discontinuation of the drug and recurred on rechallenge [64]. None of these three patients, however, had a positive ANA and the articular symptoms were milder than those usually seen with quinidine-related lupus. The duration of therapy was shorter and the symptoms resolved within 1 week of discontinuation of the drug. It is unclear whether these may represent a variant of quinidine-related lupus.

Quinine, an antimalarial drug analog of quinidine, has also been reported to be associated with the development of autoantibodies and a lupus-like syndrome [98].

Anticonvulsants

Many anticonvulsants have been implicated in the development of DRL, of which the most common are the hydantoins, ethosuximide, trimethadione, and carbamazepine [16, 17, 132–135]. There have also been reports implicating primidone and valproate [136]. The demographic features of anticonvulsant-related lupus differ from the other forms of DRL in that the majority of patients have been children and young females. Problems arise in deciding whether the development of lupus-like symptoms in patients receiving anticonvulsants represent a form of DRL or are in fact a manifestation of idiopathic lupus of which the seizure disorder is the presenting feature. This may pose particular diagnostic and management problems in adults as there may be a history of continuing convulsions and possibly psychiatric problems.

Some studies have reported a higher frequency of ANAs in children receiving anticonvulsants. Beernik and Miller [16] reported that 11 of 48 children receiving anticonvulsant therapy at a pediatric neurology clinic had anti-nuclear antibodies although none had any symptoms suggestive of lupus. They also reported 5 patients with anti-nuclear antibodies and lupus-like

symptoms. Singsen and colleagues reported that 14 of 70 patients receiving anticonvulsants associated with DRL had high titers of ANA compared with low titers in 5 of 23 receiving phenobarbitol and 1 of 50 controls [17]. In addition, they reported 5 patients who developed a lupus-like syndrome while receiving ethosuximide characterized by fever, malaise, rash, arthritis, and serositis. There was resolution of these symptoms in 4 of 5 children following discontinuation of ethosuximide although 3 did require prednisone. Histone antibodies have been described in a patient who developed DRL secondary to ethosuximide [137]. Interestingly, there does not appear to be a relationship between phenobarbital and DRL and there is only one case report suggesting a relationship with one of the benzodiazepines. The report described a photosensitive skin eruption resembling discoid lupus, mild leukopenia, and positive ANA and rheumatoid factor in a 7-year-old girl during clobazam therapy [138]. There was resolution of the cutaneous lesions and autoantibody disappearance following discontinuation of this drug. A study by Verrot *et al.* of 163 patients attending a medical center specializing in epilepsy found that 41 patients (25%) had anti-nuclear antibodies compared with 10% of the controls [139]. But no statistical association was found between the presence of anti-nuclear antibodies and the type of epilepsy, the kind of antiepileptic drug, or the age or sex of the patient. However, the numbers were relatively small and hence, may not have allowed for an association to be detected. IgG class anti-cardiolipin antibodies were found in 31 patients, although no patient had a history of a thrombotic event.

Chlorpromazine

Anti-nuclear antibodies occur in 20–50% of patients treated with chlorpromazine [140], but a lupus-like syndrome develops in less than 1%. Typical features are fever, arthralgias/arthritis, serositis, and cutaneous features [87, 141]. Photosensitivity and malar rashes have been reported which have resolved on discontinuation of the drug. Over 50% of patients have been reported to have splenomegaly. Significant elevation of IgM, prolongation of PTT, and both lupus anticoagulant and anti-cardiolipin antibodies have been described in patients receiving long-term chlorpromazine therapy [84, 101]. Canoso and de Oliveira [84] noted that 54 of 96 of these patients had IgM lupus anticoagulants and, of these, 31 had IgM and 4 had IgG anti-cardiolipin antibodies; 5 patients had anti-cardiolipin antibodies alone. Only 3 patients developed thrombotic episodes over a median follow-up period of 5 years suggesting a low risk for thrombosis. A study by Zarrabi *et al.* [59] noted that 41 of 47 patients receiving chlorpromazine for greater than 2.5 years had a circulating anticoagulant. It should be noted that many of the patients studied in the reports were hospitalized for psychiatric and psychological disorders raising the question of whether or not these disorders may have represented a manifestation of SLE. This points out the great importance of follow-up studies for all drugs and environmental agents to observe the consequences of discontinuation of the possible offending agent.

Beta Blockers

Anti-nuclear antibodies occur in 10–30% of patients receiving beta blockers. Of these, acebutolol has been the drug most commonly implicated followed by labetalol (14–16%), oxprenolol (13%), metoprolol (12%), propranolol (10%), and pindolol (10%) [142–146]. ANA titers tend to rise with duration of therapy and decrease following discontinuation of the drugs. Lupus-like syndromes rarely occur with these agents although there are reports implicating practolol, acebutolol, labetalol, and pindolol. There is also a case report of a lupus-like syndrome developing in a patient receiving ophthalmic timolol which was associated with markedly elevated IgG antibodies to the (H2A–2B)–DNA complex [76].

ACE Inhibitors

Anti-nuclear antibodies have been reported in patients receiving captopril and enalapril [147–153]. Reidenberg *et al.* [147] noted that 10 of 37 patients developed anti-nuclear antibodies while receiving high doses of captopril. Three of 9 patients receiving enalapril developed a positive ANA test (titer greater than 1:160) during a 6-week follow-up study by Schwartz *et al.* [151]. Of interest was the fact that none of these patients were ANA-positive prior to starting the drug. There have been a few case reports implicating captopril as a cause of DRL. Two patients developed serositis and anti-nuclear antibodies which resolved on discontinuation of the drug [149, 152]. Another patient developed myalgia, arthralgia, photosensitivity, anti-nuclear antibodies, and IgG antibodies to H2A–2B dimer after taking captopril for 2.5 years; there was significant improvement in the clinical symptoms following withdrawal of the medication [153].

Sulfasalazine

Sulfasalazine was first implicated in drug-related lupus in 1965 when Alarcon-Segovia *et al.* described the development of a lupus-like syndrome and LE cells in 5 patients receiving sulfasalazine for treatment of

ulcerative colitis [154]. Since then, there have been a number of reports on the development of autoantibodies and lupus-like syndromes in patients receiving this drug for treatment of inflammatory bowel disease and arthropathies [69, 75, 100, 154–163]. Dekeyser *et al.* reported the development of anti-nuclear antibodies in 13 of 77 patients with spondyloarthropathy while receiving sulfasalazine [155]. The most common clinical manifestations of sulfasalazine-related lupus are arthralgias, arthritis, pleuritis, pericarditis, and pleural effusions. There have been two reports of a patient developing cardiac tamponade [157, 161]. Fevers and a variety of cutaneous lesions have also been described. Anti-histone antibodies including IgG antibodies to the H2A–2B dimer and (H2A–2B)–DNA complex have been noted [75]. Some patients have had anti-cardiolipin and anti-neutrophil cytoplasmic antibodies [100, 163]. Mielke *et al.* [69] reported that 20 of 24 patients with inflammatory arthritis treated with sulfasalazine developed double-stranded DNA antibodies although only one developed symptoms of DRL. The occurrence of these antibodies was transient in the majority of patients.

Gordon *et al.* evaluated 100 patients with rheumatoid arthritis who were enrolled in a prospective randomized trial comparing sulphasalazine and auranofin for the development of ANAs and lupus-like syndromes [164]. Fourteen (19%) of 72 patients treated with sulphasalazine who were either ANA-negative or weakly ANA-positive at the start of the study became strongly ANA-positive during the study. This compared with an incidence of 14% among 80 patients randomized to receive auranofin. Despite the development of ANA positivity no patient developed a lupus-like syndrome. This suggests that the occurrence of ANA positivity either prior to or during treatment with sulphasalazine does not preclude treatment with this medication.

Gunnarrson *et al.* evaluated the prevalence of lupus-like reactions among 41 consecutive patients with early rheumatoid arthritis who were treated with sulphasalazine as single therapy for at least 6 months [165]. Two patients developed anti-dsDNA antibodies after approximately 12 months of treatment and 1 of these patients also developed a photosensitive rash. Another patient developed biopsy proven membranous nephritis, compatible with lupus nephritis, after 26 months of treatment with sulphasalazine. One patient developed a sun rash and increasing titers of ANA after 6 months of therapy and a fourth patient, who developed anti-dsDNA antibodies remained asymptomatic. All 4 patients were ANA-positive. Sixteen (39%) patients had ANAs prior to institution of sulphasalazine and 13 (32%) were ANA-positive following 6 months of therapy. Patients who developed lupus-like features did not have a significantly higher ANA titer. However, there was a correlation between ANA positivity and a speckled pattern prior to initiation of treatment, regardless of titer, and the development of lupus-like features. Three of the 4 patients who developed lupus-like features had the HLA DR 0301 haplotype compared with 4 out of 37 patients without lupus-like symptoms. The occurrence of this SLE-related HLA haplotype among those patients who developed a lupus-like syndrome suggests a genetic role for the development of sulphasazine-induced lupus. Increased interleukin (IL)-10 levels were also seen in these patients.

Since increasing numbers of patients are being treated with sulfasalazine for inflammatory arthropathies, such as rheumatoid arthritis and spondyloarthropathies, one must consider the possibility of development of DRL in any patient who has a significant increase in their articular symptoms.

It has been suggested that it is the sulfonamide component of sulfasalazine that is responsible for the development of the autoimmunity since sulfonamides have previously been associated with DRL. However, there are a number of reports implicating mesalazine as a cause of DRL suggesting that the presence of sulfapyridine is not necessary. Dent *et al*. described the development of pleuropericarditis, fever, and a positive ANA in a patient receiving mesalazine [26]. Kirkpatrick *et al*. reported that four patients with inflammatory bowel disease, treated with 5-aminosalicylic acid (5-ASA), developed ANA-positive-arthralgias and inflammation which resolved rapidly following discontinuation of the 5-ASA compounds [166]. Timsit *et al*. reported the case of a patient with Crohn's disease who developed polyarthritis, alopecia, lymphoneutropenia, positive ANA, and antibodies to histones, Smith, and RNP while being treated with mesalazine [167]. There was rapid resolution of the clinical features and a decrease in the levels of autoantibodies with discontinuation of the medication. Vayre *et al*. described the case of a 53-year-old male with Crohn's disease, for which he had been treated for approximately 8 years with mesalazine, who developed chest pain, ST segment elevation, and a small pericardial effusion [27]. Resolution of these features occurred following discontinuation of mesalazine. Molnar *et al*. reported the case of a 29-year-old woman with inflammatory bowel disease, treated with mesalazine for approximately 2 months, who developed pericarditis and a moderate size pericardial effusion which responded to the institution of steroids, despite the fact that she remained on mesalazine [28]. She also had a positive p-ANCA. Sentongo and Piccoli reported the case of a child who developed pericardits, believed to be secondary to treatement of mesalazine [29]. Gunnarsson *et al*. described the case of a 43-year-

old female with Crohn's disease who developed polyarthritis involving the small joints of her hands, wrists, ankles, and MTP joints [168]. ANA and IgG anti-cardiolipin antibodies were markedly positive, anti-histone antibodies were positive, and antibodies to dsDNA were borderline elevated. Her clinical features resolved within 2–3 weeeks of discontinuation of the medication, although her ANA and IgG anti-cardiolipin antibodies were still positive 1 year later. She was noted to be DQA1*0501 haplotype positive. This haplotype has previously been reported to occur more frequently among patients with sulphasalazine-induced lupus syndrome suggesting a genetic predisposition [169].

Minocycline

Minocycline hydrochloride is a semisynthetic tetracycline used for a variety of infections and for the long-term treatment of acne vulgaris. It was first associated with DRL in 1992 by Matsuura *et al*. [170]. There have been at least 56 further cases reported in the literature since then [35, 36, 38, 51, 70, 171–177]. Minocycline has also been implicated in the development of autoimmune hepatitis [38]. In addition to case reports, there have been a number of case series of lupus-like syndromes developing among patients receiving minocycline [171, 177]. In contrast to other forms of DRL, a similar gender profile to that of idiopathic SLE has been observed since the great majority of these cases have occurred in young women who were being treated for acne, although it may occur in men. Autoimmune hepatitis has been reported to occur in the majority of men who develop minocycline-related lupus [35].

The most commonly reported features include constitutional symptoms, rash, polyarthralgias, an inflammatory arthritis which tends to affect the small joints of the hands, wrists, shoulders, and ankles in a symmetrical fashion, and autoimmune hepatitis [36, 175, 176, 178]. Pleuropulmonary involvement has been described in some patients, and livedo reticularis has also been noted [35, 38, 174]. Elkayam *et al*. reported cutaneous involvment among five of seven patients with minocycline-related lupus, of whom three had livedo reticularis and two had subcutaneous nodules [35]. None of the three patients with livedo had anti-cardiolipin antibodies and only two had positive ANAs. Dunphy *et al*. reported that two patients treated with minocycline developed erythema on their legs, two an urticarial eruption, one patient thrombocytopenia, and another patient anemia [178].

Anti-nuclear antibodies and p-ANCAs with titers varying from 1:80 to 1:1280 are a common finding [35, 51, 178]. Anti-dsDNA and anti-cardiolipin antibodies occur less often and anti-histone antibodies, which are a common finding in other forms of drug-related lupus, are not commonly seen in patients with minocycline-induced lupus. Duration of minocycline therapy prior to the onset of symptoms varies from a few weeks to up to 10 years. As with other forms of drug-related lupus, there is resolution or significant improvement of the clinical features in most cases following the drug withdrawal although some patients have required institution of steroids. Serological features also improve following discontinuation of minocycline, although they can persist for several months following discontinuation. The autoimmune hepatitis tends to resolve more slowly [35]. There are reports of an allergic type fulminant type hepatitis and of hypersensitivity-like reactions consisting of fever, lymphadenopathy, and skin eruption developing in patients receiving minocycline [175].

Gordon and Porter [70] reported the clinical and serological features of a case series of 20 patients diagnosed with minocycline-related lupus over a 5-year period in the west of Scotland. Minocycline had been prescribed for a mean of 25 months, range of 3–60 months, prior to the onset of symptoms. Fifteen patients were female and 5 were male, with the mean age of 24 years. Twenty patients had arthritis with some patients complaing of lethargy, myalgia, and fever. Discontinuation of minocycline led to resolution of symptoms in all patients after a mean of 15.7 weeks (range of 2 to 56 weeks). All patients had a positive ANA, 2 patients had anti-cardiolipin antibodies, and 1 patient had a positive p-ANCA. Eight of 20 (40%) patients had antibodies to dsDNA, of which 2 were positive using the *Crithidia* assay. Antibodies to dsDNA had disappeared and ANA titers decreased during the follow-up period. Six of the patients who had initially denied talking minocycline developed a recurrence of symptoms after a mean of 4.5 days (range: 2–7 days) of restarting minocycline. Two of these patients required treatment with steroids and 5 received hydroxychloroquine. Thus, denial of previous use of minocycline does not rule out the possibility of minocycline-related lupus and rechallenge with the medication may be helpful in establishing the diagnosis.

Some patients have been rechallenged with minocycline and have developed a recurrence of their joint symptoms within a few days. Lawson *et al*. reported on the series of 23 patients with minocycline-induced lupus [51]. All the patients complained of polyarthralgias with 3 patients having synovitis of the MCP and PIP joints and one patient an effusion. Cutaneous vasculitis occurred in 2 patients. Five patients complained of impaired concentration and poor memory and 1 patient had neuropathy. Nineteen patients had positive ANAs with titers varying from 1:40 to greater than 1:16,000. Four patients had antibodies to ds-DNA by ELISA and

Crithidia assays and 10 patients had positive p-ANCAs, with the majority having titers of 1:160 or greater. Of these 10 patients, 7 had antibodies to myeloperoxidase. Anti-histone antibodies were not detected in the 9 patients in whom they were sought. Following discontinuation of minocycline, 10 patients became asymptomatic within 1 month, 9 patients had improved within 1–6 months, and 3 within 6–12 months; it took 2 years for the symptoms to resolve in 1 patient. Four patients with severe disease required treatment with corticosteroids. There was no correlation between duration of treatment with minocycline and the time taken for resolution of the symptoms following discontinuation of the drug. Anti-nuclear antibodies remained strongly positive in 4 patients but became negative in the remainder of patients within 2 years of withdrawal of minocycline. Recurrence of symptoms occurred within hours of restarting minocycline in 5 patients. Ten additional patients underwent a supervised rechallenge with minocycline. There was a recurrence of symptoms in 7 patients within 12 h and in the remaining 3 patients within 72 h of ingesting a single 100-mg dose of minocycline. Two patients who were rechallenged required a short course of steroids; all subjects were asymptomatic again within 2 weeks. Elkayam *et al*. rechallenged 5 patients with a single dose of 50 mg of minocycline: all 5 patients developed arthralgias within 24 h that subsequently resolved spontaneously [35]. Thus, supervised rechallenge may be helpful in confirming the diagnosis in some patients.

In a case control study of 27,688 acne patients, Sturkenboom *et al*. reported an 8.5 risk of developing a lupus-like syndrome among patients who were current users of minocycline compared with previous and nonusers of tetracycline combined [179]. Current exposure to minocycline and a cumulative dose above 100 defined daily doses increased the risk 16-fold. Among females, there were 17.2 cases of lupus-like syndrome per 100,000 prescriptions for oxytetracycline and 52.8 cases per 100,000 prescriptions for minocycline, suggesting that minocycline is more likely to induce lupus-like syndromes compared with other forms of tetracycline. There are early reports implicating tetracycline in DRL. However, it is generally considered that the association with tetracyclines is weak and reported cases may in fact represent an exacerbation of SLE. Work by McHugh *et al*. suggests that minocycline-induced toxicity may be mediated through an oxidized metabolite and be myeloperoxidase dependent [180]. The enzymes involved in the metabolism of minocycline may bind to a reactive intermediate acting as a hapten, leading to the formation of a neoantigen with consequent induction of an autoimmune response. Herzog *et al*. suggested that there may be an antibody reaction to a metabolite of minocycline which cross-reacts with microsomal chromosomes in the liver [181]. They reported that serum obtained from a patient with minocycline induced autoimmune hepatitis reacted with a 50-kDa protein from rat liver microsomes. Minocycline is known to have immunomodulating properties and has been demonstrated to affect production of cytokines, including TNF-α [182]. These effects on cytokine production may play a role in the development of minocycline-related autoimmunity.

Reports have also suggested a role for MHC Class II genes in the development of minocycline-related lupus. Dunphy *et al*. reported a case series of 14 patients who developed minocycline-induced lupus after using minocycline for a median of 3.8 years (range: 1–10 years) [178]. Discontinuation of minocycline led to resolution of symptoms in all patients within days to 6 months. Recurrence of symptoms was seen among patients, who were rechallenged with minocycline. Sera from all 14 patients had ANCAs giving a perinuclear pattern on indirect immunofluorescence. Eleven patients had elevated anti-myeloperoxidase antibodies and 10 had anti-elastase antibodies which decreased following discontinuation of medication. All 13 patients who were tested were either HLA-DR4 (9 patients) or HLA-DR 2 (4 patients) -positive and all had an HLA-DQB1 allele. The authors suggested that the presence of p-ANCA may be a marker for the development of lupus-like symptoms in genetically susceptible individuals prescribed minocycline. Elkayam *et al*. reported that 4 out of 6 patients whom they had HLA typed had HLA DRβ1*0104 that compared to an expected frequency of 12% in the general Israeli population [35].

Lipid Lowering Agents

There have been some case reports associating the use of HMG-CoA reductase agents with the development of lupus-like syndromes. Lupus-like syndromes have been associated with the use of lovastatin, simvastatin, atorvastatin, and fluvastatin. Ahmad [183] described two patients who developed malaise, inflammatory polyarthritis, and ANAs while receiving lovastatin. Anti-histone antibodies were detected in one patient. Both patients had resolution of these symptoms following discontinuation of the drug and a short course of prednisone therapy. Bannwarth *et al*. described the development of a symmetric polyarthritis, fatigue, and positive anti-nuclear antibodies in a patient receiving simvastatin [184]. There was resolution of the polyarthritis following discontinuation of the drug and a short course of prednisone. Kaur *et al*. reported the development of left-sided chest pain, low grade fevers, and a minimal left pleural infusion and mild pericardial

effusion in a 73-year-old Caucasian female, who had been receiving simvastatin 80 mg/day [185]. She also had a markedly elevated ESR at 111 mm/h, positive CRP at 3.5, and a positive ANA at 1 : 160 homogeneous pattern. Anti-histone antibodies were strongly positive at a titer of 5.4 (normal < 0.8). Discontinuation of simvastatin and institution of prednisone resulted in resolution of clinical features and normalization of the serological abnormalities within 8 weeks. Ahmad *et al*. reported the case of a patient who developed pleurisy and arthralgia after commencement of simvastatin [186].

Hanson and Bossingham reported the development of Raynaud's phenomenon and some "occasional arthralgias" in a 39-year-old male who had been receiving simvastatin (20 mg) daily for 4 years [187]. He also had a positive ANA at a titer >1 : 2560, speckled pattern, but other autoantibodies were negative. Simvastatin was discontinued with improvement in his symptoms although his ANA was still positive at the same titer 1 year later. Khosla *et al*. reported the case of a 79-year-old male who developed fatigue, mayalgia, and pleuropericarditis 3 months after initiation of the therapy with simvastatin [188]. Sridhar and Abdulla reported the development of polyarthritis affecting the hands and right knee, myalgias, and a generalized rash in a 67-year-old woman approximately 1 week after she started fluvastatin (20 mg per day) [189]. However, symptoms did not resolve in spite of discontinuation of medication for more than a month. Initial testing was negative for anti-dsDNA antibodies but subsequent tests were positive for anti-dsDNA antibodies although anti-histone antibodies were negative. The patient subsequently went to develop ARDS syndrome and died. Although this patient was reported as a case of a fluvastatin-induced lupus the features are somewhat atypical in that the symptoms developed relatively quickly after starting the medication and did not resolve with discontinuation.

Obermoserg *et al*. reported the case of a patient who developed severe autoimmune hepatitis, rash, polyarthralgias, and a positive ANA with a titer of 1 : 640 while receiving atorvastatin [190]. Biopsy of the rash confirmed the diagnosis of lupus. The patient also had anti-histone and also strongly positive anti-dsDNA antibodies. Liver biopsy revealed highly active chronic hepatitis which initially did not respond to high-dose steroid therapy but improved when treated with mycophenolate mofetil and tacrolimus.

D-Penicillaimine

D-Penicillaimine-related lupus was first described in patients with rheumatoid arthritis by Harkcom *et al*. in 1978 [191] but has also been reported in those with other autoimmune diseases such as scleroderma and in patients with Wilson's disease and cystinuria. The frequency of D-penicillaimine-related lupus has been reported as high as 2% of rheumatoid arthritis [192] patients and up to 7% of patients with Wilson's disease [193]. The most common clinical manifestations include polyarthritis, pleurisy, leukopenia, thrombocytopenia, and cutaneous involvement. In addition to antinuclear antibodies, some patients have been reported to develop antibodies to double-stranded DNA and hypocomplementemia [191, 193–195]. Penicillaimine therapy has also been associated with the development of other autoimmune phenomena such as myasthenia gravis and polymyositis.

HORMONAL FACTORS

Hormonal factors are believed to play a role in the development of SLE. Bae *et al*. suggested that growth hormone may have precipitated a flare in a 19-year-old male with a 11-year-old history of lupus after he had been treated with a growth hormone for 9 months because of growth retardation [196]. The flare was manifested by a decrease in C3 and C4, elevated anti-dsDNA antibodies, and the development of nephrotic range proteinuria at 1.5 g/24 h. He required treatment with monthly IV bolus doses of cyclophosphamide and high doses of prednisolone; in addition his growth hormone was discontinued. It has been postulated that growth hormone may play a role in preventing apoptosis of mononuclear cells with consequent increased survival of autoreactive immune cells. A study by Ogueta *et al*. reported that transgenic mice that expressed bovine growth hormone developed arthritis and autoantibodies [197]. Higher basal prolactin levels have been found and hyperprolactinemia has been reported to play a role in the development and progression of lupus [198, 199]. These findings are of interest since both growth hormone and prolactin belong to the same receptor family.

BIOLOGICAL AGENTS

Biological agents are being used increasingly to treat a variety of diseases including malignancies, infectious and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, multiple sclerosis, and psoriasis. These agents include a number of cytokines, such as interferon α, β, γ and interleukin-2 (IL-2), which have immunomodulating affects. Despite considerable therapeutic success, many of the patients receiving these agents develop autoanti-

bodies and in some cases autoimmune-like diseases [200–205]. Treatment with interferon α and β and interleukin-2 (IL-2) has been associated with the development of thyroid autoantibodies, anti-DNA, and anti-nuclear antibodies [201, 205]. In most cases, these autoantibodies disappear following discontinuation of treatment. Anti-nuclear and anti-DNA antibodies may develop as early as the first month of treatment [202].

Some patients develop evidence of autoimmune-like diseases including autoimmune thyroid disease, autoimmune hemolytic anemia, autoimmune thrombocytopenia, lupus-like syndromes, pernicious anemia, and vasculitis [201, 206, 207]. Ronnblom *et al*. [201] reported that 25 (19%) of their 135 patients with carcinoid tumors developed evidence of autoimmune diseases after treatment with interferon α. Reports suggest that autoimmune thyroid disease is more likely to develop in patients with malignancies who have thyroid antibodies prior to the institution of therapy with interferon α [201, 205]. Over 60% of patients with thyroid antibodies who received interferon α were reported to develop autoimmune thyroid disease compared with 7% of those who were antibody negative [202].

Obermoser *et al*. implicated interferon α in the development of a lupus-like syndrome [208]. An 80-year-old woman with chronic myelogenous leukaemia (CML), treated with interferon α developed fatigue, fever, leukopenia, and a multiforme-like erythema on the arms and legs, biopsy of which revealed an interface dermatitis suggestive of lupus erythematosus. ANA was positive at 1:320 titer but antibodies to dsDNA and ENA were negative. Interferon was discontinued and the patient was treated with steroids with subsequent resolution of her clinical features.

Interferon α and IL-2 have been associated with the induction or exacerbation of symmetric inflammatory arthropathies [206, 209]. Three patients treated with IL-2 developed a positive rheumatoid factor, anti-nuclear antibodies, and an inflammatory arthritis [209]. It has been postulated that high-dose interleukin-2 may enhance expression of HLA Class II antigen leading in turn to autoimmunity. Reversible hypothyroidism was reported in two patients treated with granulocyte–monocyte colony stimulating factor who had antibodies to thyroid peroxidase prior to therapy [210].

TUMER NECROSIS FACTOR ALPHA INHIBITORS

Anti-nuclear antibodies and antibodies to dsDNA have been detected in a number of patients following treatment with TNF-α inhibitors. However, to date, only a few patients have been reported to develop a lupus-like syndrome [211].

DeBandt *et al*. reported the development of a lupus-like syndrome in two patients with rheumatoid arthritis treated with etanercept [32]. One patient developed cutaneous lesions on the face and scalp, fatigue, and diffuse pain 3 months after starting etanercept. She had a positive ANA, anti-DNA and anti-cardiolipin antibodies, a low C4, and elevated muscle enzymes. Cutaneous lesions resolved within 1 month with discontinuation of the etanercept. Biological abnormalities returned to normal within 4 months but her polyarthritis became more active. The second was a 50-year-old woman who developed a diffuse erythematous and purpuric eruption with fine scaling of the hands and fingers approximately 4 months after starting etanercept. She also had lymphopenia, thrombocytopenia, an elevated ESR, a positive ANA at 1:640 titer, and anti-dsDNA antibodies were negative. The cutaneous manifestations resolved following discontinuation of the etanercept.

Brion *et al*. reported the development of cutaneous lesions in two patents treated with etanercept for rheumatoid arthritis [31]. The first patient was a 78-year-old woman who developed a diffuse erythematous rash 4 days after her fourth etanercept injection. Biopsy of the rash revealed acute discoid lupus. She was negative for ANA and anti-dsDNA antibodies. The second patient was a 58-year-old woman who developed a widespread rash after her seventh etanercept injection with purpuric lesion on the lower extremities and buttocks. Biopsy revealed findings consistent with a necrotizing vasculitis. Anti-dsDNA antibodies were negative. Both patients' rashes resolved within 2 weeks after discontinuation of etanercept.

Schaible from Centocor reported that low titers of autoantibodies developed in less than 10% of a total of 777 patients who received infliximab as part of several placebo-controlled, randomized clinical, and open trails [212]. He also reported that drug-induced lupus had occurred in less than 1% of these patients and that these lupus-like syndromes resolved on discontinuation of infliximab infusions. A review by Markham and Lamb reported that ANAs were detected in 23% of patients receiving infliximab compared with 6% of placebo recipients during the ATTRACT study [213]. Anti-dsDNA antibodies were found in 16% (54 of 342) of patients receiving infliximab but were not detected in patients who received placebo. Antibodies tended to appear 4–10 weeks (mean of 6.5 weeks) following initiation of treatment. They disappeared spontaneously during or after treatment. No correlation was found between the development of autoantibodies and

infliximab dosages. Three (<0.5%) of approximately 388 patients who have been treated with infliximab as part of clinical trails have developed drug-induced lupus. One patient had Crohn's disease. The other 2 patients had rheumatoid arthritis and had received 10 mg/kg every 8 weeks or 3 mg/kg approximately every 4 weeks in addition to methotrexate. One of these patients developed serositis, fever, and high levels of both ANA and anti-dsDNA antibodies and the other patient developed a rash with evidence of mild perivascular infiltrate. These features resolved on discontinuation of infliximab and treatment with steroids.

Anti-nuclear antibodies developed in 34% of patients treated with infliximab for Crohn's disease although no patient was ANA-positive prior to treatment. Anti-dsDNA antibodies developed in 9% of patients after approximately 4–12 weeks of treatment and disappeared when infliximab was discontinued. More recently, combined verifiable data from the Crohn's disease and rheumatoid arthritis trials indicated that 4 of 987 (0.2%) patients developed a lupus-like syndrome, primarily manifested by rash and fevers with 1 patient developing pleuropericarditis. Of the patients, 3 had rheumatoid arthritis and one had Crohn's disease. Postmarketing surveillance by Centocor revealed a 0.04% incidence among 170,000 patients.

According to FDA data, there have been 14 cases of patients with Crohn's disease who have developed ANAs and lupus-like syndromes while being treated with infliximab. Eight patients developed antibodies to dsDNA. Following discontinuation of the infliximab infusions there was resolution of the lupus-like syndrome in six patients but one patient failed to improve. There were no data available as to whether the other seven patients had resolution of their symptoms following discontinuation of infliximab. Patients had received a median of three infusions of infliximab prior to the development of the symptoms.

Charles *et al*. evaluated sera obtained from 156 patients before and after treatment with infliximab for the presence of anti-dsDNA antibodies using three methods, which included the *Crithidia luciliae* indirect immunoflourescence assay, a commercial Farr assay using mammalian DNA, and a Farr assay that utilized ^{125}I-labeled circular plasmid DNA [71]. Anti-nuclear antibodies were present in 29% of the patients pretreatment, compared with 30% of the controls, and in 53% of the patients after treatment with infliximab. Twenty-two (14%) patients treated with infliximab developed antibodies to dsDNA after a mean of 6.3 weeks, range of 4–10 weeks. No patient had anti-dsDNA antibodies prior to treatment. The anti-dsDNA antibodies were of the IgM class in all patients except for 1 patient who also had IgG and IgA antibodies to dsDNA. One of the patients developed a lupus-like syndrome characterized by fever, dry cough, rash, chronic chest pain, and a pericardial effusion approximately 11 weeks after the initiation of treatment with infliximab. This patient had antibodies to dsDNA detected by all three assays: she was the only patient who had anti-dsDNA antibodies detected by the Farr assay that utilized circular plasma DNA as the antigen. It is believed that this assay only detects high affinity anti-dsDNA antibodies. All of the clinical features and the anti-dsDNA antibodies disappeared within 8 weeks of discontinuation of infliximab.

There has been a speculation that the development of ANAs and anti-dsDNA antibodies among rheumatoid arthritis patients receiving infliximab may result from increased amounts of nucleosomal antigens in the synovium as a result of a rapid decrease in C-reactive protein that occurs during the early phase of treatment with infliximab. C-reactive protein has been postulated to be important in the clearance of this apoptotic material and a sudden decrease in its level may lead to impaired clearance of nucleosomal antigens with subsequent induction of an autoantibody response.

ENVIRONMENTAL AGENTS

A number of chemical agents, heavy metals, and dietary factors have been associated with the development of lupus-like syndromes (Table 3).

TABLE 3 Environmental Factors Reported to Be Associated with the Development of Autoantibodies and Lupus-Like Syndromes

Hydrazines
Tartrazine
Hair dyes
Chemicals used in computer manufacturing
? Trichlorethylene
? Industrial emissions and hazardous wastes
Silica (Quartz)
Paraffin/Silicone
Mercury
Cadmium
Gold
L-Canavanine
Rapeseed oil—toxic oil syndrome
L-Tryptophan—eosinophilia myalgia syndrome

Chemical Agents

Chemical agents that have been implicated include hydrazines and their derivatives, which are present in tobacco, mushrooms, and penicillin and in a variety of compounds used in agriculture and industry, and aromatic amines, which are present in the dyes as azo food dyes such as tartrazine and paraphenylenediamine [214–216]. Reidenberg *et al*. reported the development of a lupus-like syndrome characterized by recurrent episodes of arthralgias, fever, photosensitivity, and a positive ANA in a 25-year-old technician exposed to hydrazine sulfate during her work [214]. Resolution of these features occurred following avoidance of further exposure to hydrazine. Pereyo reported the development of a lupus-like syndrome characterized by arthralgias, myalgias, and photosensitivity following ingestion of tartrazine [215]. Of interest, this patient had previously developed a lupus-like syndrome following treatment with procainamide. A similar lupus-like syndrome consisting of arthralgias, myalgias, malaise, photosensitivity, and a positive ANA developed in another patient following oral challenge with tartrazine. Pereyo also noted that some SLE patients developed exacerbations of their cutaneous disease following ingestion of tartrazine [216].

Paraphenylenediamine is an aromatic amine used commercially as a hair dyeing compound which has been associated with the development of autoimmune-like diseases in animal studies. Studies seeking an association between the use of hair dyes and other compounds in the development of systemic lupus erythematosus in humans have provided conflicting results [217–220]. Hochberg and Kaslow reported an increase in the prior use of hair dyes among 74 patients with SLE in their case control study; however, this increase was not statistically significant [217]. In a subsequent case control study, Freni-Titulaer *et al*. reported an association between the use of hair care products and the development of a variety of connective tissue diseases including SLE [218]. Although crude analysis found an association between the development of connective tissue diseases and the use of a variety of hair care products, including hair dyes, hair permanent solutions, and hair spray, only the association with hair dyes remained statistically significant after multivariate analyses were performed. Petri and Allbritton failed to find a statistically significant association between the development of SLE and prior exposure to hair permanents or dyes in their case-controlled study of 218 lupus patients [219]; nor did they find a statistical significant association between subsequent usage of hair products and lupus activity. A further case-controlled study of 159 patients by Reidenberg *et al*. did not find a statistically significant association between environmental exposures to amines, including the use of hair dyes, and the development of SLE [220]. In a cohort study Sanchez-Guerrero *et al*. investigated the possible role of permanent hair dye usage in the development of SLE using data obtained from the Nurses health study, which is a prospective cohort study involving 106,390 women as participants who have been followed for up to 14 years [221]. The age-adjusted relative risk for development of SLE among users of permanent hair dye was 0.96 which was not significantly different from that of the never-users. In addition, the risk of developing SLE was not related to the duration of use of the hair dye, age at first use, or frequency of uses of these dyes. Similarly, a more recent study by Hardy *et al*. did not find a significant association between various hair treatments, including permanent and nonpermanent dyes, bleach, highlights, and lowlights in the development of SLE [222]. These authors evaluated 150 SLE patients by interviewed administered questionnaire and compared the results to 300 controls from the same geographical region. Of interest, a significant negative association was noted between ever having used highlights and SLE with cases having used highlights less frequently than the healthy controls. The reason for this is unclear although it did not appear to be an artifact of analysis since there was not an unusually low number of responses from participants.

Cooper *et al*. conducted a population based case control study that assessed smoking and hair treatments as risk factors for the development of systemic lupus erythematosus [223]. Two hundred and sixty-five patients who had been diagnosed with SLE by rheumatologists in eastern North Carolina and South Carolina and 355 age-, sex-, and state-matched controls were evaluated. Data collection consisted of a structured 60-min in person interview that obtained information on the use of smoking and hair treatments, which included use of permanents to curl or straighten hair, permanent hair dyes, and temporary hair dyes. A positive history was defined as lifetime use of 5 or more times. Analysis was limited to experiences that occurred prior to the age of diagnosis of SLE or reference age for controls. There was no association between history of smoking and risk of developing SLE. There was a small increase in the risk of developing SLE among women who had used permanent hair dyes. The association was somewhat higher with longer duration of use. There was little evidence of an association with use of temporary dyes or of permanents or straighteners and risk of developing SLE. These results would suggest at the most a weak association between the usage of permanent hair dyes and risk of developing SLE.

Thus, while some smaller studies had suggested an association between hair dye usage and the development of SLE, the more recent larger studies have failed to show a significant risk.

Vojdani *et al*. reported evidence of immune dysregulation, including development of autoantibodies such as IgM rheumatoid factor and anti-nuclear nuclear antibodies, in a significant number of workers who had been chronically exposed to a variety of different chemicals in computer manufacturing plants [224]. Their study population included 289 subjects with a history of 10 years or greater exposure to industrial chemicals including phthalic anhydride, formaldehyde, isocyanate, trimellitic anhydride, aliphatic, and aromatic hydrocarbons who were compared to a group of 120 nonexposed individuals. They noted significant elevation of both IgG and IgM chemical-hapten antibodies directed against some of these chemicals in addition to significantly elevated levels of antibodies to myelin basic protein, anti-nuclear antibodies, IgM rheumatoid factors, and immune complexes. As of this time, no further follow-up reports or confirmation of this study has been published.

Kilburn and Warshaw reported an increased prevalence of anti-nuclear antibodies and lupus-like syndromes among people chronically exposed to well water contaminated by trichlorethylene and other chemicals in the Tucson, Arizona area [225]. Their study group of 362 subjects exposed to contaminated well water was compared to a control group of 158 nonexposed individuals living in southwest Phoenix. They used the 1971 ARA preliminary criteria for SLE and found a statistically significant increase in the frequency of arthritis/arthralgias, Raynauds phenomenon, malar rash, "heliotropic skin lesions" and seizure/convulsions among the study group compared with the controls. ANAs in a titer of 1:160 or greater were seen in 10.7% of the study group compared with 4.7% of the controls. However, some of the subjects with the high ANA titers had other predisposing factors for the development of ANA, the clinical data was collected by questionnaires and the 1971 rather than the 1982 ARA criteria for SLE were used. A higher than expected prevalence of SLE was subsequently reported among individuals living in Nogales, Arizona [226] and among an African-American community in north Georgia who had a long-standing exposure to industrial emissions and hazardous wastes [227]. However, all these reports have yet to be confirmed. Studies by Sanchez-Roman *et al*. [228] and Conrad *et al*. [229] have suggested an association between the development of lupus syndromes and heavy exposure to quartz (offending agent probably silica), in Silesian miners who were mostly male. Previous studies have linked exposure to silica with the development of scleroderma [230].

Autoimmune diseases including SLE have been reported to occur after the injection or implantation of paraffin or silicone [231]. There have been at least 100 reported cases of patients developing autoimmune-like diseases up to 25 years after augmentation mammoplasty procedures. In some cases, there has been resolution of the clinical features following removal of the implant [232]. However, most of the evidence has been based on case reports which provide very weak evidence for a causal relationship. More recently, there have been a number of large studies which have failed to confirm an association between the development of autoimmune diseases and silicone breast implants [233–235]. Hennekens *et al*. [236] reported on a retrospective cohort study of 395,543 female health professionals who completed questionnaires as part of the women's health study. Of these, 10,830 women reported having breast implants and 11,805 reported connective tissue diseases. Although they reported a statistically significant association between breast implants and "any connective tissue disease," no association was found with any specific connective tissue disease including lupus. Other studies have also evaluated the risk of implants in the development of connective tissue disease but have not found an association. However, the smaller number of subjects included in these studies would not allow for the detection for relative risks less than 2.0. Hochberg *et al*. in a multicenter case-controlled study examined the possible association of augmentation mammoplasty with scleroderma [237]. Eight hundred and thirty-seven women with a clinical diagnosis of scleroderma recruited from three university-based tertiary care scleroderma clinical research centers were compared to 2507 race-matched local-controlled women. Eleven (1.31%) of the scleroderma patients reported a history of augmentation mammoplasty prior to the diagnosis of their disease compared with 31 (1.24%) of the controls. No statistically significant association between the augmentation mammoplasty and scleroderma was demonstrated.

In a more recent study, Edworthy *et al*. evaluated 1576 women who had undergone breast implantation between 1978 to 1986 for possible connective tissue disease [238]. The women were initially evaluated by an extensive questionnaire and by blood sample and those with features suggestive of connective tissue disease were subsequently assessed blindly by a rheumatologist. Age- and sex-adjusted prevalence rates for rheumatoid arthritis, SLE, scleroderma, and Sjögren's syndrome among the study group were consistent with those published reports for Caucasian women. Breast implant recipients reported significantly greater frequen-

cies of symptoms and in particular "thought problems," "numbness in extremities," "muscle pain," "headache," and "hand pain" than the controls. However, there was no increase in the postsurgical incident rates for any of the connective tissue diseases compared with the control population. Hence, similar to other studies, no significant association was noted between silicone gel filled implants and the development of connective tissue diseases. In addition, serological analysis did not reveal any significant differences between the breast implant recipients and controls when adjusted for age.

Similar results were reported by Karlson *et al*. who evaluated 200 randomly selected women who had had silicone breast implants but did not have a history of connective tissue disease and 500 age-matched nonexposed women, of whom 100 had a connective tissue disease, for the presence of a variety of autoantibodies, including ANAs, antibodies to dsDNA, ssDNA, Smith, RNP, Ro, La, Scl-70, cardiolipin, thyroglobulin, thyroid microsomal, and silicone, rheumatoid factor, and immunoglobulins [239]. Anti-nuclear antibodies were present in 14% of the women with silicone breast implants compared to 20% of healthy controls. There was a higher frequency of anti-ssDNA antibodies at 41% among silicone breast implants compared with 29% for the control group. However, there were no other significant differences in the frequency of antibodies between the two groups. Since anti-ssDNA antibodies are generally considered to have little clinical relevance, the results of these studies would suggest a lack of association between autoimmunity and silicone breast implants. Taken in combination, the results of the aforementioned epidemiological studies would rule out a large increase in the risk for the development of connective tissue diseases as a result of silicone breast implantation. Although associations cannot be definitively ruled out as yet, given the relatively low prevalence of some connective tissue diseases (e.g., scleroderma), any association with the more common diseases (e.g., SLE and rheumatoid arthritis) is unlikely.

A report of the National Science panel established by a U.S. district court order to evaluate whether existing studies, research, and reported observations provided a scientific evidence of an association between silicone breast implants and "classic" connective tissue diseases was recently published [240]. The group evaluated 24 studies which consisted of either human cohort, case-controlled, or cross-sectional studies with at least 10 participants and the appropriate controls. No association between silicone breast implants and any established or atypical connected tissue disease was evident. There was discordance in the reporting of arthralgias, lymphadenopathy, myalgias, sicca symptoms, skin changes, and stiffness between the studies which may reflect differences in symptoms included in the various categories and the small number in some of the studies.

Sclerodermatous-like disease and development of autoantibodies were included among the features of the toxic oil syndrome which first appeared in Spain in 1981 and was related to the ingestion of denatured rapeseed oil, although the specific agent was not identified [241, 242]. Approximately 20,000 people were affected with this syndrome with a marked predominance of females among those with more severe and chronic disease. Patients generally presented with nonspecific symptoms such as fever, malaise, headache, dyspnea, and myalgias associated with peripheral eosinophilia. Subsequently some developed joint contractures, severe myalgias associated with weakness and atrophy, sclerodermatous-like cutaneous lesions, alopecia, Raynaud's, peripheral neuropathy, and sicca syndrome. Many patients were noted to have elevated titers of anti-nuclear and anti-histone antibodies which subsequently decreased and became negative in most cases; anti-dsDNA antibodies were not found. The majority had improvement in their symptoms with less than 10% of patients having evidence of functional limitation or severe organ involvement at long-term follow-up [243]. No further cases have been noted following that one major outbreak.

The eosinophilia myalgia syndrome, which had similar manifestations to the toxic oil syndrome including the development of anti-nuclear antibodies in some patients [244, 245] was first described by the Centers for Disease Control and Prevention in 1989. It was linked to the ingestion of L-tryptophan from certain, most likely contaminated, batches from a particular manufacturer [244, 245]. There was a significant decrease in the incidence of new cases following withdrawal of these L-tryptophan containing products. Fatigue, myalgias, and arthralgias were the most frequent presenting complaints with some patients also having respiratory symptoms. Typically, the patients had peripheral blood esinophilia and muscle biopsies revealed perivascular, primarily mononuclear cell infiltrates. Many patients subsequently developed sclerodermatous-like cutaneous involvement, sensory neuropathies, and pulmonary involvement. As with the toxic oil syndrome, anti-nuclear antibodies were detected but antibodies to double-stranded DNA and other nuclear and cytoplasmic antigens were negative.

Heavy Metals

Chronic exposure to certain metals such as mercury, gold, and cadmium has been implicated in the induction of autoantibodies and immune complex mediated

glomerulonephropathies in both experimental animals and humans [246]. Mercuric chloride has been reported to induce the development of autoantibodies and immune complex mediated glomerulonephropathies in susceptible strains of rats and mice. Anti-nuclear antibodies with specificity for the nucleolar protein, fibrillarin, have been detected in certain strains of mice treated with mercuric chloride for 10 weeks [247]. Observations by Pollard *et al*. suggests that mercury can alter the physicochemical properties of fibrillarin, possibly by chemical modification of the exposed cysteines, which may help explain the antigenic specificity of mercury-induced murine autoimmunity [248]. Other *in vitro* studies by Pollard *et al*. have indicated that modification of fibrillarin by mercuric chloride accompanies cell death; mercury can, then, elicit a specific protease activity that results in cleavage of fibrillarin producing novel protein fragments which may be released and act as sources of antigenic determinants for self-reactive T cells [249]. Other studies by this group indicated that exposure to mercury may significantly accelerate the onset of systemic autoimmune disease in a strain-dependant manner suggesting that genetic susceptibility may predispose to mercury-induced exacerbations of autoimmunity [250]. Genetic predisposition may be important in the development of xenobiotic-induced autoimmunity in humans as well.

Deposits of immunoglobulins and complement have been reported in the glomerular basement in patients exposed to mercury through the use of skin lightening creams containing this metal [251]. Diffuse granular deposits of IgM, IgG, and complement have been noted in the glomerular lesions of patients receiving chronic administration of gold.

Dietary Factors

The ingestion of L-canavanine, a common amino acid found in alfalfa seeds and sprouts, has been associated with a lupus-like syndrome in both humans and monkeys. Adult female cynomolgus macaques fed alfalfa sprouts or seeds were reported to develop anti-nuclear and anti-dsDNA antibodies, hypocomplementemia, and lupus-like features such as antiglobulin positive anemia and glomerulonephropathy [252, 253]. Resolution of the clinical features occurred in some monkeys following discontinuation of alfalfa in their diets with recurrence in three monkeys following the readdition of L-canavanine [254]. Autoimmune-like features including anti-nuclear antibodies have been reported in humans ingesting significant amounts of alfalfa [254]. Two patients had reactivation of their SLE following ingestion of alfalfa tablets [255]. As already mentioned, with the increase in the use of health food products physicians will need to be alert to the possibility of these syndromes occurring in the general population.

CONCLUSION

The cause(s) of systemic lupus erythematosus remains unknown. Genetic factors are believed to play a significant role but results from monozygotic twin studies suggest that other factors are also important. It is therefore likely that exposure to some environmental agent is required to induce the development of the disease. As discussed in this chapter, there is considerable evidence to support a role for environmental agents, including drugs, in the induction of lupus-like and other autoimmune syndromes [256]. Continued study into the possible roles for environmental and infectious agents, most likely viral, in the etiopathogenesis of SLE is required.

References

1. Deapen, D., Escalante, A., Weinrib, L., Horwitz, D., Bachman, B., Roy-Burman, P., Walker, A., and Mack, T. M. (1992). A revised estimate of twin concordance in systemic lupus erythematosus. *Arthritis Rheum.* **35**, 311–318.
2. Hoffman, B. J. (1945). Sensitivity of sulfadiazine resembling acute disseminated lupus erythematosus. *Arch. Dermatol. Syph.* **51**, 190–192.
3. Gold, S. (1951). Role of sulphonamides and penicillin in the pathogenesis in systemic lupus erythematosus. *Lancet* **1**, 268–272.
4. Walsh, J. R., and Zimmerman, J. H. (1953). The demonstration of the "LE" phenomenon in patients with penicillin hypersensitivity. *Blood* **8**, 65–71.
5. Paull, A. M. (1955). Occurrence of the "LE" phenomenon in a patient with a severe penicillin reaction. *N. Engl. J. Med.* **252**, 128–129.
6. Honey, M. (1956). SLE presenting with sulphonamide hypersensitivity. *Br. Med. J.* **1**, 1272–1275.
7. Morrow, J. D., Schroeder, H. A., and Perry, H. M., Jr. (1953). Studies on the control of hypertension by Hyphex: II. Toxic reactions and side effects. *Circulation* **8**, 829–839.
8. Perry, H. M., and Schroeder, H. A. (1954). Syndrome simulating collagen disease caused by hydralazine (Apresoline). *JAMA* **154**, 670–673.
9. Lee, S. L., and Chase, P. H. (1975). Drug-induced systemic lupus erythematosus: A critical review. *Semin. Arthritis Rheum.* **5**, 83–103.
10. Lindqvist, T. (1957). Lupus erythematosus disseminatus after administration of mesantoin. Report of two cases. *Acta Med. Scand.* **158**, 131–138.
11. Ladd, A. T. (1962). Procainamide-induced lupus erythematosus. *N. Engl. J. Med.* **267**, 1357.

12. Cannat, A., and Seligmann, M. (1966). Possible induction of antinuclear antibodies by isoniazid. *Lancet* **1**, 825–827.
13. Rothfield, N. F., Bierer, W. G., and Garfield, J. W. (1978). Isoniazid induction of antinuclear antibodies: A prospective study. *Ann. Intern. Med.* **88**, 650–652.
14. Fabius, A. J. M., and Gaulhofer, W. K. (1971). Systemic lupus erythematosus induced by psychotropic drugs. *Acta Rheumatol. Scand.* **17**, 137.
15. Tan, E. M., Cohen, A. S., Fries, J. F., Masi, A. T., McShane, D. J., Rothfield, N. F., Schaller, J. G., Talal, N., and Winchester, R. J. (1982). The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rhuem.* **25**, 1271–1277.
16. Beernink, D. H., and Miller, J. J. (1973). Anticonvulsant-induced antinuclear antibodies and lupus-like disease in children. *J. Pediatr.* **82**, 113–117.
17. Singsen, B. H., Fishman, L., and Hanson, V. (1976). Antinuclear antibodies and lupus-like syndromes in children receiving anticonvulsants. *Pediatrics* **57**, 529–534.
18. Batchelor, J. R., Welsh, K. I., Tinoco, R. M., *et al*. (1980). Hydralazine-induced systemic lupus erythematosus: Influence of HLA-DR and sex on susceptibility. *Lancet* **1**, 1102–1109.
19. Miller, F. W., Hess, E. V., Clauw, D. J., Hertzman, P. A., Pincus, T., Silver, R. M., Mayes, M., Varga, J., Medsger, T. A., and Love, L. A. (2000). Approaches for identifying and defining environmentally associated rheumatic disorders. *Arthritis Rheum.* **43**, 243–249.
20. Vivino, F. B., and Schumacher, H. R. (1989). Synovial fluid characteristics and the lupus erythematosus cell phenomenon in drug-induced lupus. *Arthritis Rheum.* **32**, 560–568.
21. Cush, J. J., and Goldings, E. A. (1985). Drug-induced lupus: Clinical spectrum and pathogenesis. *Am. J. Med. Sci.* **290**, 36–45.
22. Goldberg, M. J., Hsain, M., Wajszczuk, W. J., and Rubenfire, M. (1980). Procainamide-induced lupus erythematosus pericarditis encountered during coronary bypass surgery. *Am. J. Med.* **69**, 159–162.
23. Carey, A. M., Coleman, M., and Feder, A. (1973). Pericardial tamponade: A major presenting manifestation of hydralazine induced lupus syndrome. *Am. J. Med.* **54**, 84–87.
24. Ghose, M. K. (1975). Pericardial tamponade. A presenting manifestation of procainamide-induced lupus erythematosus. *Am. J. Med.* **58**, 581–585.
25. Sunder, S. K., and Shah, A. (1975). Constrictive pericarditis in procainamide-induced lupus erythematosus syndrome. *Am. J. Cardiol.* **36**, 960–962.
26. Dent, M. T., Ganapathy, S., Holdsworth, C. D., and Channer, K. C. (1992). Mesalazine induced lupus-like syndrome. *Br. Med. J.* **305**, 159.
27. Vayre, F., Vayre-Oundjian, L., and Monsuez, J. J. (1999). Pericarditis associated with longstanding mesalazine administration in a patient. *Int. J. Cardiol.* **68**, 243–245.
28. Molnar, T., Hogye, M., Nagy, F., and Lonovics, J. (1999). Pericarditis associated with inflammatory bowel disease: Case report. *Am. J. Gastroentrol.* **94**, 1099–1100.
29. Sentongo, T. A., and Piccoli, D. A. (1998). Recurrent pericarditis due to mesalazine hypersensitivity: A pediatric case report and review of the literature. *J. Pediatric. Gastroenterol. Nutr.* **27**, 344–347.
30. Alexander, S. (1962). Lupus erythematosus in two patients after griseofulvin treatment of trichophyton rubrum infection. *Br. J. Dermatol.* **74**, 72.
31. Brion, P. H., Mittal-Henke, A., and Kalunian, K. C. (1999). Autoimmune skin rashes associated with etanercept for rheumatoid arthritis. *Ann. Intern. Med.* **131**, 634.
32. DeBandt, M., Descamps, V., and Meyer, O. (2001). Etanercept induced systemic lupus erythematosus: Two patients with rheumatoid arthritis. *Lupus* **10**, S118.
33. Servitje, O., Ribera, M., Juanola, X., and Rodriguez-Moreno, J. (1987). Acute neutrophilic dermatosis associated with hydralazine-induced lupus. *Arch. Dermatol.* **123**, 1435–1436.
34. Sequeira, W., Polisky, R. B., and Alrenga, D. P. (1986). Neutrophilic dermatosis (Sweet's syndrome): Association with hydralazine-induced lupus syndrome. *Am. J. Med.* **81**, 558–560.
35. Elkayam, O., Levarovosky, D., Brautbar, C., *et al*. (1998). Clinical and immunological study of 7 patients with minocycline-induced autoimmune phenomena. *Am. J. Med.* **105**, 484–487.
36. Tournigand, C., Genereau, T., Prudent, M., Dimert, M.-C., Herson, S., and Chosidow, O. (1999). Minocycline-induced clinical and biological lupus-like disease. *Lupus* **8**, 773–774.
37. Hope, R. R., and Bates, L. A. (1972). The frequency of procainamide-induced systemic lupus erythematosus. *Med. J. Aust.* **2**, 298–303.
38. Gough, A., Chapman, S., Wagstaff, K., Emery, P., and Elias, E. (1996). Minocycline induced autoimmune hepatitis and systemic lupus erythematosus-like syndrome. *Br. Med. J.* **312**, 169–172.
39. Sheikh, T. K., Charron, R. C., and Katz, A. (1981). Renal manifestations of drug-induced systemic lupus erythematosus. *Am. J. Clin. Pathol.* **75**, 755–762.
40. Shapiro, K. S., Pinn, V. W., Harrington, J. T., *et al.* (1984). Immune complex glomerulonephritis in hydralazine-induced SLE. *Am. J. Kidney Dis.* **3**, 270–274.
41. Dustan, H. P., Taylor, R. D., Corcoran, A. C., *et al*. (1954). Rheumatic and febrile syndromes during prolonged hydralazine therapy. *JAMA* **154**, 23–29.
42. Sturman, S. G., Kumararatne, D., and Beevers, D. G. (1988). Fatal hydralazine-induced systemic lupus erythematosus [letter]. *Lancet* **2**, 1304.
43. Bjorck, S., Svalander, C., and Westberg, G. (1985). Hydralazine-associated glomerulonephritis. *Acta Med. Scand.* **218**, 261–269.
44. Alarcon-Segovia, D., Wakim, K. G., Worthington, J. W., *et al.* (1967). Clinical and experimental studies on the hydralazine syndrome and its relationship to systemic lupus erythematosus. *Medicine* **46**, 1–33.
45. Sahenk, Z., Mendell, J. R., Rossio, J. L., *et al.* (1977). Polyradiculoneuropathy accompanying procainamide-induced lupus erythematosus: Evidence for drug-

induced enhanced sensitization to peripheral nerve myelin. *Ann. Neurol.* **1**, 378–384.
46. Ahmad, S. (1981). Procainamide and peripheral neuropathy. *South Med. J.* **74**, 509–510.
47. Bernstein, R. M., Egerton-Vernon, J., and Webster, J. (1980). Hydralazine-induced cutaneous vasculitis. *Br. Med. J.* **280**, 156–157.
48. Cameron, H. A., and Ramsay, L. E. (1984). The lupus syndrome induced by hydralazine: A common complication with low dose treatment. *Br. Med. J.* **289**, 410–412.
49. Knox, J. P., Welykyj, S. E., Grandini, R., *et al.* (1988). Procainamide-induced urticarial vasculitis. *Cutis* **42**, 469–472.
50. Rosin, J. M. (1967). Vasculitis following procaineamide therapy. *Am. J. Med.* **42**, 625–629.
51. Lawson, T. M., Amos, N., Bulgen, D., and William, B. D. (2001). Minocycline induced lupus: Clinical features and response to rechallenge. *Rheumatology* **40**, 329–335.
52. Choi, H. K., Merkel, P. A., Walker, A. M., and Niles, J. L. (2000). Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis. *Arthritis Rheum.* **43**, 405–413.
53. Alarcon-Segovia, D. (1969). Drug-induced lupus syndromes. *Mayo Clin. Proc.* **44**, 664–681.
54. Dupont, A., and Six, R. (1982). Lupus-like syndrome induced by methyldopa. *Br. Med. J.* **285**, 693–694.
55. Sherman, J. D., Love, D. E., and Harrington, J. F. (1967). Anemia, positive lupus and rheumatoid factors with methyldopa. A report of three cases. *Arch. Intern. Med.* **120**, 321–326.
56. Harth, M. (1968). L. E. cells and positive direct Coomb's test induced by methyldopa. *Can. Med. Assoc. J.* **99**, 277–280.
57. Blomgren, S. E., Condemi, J. J., and Vaughan, J. H. (1972). Procainamide-induced lupus erythematosus. Clinical and laboratory observations. *Am. J. Med.* **52**, 338–348.
58. Kleinman, S., Nelson, R., Smith, L., and Goldfinger, D. (1984). Positive direct antiglobulin tests and immune hemolytic anemia in patients receiving procainamide. *N. Engl. J. Med.* **311**, 809–812.
59. Zarrabi, M. H., Zucker, S., Miller, F., Derman, R. M., Romano, G. S., Hartnett, J. A., and Varma, A. O. (1979). Immunologic and coagulation disorders in chlorpromazine-treated patients. *Ann. Intern. Med.* **91**, 194–199.
60. Nordstrom, D. M., West, S. G., and Rubin, R. L. (1989). Methyldopa-induced systemic lupus erythematosus. *Arthritis Rheum.* **32**, 205–208.
61. Becker, M., Klajman, A., Moalem, T., *et al.* (1979). Circulating immune complexes in sera from patients receiving procainamide. *Clin. Immunol. Immunopathol.* **12**, 220–227.
62. Mitchell, J. A., Batchelor, J. R., Chapel, H., Spiers, C. N., and Sim, E. (1987). Erythrocyte complement receptor type I (CRI) expression and circulating immune complex (CIC) levels in hydralazine-induced SLE. *Clin. Exp. Immunol.* **68**, 446–456.
63. Utsinger, P. D., Zvaifler, N. J., and Bluestein, H. G. (1976). Hypocomplementemia in procainamide-associated systemic lupus erythematosus. *Ann. Intern. Med.* **84**, 293.
64. Cohen, M. G., Kevat, S., Prowse, M. V., *et al.* (1988). Two distinct quinidine-induced rheumatic syndromes. *Ann. Intern. Med.* **108**, 369–371.
65. Grossman, J., Callerame, M. L., and Condemi, J. J. (1974). Skin immunofluorescence studies on lupus erythematosus and other antinuclear-antibody-positive diseases. *Ann. Intern. Med.* **80**, 496–500.
66. Kirby, J. D., Dieppe, P. A., Huskisson, E. C., *et al.* (1979). D-Penicillamine and immune complex deposition. *Ann. Rheum. Dis.* **38**, 344–346.
67. Naparstek, Y., Kopolovic, J., Tur-Kaspa, R., *et al.* (1984). Focal glomerulonephritis in the course of hydralazine-induced lupus syndrome. *Arthritis Rheum.* **27**, 822–825.
68. Fritzler, M. J., and Tan, E. M. (1978). Antibodies to histones in drug-induced and idiopathic lupus erythematosus. *J. Clin. Invest.* **62**, 560–567.
69. Mielke, H., Wildhagen, K., Mau, W., and Zeidler, H. (1993). Follow-up of patients with double-stranded DNA antibodies induced by sulfasalazine during the treatment of inflammatory rheumatic diseases. *Rheumatology* **22**, 229–301.
70. Gordon, M.-M., and Porter, D. (2001). Minocycline related lupus: Case series in the West of Scotland. *J. Rheumatol.* **28**, 1004–1006.
71. Charles, P. J., Smeenk, R. J. T., DeJonj, J., Feldmann, M., and Manie, R. N. (2001). Assessment of antibodies to double-stranded DNA induced in Rheumatoid Arthritis patients following treatment with Infliximab, a monoclonal antibody to tumor necrosis factor α: Findings in open-label and randomized placebo-controlled trials. *Arthritis Rheum.* **43**, 2383–2390.
72. Mongey, A. B., Donovan-Brand, R., Thomas, T. J., *et al.* (1992). Serologic evaluation of patients receiving procainamide. *Arthritis Rheum.* **35**, 219–223.
73. Burlingame, R. W., and Rubin, R. L. (1991). Drug-induced anti-histone autoantibodies display two patterns of reactivity with substructures of chromatin. *J. Clin. Invest.* **88**, 680–690.
74. Rubin, R. L., Bell, S. A., and Burlingame, A. W. (1992). Autoantibodies associated with lupus induced by diverse drugs target a similar epitope in the (H2A-2B)-DNA complex. *J. Clin. Invest.* **90**, 165–173.
75. Bray, V. J., West, S. G., Schultz, K. T., Boumpas, D. T., and Rubin, R. L. (1994). Antihistone autobody profile in sulfasalazine induced lupus. *J. Rheumatol.* **21**, 2157–2158.
76. Zamber, R., Martens, H., Rubin, R. L., and Starkebaum, G. (1992). Drug induced lupus due to ophthalmic timolol. *J. Rheumatol.* **19**, 977–979.
77. Gohill, J., Cary, P. D., Couppez, M., and Fritzler, M. J. (1985). Antibodies from patients with drug-induced and idiopathic lupus erythematosus react with epitopes restricted to the amino and carboxyl termini of histone. *J. Immunol.* **135**, 3116–3121.
78. Craft, J. E., Radding, J. A., Harding, M. A., Bernstein, R. M., and Hardin, J. A. (1987). Autoantigenic histone epitopes: A comparison between procainamide- and hydralazine-induced lupus. *Arthritis Rheum.* **30**, 689–694.
79. Portanova, J. P., Rubin, R. L., Joslin, F. G., *et al.* (1982). Reactivity of anti-histone antibodies induced by

procainamide and hydralazine. *Clin. Immunol. Immunopathol.* **25**, 67–79.
80. Portanova, J. P., Arndt, R. E., Tan, E. M., *et al.* (1987). Anti-histone antibodies in idiopathic and drug-induced lupus recognize distinct intrahistone regions. *J. Immunol.* **138**, 446–451.
81. West, S. G., McMahon, M., and Portanova, J. P. (1984). Quinidine-induced lupus erythematosus. *Ann. Intern. Med.* **100**, 840–842.
82. Totoritis, M. C., Tan, E. M., McNally, E. M., *et al.* (1988). Association of antibody to histone complex H2A-H2B with symptomatic procainamide-induced lupus [published erratum appears in *N. Engl. J. Med.* **319**, 256, 1988]. *N. Engl. J. Med.* **318**, 1431–1436.
83. Shoenfeld, Y., and Segol, O. (1989). Anti-histone antibodies in SLE and other autoimmune diseases. *Clin. Exp. Rheumatol.* **7**, 265–271.
84. Canoso, R. T., and de Oliveira, R. M. (1988). Chlorpromazine-induced anticardiolipin antibodies and lupus anticoagulant: Absence of thrombosis. *Am. J. Hematol.* **27**, 272–275.
85. Canoso, R. T., Lewis, M. E., and Yunis, E. J. (1982). Association of HLA-Bw44 with chlorpromazine-induced autoantibodies. *Clin. Immunol. Immunopathol.* **25**, 278–282.
86. Tollefson, G., Rodysill, K., and Cusulos, M. (1984). A circulating lupus-like coagulation inhibitor induced by chlorpromazine. *J. Clin. Psych. Pharmacol.* **4**, 49–51.
87. Steen, V. D., and Ramsey-Goldman, R. (1988). Phenothiazine-induced systemic lupus erythematosus with superior vena cava syndrome: Case report and review of the literature. *Arthritis Rheum.* **31**, 923–926.
88. Weber, M. T., and Hocking, W. G. (1988). Procainamide induced lupus anticoagulant. *Wis. Med. J.* **87**, 30–32.
89. Asherson, R. A., Zulman, J., and Hughes, G. R. V. (1989). Pulmonary thromboembolism associated with procainamide-induced lupus syndrome and anticardiolipin antibodies. *Ann. Rheum. Dis.* **48**, 232–235.
90. Chokron, R., Robert, A., and Rozensztajn, L. (1982). Procainamide-induced lupus with circulating anticoagulant [letter]. *Nouv. Presse Med.* **11**, 2568.
91. Davis, S., Furie, B. C., Griffin, J. H., and Furie, B. (1978). Circulating inhibitors of blood coagulation associated with procainamide-induced lupus erythematosus. *Am. J. Hematol.* **4**, 401–407.
92. Edwards, R. L., Rick, M. E., and Wakem, C. J. (1981). Studies on a circulating anticoagulant in procainamide-induced lupus erythematosus. *Arch. Intern. Med.* **141**, 1688–1690.
93. List, A. F., and Doll, D. C. (1989). Thrombosis associated with procainamide-induced lupus anticoagulant. *Acta Haematol.* **82**, 50–52.
94. Merrill, J. T., Shen, C., Gugnani, M., Lahita, R. G., and Mongey, A.-B. (1997). High prevalence of antiphospholipid antibodies in patients taking procainamide. *J. Rheumatol.* **24**, 1083–1088.
95. Gastineau, D. A., Kazmier, F. J., Nichols, W. L., *et al.* (1985). Lupus anticoagulant: An analysis of the clinical and laboratory of 219 cases. *Am. J. Hematol.* **19**, 265–275.
96. Lavie, C. J., Biundo, J., Quinet, R. J., *et al.* (1985). Systemic lupus erythematosus (SLE) induced by quinidine. *Arch. Intern. Med.* **145**, 446–448.
97. Bird, M. R., O'Neill, A. I., Buchanan, R. R. C., Ibrahim, K. M., and Parkin, J. D. (1995). Lupus anticoagulant in the elderly may be associated with both quinine and quinidine usage. *Pathology* **27**, 136–139.
98. Rosare, D., Garcia, F., Gascon, J., Angrill, J., and Cervera, R. (1996). Quinine induced lupus-like syndrome and cardiolipin antibodies. *Ann. Rheum. Dis.* **55**, 559–560.
99. Anderson, B., and Stillman, M. T. (1978). False-positive FTA-ABS in hydralazine induced lupus. *JAMA* **239**, 1392–1393.
100. Vyse, T., and So, A. K. (1992). Sulphasalazine induced autoimmune syndrome. *Br. J. Rheumatol.* **31**, 115–116.
101. Canoso, R. T., and Sise, H. S. (1982). Chlorpromazine-induced lupus anticoagulant and associated immunologic abnormalities. *Am. J. Hematol.* **13**, 121–129.
102. Nassberger, L., Johansson, A. C., Bjorck, S., *et al.* (1991). Antibodies to neutrophil granulocyte myeloperoxidase and elastase: Autoimmune responses in glomerulonephritis due to hydralazine treatment. *J. Intern. Med.* **229**, 261–265.
103. Nassberger, L., Sjoholm, A. G., Jonsson, H., *et al.* (1990). Autoantibodies against neutrophil cytoplasm components in systemic lupus erythematosus and in hydralazine-induced lupus. *Clin. Exp. Immunol.* **81**, 380–383.
104. Cambridge, G., Wallace, H., Bernstein, R. M., and Leaker, B. (1994). Autoantibodies to myeloperoxidase in idiopathic and drug-induced systemic lupus erythematosus and vasculitis. *Br. J. Rheumatol.* **33**, 109–114.
105. Cambridge, G., Wallace, H., Bernstein, R. M., and Leaker, B. (1994). Autoantibodies to myeloperoxidase in idiopathic and drug-induced systemic lupus erythematosus and vasculitis. *Br. J. Rheumatol.* **33**, 109–114.
106. Honda, H., Shibata, T., Hara, H., Ban, Y., and Sugisaki, T. (1996). MPO-ANCA in patients with Grave's disease; strong association with propylthiouracil therapy (abstract). *Sarcoidosis Vasc. Diffuse Lung Dis.* **13**, 280.
107. Cohen Tervaert, J. W., Tel, W., de Vries, O., Links, C. A., and Stegman, C. A. (1996). The occurrence of antineutrophil cytoplasmic antibodies with specificity for proteinase 3, myeloperoxidase, and/or elastase during treatment with antithyroid drugs (abstract). *Sarcoidosis Vasc. Diffuse Lung Dis.* **13**, 280.
108. Choi, H. K., Slot, M. C., Pan, G., Weissbach, C. A., Niles, J. L., and Merkel, P. A. (2000). Evaluation of antineutrophil cytoplasmic antibody seroconversion induced by minocycline, sulfasalazine or penicillamine. *Arthritis Rheum.* **43**, 2488–2492.
109. Bluestein, H. G., Redelman, D., and Zvaifler, N. J. (1981). Procainamide-lymphocyte reactions. A possible explanation for drug-induced autoimmunity. *Arthritis Rheum.* **24**, 1019–1023.
110. Hughes, G. R., Rynes, R. I., Gharavi, A., *et al.* (1981). The heterogeneity of serologic findings and predisposing host factors in drug-induced lupus erythematosus. *Arthritis Rheum.* **24**, 1070–1073.

111. Ryan, P. F., Hughes, G. R., Bernstein, R., *et al.* (1979). Lymphocytotoxic antibodies in hydralazine-induced lupus erythematosus [letter]. *Lancet* **2**, 1248–1249.
112. Ooi, B. S., Kant, K. S., Hanenson, I. B., Pesce, A. J., and Pollack, V. E. (1977). Lymphocytotoxins in epileptic patients receiving phenytoin. *Clin. Exp. Immunol.* **30**, 56–61.
113. Hahn, B. H., Sharp, G. C., Irvin, W. S., Kantor, O. S., Gardner, C. A., Bagby, M. K., Perry, H. M., and Osterland, C. K. (1972). Immune response to hydralazine and nuclear antigens in hydralazine-induced lupus erythematosus. *Ann. Intern. Med.* **76**, 365–374.
114. Russell, A. S., and Ziff, M. (1968). Natural antibodies to procainamide. *Clin. Exp. Immunol.* **3**, 901–909.
115. Perry, H. M., Jr., Tan, E. M., Carmody, S., *et al.* (1970). Relationship of acetyl transferase activity to antinuclear antibodies and toxic symptoms in hypertensive patients treated with hydralazine. *J. Lab. Clin. Med.* **76**, 114–125.
116. Brand, C., Davidson, A., Littlejohn, G., and Ryan, P. (1984). Hydralazine-induced lupus: No association with HLA-DR4. *Lancet* **2**, 462.
117. Russell, G. I., Bing, R. F., Jones, J. A., Thurston, H., and Swales, J. D. (1987). Hydralazine sensitivity: Clinical features, autoantibody changes and HLA-DR phenotype. *Q. J. Med.* **65**, 845–852.
118. Speirs, C., Fielder, A. H., Chapel, H., *et al.* (1989). Complement system protein C4 and susceptibility to hydralazine-induced systemic lupus erythematosus. *Lancet* **1**, 922–924.
119. Litwin, A., Adams, L. E., Zimmer, H., and Hess, E. V. (1981). Immunologic effects of hydralazine in hypertensive patients. *Arthritis Rheum.* **24**, 1074–1077.
120. Thomas, T. J., Seibold, J. R., Adams, L. E., and Hess, E. V. (1993). Hydralazine induces Z-DNA confirmation in a polynucleotide and elicits anti (Z-DNA) antibodies in treated patients. *Biochem. J.* **294**, 419–425.
121. Woosley, R. L., Drayer, D. E., Reidenberg, M. D., Nies, A. S., Carr, K., *et al.* (1978). Effector of acetylator phenotype on the rate at which procainamide induces antinuclear antibodies and the lupus syndrome. *N. Engl. J. Med.* **298**, 1157–1179.
122. Sonnhag, C., Karlsson, E., and Hed, J. (1979). Procainamide-induced lupus erythematosus-like syndrome in relation to acetylator phenotype and plasma levels of procainamide. *Acta Med. Scand.* **206**, 245–251.
123. Mongey, A.-B., Sim, E., Risch, A., and Hess, E. V. (1999). Acetylation status is associated with serological changes but not clinically significant disease in patients receiving procainamide. *J. Rheumatol.* **26**, 1721–1726.
124. Adams, L. E., Balakrishnan, K., Roberts, S. M., Belcher, R., Mongey, A.-B., Thomas, T. J., and Hess, E. V. (1993). Genetic, immunologic and biotransformation studies of patients on procainamide. *Lupus* **2**, 89–98.
125. Rothfield, N. F., Bierer, W. F., and Garfield, J. W. (1978). Isoniazid induction of antinuclear antibodies. A prospective study. *Ann. Intern. Med.* **88**, 650–652.
126. Salazar-Proamo, M., Rubin, R. L., and Garcia-de la Torre, I. (1987). Isoniazid-induced systemic lupus erythematosus. *Ann. Rheum. Dis.* **51**, 1085–1087.
127. Alarcon-Segovia, D., Fishbein, E., and Alcala, H. (1971). Isoniazid acetylation rate and development of antinuclear antibodies upon isoniazid treatment. *Arthritis Rheum.* **14**, 748–752.
128. Evans, D. A., Bullen, M. F., Houston, J., *et al.* (1972). Antinuclear factor in rapid and slow acetylator patients treated with isoniazid. *J. Med. Genet.* **9**, 53–56.
129. Kendall, M. J., and Hawkins, C. F. (1970). Quinidine-induced systemic lupus erythematosus. *Postgrad. Med. J.* **46**, 729–731.
130. Alloway, J. A., and Salata, M. P. (1995). Quinidine-induced rheumatic syndromes. *Semin. Arthritis Rheum.* **24**, 315–322.
131. Amadio, P., Jr., Cummings, D. M., and Dashow, L. (1985). Procainamide, quinidine, and lupus erythematosus [letter]. *Ann. Intern. Med.* **102**, 419.
132. De Giorgio, C. M., Rabinowicz, A. L., and Olivas, R. D. (1991). Carbamazepine-induced antinuclear antibodies and systemic lupus erythematosus-like syndrome. *Epilepsia* **32**, 128–129.
133. Schmidt, S. T., Welcker, M., Greil, W., and Schattenkirchner, M. (1992). Carbamazepine-induced systemic lupus erythematosus. *Br. J. Psych.* **161**, 560–561.
134. Alballa, S., Fritzler, M., and Davis, P. (1987). A case of drug induced lupus due to carbamazepine. *J. Rheumatol.* **14**, 599–600.
135. Drory, V. E., and Korczyn, A. D. (1993). Hypersensitivity vasculitis and systemic lupus erythematosus induced by anticonvulsants. *Clin. Neuropharmacol.* **16**, 19–29.
136. Asconape, J. J., Manning, K. R., and Lancman, M. E. (1994). Systemic lupus erythematosus associated with use of valproate. *Epilepsia* **35**, 162–163.
137. Ansell, B. M. (1993). Drug-induced systemic lupus erythematosus in a nine-year-old boy. *Lupus* **2**, 193–194.
138. Caramaschi, P., Biasi, D., Carletto, A., Manzo, T., and Bambara, L. M. (1994). Clobazam induced lupus erythematosus [letter]. *Lupus* **3**, 69.
139. Verrot, D., San-Marco, M., Dravet, C., *et al.* (1997). Prevalence and signification of antinuclear and anticardiolipin antibodies in patients with epilepsy. *Am. J. Med.* **103**, 33–37.
140. Quismorio, F. P., Bjarnason, D. F., Kiely, W. F., *et al.* (1975). Antinuclear antibodies in chronic psychotic patients treated with chlorpromazine. *Am. J. Psychiatry* **132**, 1204–1206.
141. Pavlidakey, G. P., Hashimoto, K., Heller, G. L., and Daneshvar, S. (1985). Chlorpromazine-induced lupus-like disease. Case report and review of the literature. *Am. J. Acad. Dermatol.* **13**, 109–115.
142. Wilson, J. D. (1980). Antinuclear antibodies and cardiovascular drugs. *Drugs* **19**, 292–305.
143. Record, N. B., Jr. (1981). Acebutolol-induced pleuropulmonary lupus syndrome. *Ann. Intern. Med.* **95**, 326–327.
144. Raferty, E. B., and Denman, A. M. (1973). Systemic lupus erythematosus induced by practolol. *Br. Med. J.* **2**, 452.
145. Bensaid, J., Aldigier, J. C., and Gaulde, N. (1979). SLE syndrome induced by pindolol. *Br. Med. J.* **1**, 1603–1604.

146. Brown, R. C., Cooke, J., and Losowsky, M. S. (1987). SLE syndrome probably induced by labetalol. *Postgrad. Med. J.* **57**, 189–190.
147. Reidenberg, M. M., Case, D. B., Drayer, D. E., Reis, S., and Lorenzo, B. (1984). Development of antinuclear antibody in patients treated with high doses of captopril. *Arthritis Rheum.* **27**, 579–581.
148. Kallenberg, C. G. (1985). Antibodies during captopril treatment. *Arthritis Rheum.* **28**, 597–598.
149. Sieber, C., Grimm, E., and Follath, F. (1990). Captopril and systemic lupus erythematosus. *Br. Med. J.* **301**, 669.
150. Pigott, P. V. (1982). Captopril and drug-induced lupus. *Br. Med. J.* **284**, 1786.
151. Schwartz, D., Pines, A., Averbuch, M., and Levo, Y. (1990). Enalapril-induced antinuclear antibodies. *Lancet* **336**, 187.
152. Pelayo, M., Vargas, V., Gonzales, A., Vallano, A., Esteban, R., and Guardia, J. (1993). Drug-induced lupus-like reaction and captopril. *Ann. Pharmacother.* **27**, 1541–1542.
153. Bertin, P., Kamdem, J., Bonnet, C., Arnaud, M., and Treves, R. (1993). Captopril induced lupus. *Clin. Exp. Rheumatol.* **11**, 695.
154. Alarcon-Segovia, D., Herslovic, T., Dearing, W. H., Bartholomew, L., Cain, J., and Shorter, R. (1965). Lupus erythematosus cell phenomenon in patients with chronic ulcerative colitis. *Gut* **6**, 39–47.
155. Dekeyser, F., Mielants, H., Praet, J., Goemaere, S., and Veys, E. M. (1993). Changes in antinuclear serology in patients with spondyloarthropathy under sulphasalazine treatment. *Br. J. Rheumatol.* **32**, 521.
156. Siam, A. R., and Hammoudeh, M. (1993). Sulfasalazine induced systemic lupus erythematosus in a patient with rheumatoid arthritis [letter]. *J. Rheumatol.* **20**, 207.
157. Clementz, G. L., and Dolin, B. J. (1988). Sulfasalazine-induced lupus erythematosus. *Am. J. Med.* **84**, 535–538.
158. Laversuch, C. J., Collins, D. A., Charles, P. J., and Bourke, B. E. (1995). Sulphasalazine-induced autoimmune abnormalities in patients with rheumatic disease. *Br. J. Rheumatol.* **34**, 435–439.
159. Walker, E. M., and Carty, J. E. (1994). Sulphasalazine-induced systemic lupus erythematosus in a patient with erosive arthritis. *Br. J. Rheumatol.* **33**, 175–176.
160. Veale, D. J., Ho, M., and Morley, K. D. (1995). Sulphasalazine-induced lupus in psoriatic arthritis. *Br. J. Rheumatol.* **34**, 383–384.
161. Deboever, G., Devogelaere, R., and Holvoet, G. (1989). Sulphasalazine-induced lupus-like syndrome with cardiac tamponade in a patient with ulcerative colitis. *Am. J. Gastroenterol.* **84**, 85–86.
162. Carr-Locke, D. L. (1982). Sulfasalazine-induced lupus syndrome in a patient with Crohn's disease. *Am. J. Gastroenterol.* **77**, 614–616.
163. Caulier, M., Dromer, C., Andrieu, V., LeGuennec, P., and Fourne, B. (1994). Sulfasalazine induced lupus in rheumatoid arthritis. *J. Rheumatol.* **21**, 750–751.
164. Gordon, M.-M., Porter, D. R., and Capell, H. A. (1999). Does sulphasalazine cause drug induced lupus erythematosus? No effect evident in a prospective randomized trial of 200 rheumatoid patients treated with sulphasalazine or auranofin over five years. *Ann. Rheum. Dis.* **58**, 288–290.
165. Gunnarrson, I., Nordmark, B., Hassan Bakri, A., Grondal, G., Larsson, P., Forslid, J., Klareskog, and Ringertz, B. (2000). Development of lupus-related side-effects in patients with early RA during sulphasalazine treatment—the role of IL-10 and HLA. *Rheumatology* **39**, 886–893.
166. Kirkpatrick, A. W., Bookman, A. A., and Habal, F. (1999). Lupus-like syndrome caused by 5-aminosalicylic acid in patients with inflammatory bowel like disease. *Can. J. Gastroenterol.* **13**, 159–162.
167. Timsit, M. A., Anglicheau, D., Liote, F., Marteau, P., and Dryll, A. (1997). Mesalazine-induced lupus. *Rev. Rheum. Engl. Ed.* **64**, 586–588.
168. Gunnarsson, I., Forslid, J., and Ringertz, B. (1999). Mesalazine-induced lupus syndrome. *Lupus* **8**, 486–491.
169. Gunnarsson, I., Kanerud, L., Pettersson, E., *et al*. (1997). Predisposing factors in sulphasalazine-induced systemic erythmatosus. *Br. J. Rheumatol.* **36**, 1089–1094.
170. Matsuura, T., Shimizu, Y., Fujimoto, H., Miyazaki, T., and Kano, S. (1992). Minocycline-related lupus. *Lancet* **340**, 1553.
171. Byrne, P. A. C., Williams, B. D., and Pritchard, M. H. (1994). Minocycline-related lupus. *Br. J. Rheumatol.* **33**, 674–676.
172. Quilty, B., and McHugh, N. (1994). Lupus-like syndrome associated with the use of minocycline. *Br. J. Rheumatol.* **33**, 1197–1198.
173. Bulgen, D. Y. (1995). Minocycline-related lupus. *Br. J. Rheumatol.* **34**, 398.
174. Elkayam, O., Yaron, M., and Caspi, D. (1996). Minocycline induced arthritis associated with fever, livedo reticularis and p ANCA. *Ann. Rheum. Dis.* **55**, 769–771.
175. Knowles, S. R., Shapiro, L., and Shear, N. H. (1996). Serious adverse reactions induced by minocycline. *Arch. Dermatol.* **132**, 934–939.
176. Gough, A., Chapman, S., Wagstaff, K., Emery, P., and Elias, E. (1996). Minocycline induced autoimmune hepatitis and systemic lupus erythematosus-like syndrome. *Br. Med. J.* **312**, 169–172.
177. Shapiro, L. E., Knowles, S. R., and Shear, N. H. (1997). Comparative safety of tetracycline, minocycline and doxycycline. *Arch. Dermatol.* **133**, 1224–1230.
178. Dunphy, J., Oliver, M., Rands, A. L., Lovell, C. R., and McHugh, N. J. (2000). Antineutrophil cytoplasmic antibodies and HLA class II alleles in minocycline-induced lupus like syndrome. *Br. J. Dermatol.* **142**, 461–467.
179. Sturkenboom, M. C., Meier, C. R., Jick, H., and Sticker, B. H. (1999). Minocycline and lupuslike syndrome in acne patients. *Arch. Intern. Med.* **159**, 493–497.
180. McHugh, N. J., Dunphy, J., and Rands, A. (1996). Antimyeloperoxidase antibodies in minocycline-induced lupus. *Arthritis Rheum.* **39**(Suppl.), S110.
181. Herzog, D., Hajoui, O., Russo, P., and Alvarez, F. (1997). Study of immune reactivity of minocycline-induced chronic active hepatitis. *Dig. Dis. Sci.* **42**, 1002–1103.
182. Kloppenburg, M., Binkman, B. M. N., deRooij-Dijk, H. H., *et al*. (1996). The tetracycline derivative minocy-

cline differentially affects cytokine production by monocytes and T-lymphocytes. *Antimicrob. Agents Chemother.* **40**, 934–940.
183. Ahmad, S. (1991). Lovastatin-induced lupus erythematosus. *Arch. Intern. Med.* **151**, 1667–1668.
184. Bannwarth, B., Miremont, G., and Papapietro, P.-M. (1992). Lupus like syndrome associated with simvastatin. *Arch. Intern. Med.* **152**, 1093.
185. Kaur, H., Singh, D., and Bollin, G. (2001). Simvastatin-induced lupus erythematosus. ACP-ASIM Ohio Chapter Scientific Meeting. 74.
186. Ahmad, A., Fletcher, M. T., and Roy, T. M. (2000). Simvastatin-induced lupus-like syndrome. *Tenn. Med.* **93**, 21–22.
187. Hanson, J., and Bossingham, D. (1998). Lupus-like syndrome associated with simvastatin. *Lancet* **352**, 1070.
188. Khosla, R., Butman, A. N., and Hammer, D. F. (1998). Simvastatin-induced lupus erythematosus. *South Med. J.* **91**, 873–874.
189. Sridhar, M. K., and Abdulla, A. (1998). Fatal lupus-like syndrome and ARDS induced by fluvastatin. *Lancet* **352**, 114.
190. Obermoserg, G., Graziadei, I., Sepp, N., and Bogel, W. (2001). Lupus-like syndrome associated with Atorvastatin. *Lupus* **10**, S121.
191. Harkcom, T. M., Conn, D. L., and Holley, K. E. (1978). D-Penicillamine and lupus erythematosus-like syndrome [letter]. *Ann. Intern. Med.* **89**, 1012.
192. Chalmers, A., Thompson, D., Stein, H. E., Reid, G., and Patterson, A. L. (1982). Systemic lupus erythematosus during penicillamine therapy for rheumatoid arthritis. *Ann. Intern. Med.* **97**, 659–663.
193. Walshe, J. M. (1981). Penicillamine and the SLE syndrome. *J. Rheumatol. Suppl.* **8**, 155–160.
194. Thorvaldsen, J. (1981). Penicillamine-induced lupus-like reaction in rheumatoid arthritis and vasculitis. *Dermatologica* **162**, 277–280.
195. Camus, J. P., Homberg, J. C., Crouzet, J., *et al.* (1981). Autoantibody formation in D-penicillamine-treated rheumatoid arthritis. *J. Rheumatol. Suppl.* **7**, 80–83.
196. Bae, Y.-S., Bae, S.-L., Lee, S.-W., Yoo, D.-H., Kim, T. Y., and Kim, S. Y. (2001). Lupus flare associated with growth hormone. *Lupus* **10**, 448–450.
197. Ogueta, S., Olazabal, I., Santos, I., Delgado-Baeza, E., and Garcia-Ruiz, J. P. (2000). Transgenic mice expressing bovine GH develop arthritic disorder and self-antibodies. *J. Endocrinol.* **165**, 321–328.
198. Rovensky, J., Blazickova, S., Rauoval, L., *et al.* (1998). The hypothalamic–pituitary response in SLE. Regulation of prolactin growth hormone and cortisol release. *Lupus* **7**, 409–413.
199. McMurray, R. W., Allen, S. H., Braun, A. L., Rodriguez, S., and Walker, S. E. (1994). Long-standing hyperprolactinemia associated with systemic lupus erythematosus: Possible hormonal stimulation of an autoimmune disease. *J. Rheumatol.* **21**, 843–850.
200. Wandl, U. B., Nagel-Hiemke, M., May, D., Kreuzfelder, E., Kloke, O., Kranzhoff, M., Seeber, S., and Niederle, N. (1992). Lupus-like autoimmune disease induced by interferon therapy for myeloproliferative disorders. *Clin. Immunol. Immunopathol.* **65**, 70–74.
201. Ronnblom, L. E., Alm, G. V., and Oberg, K. E. (1991). Autoimmunity after alpha-interferon therapy for malignant carcinoid tumors. *Ann. Intern. Med.* **115**, 178–183.
202. Mayet, W.-J., Hess, G., Gerken, G., Rossel, S., Voth, R., Manns, M., and Meyerzum-Buschenfelde, K. M. (1989). Treatment of chronic type B hepatitis with recombinant α-interferon induced autoantibodies not specified for autoimmune chronic hepatitis. *Hepatology* **10**, 24–28.
203. Atkins, M. B., Mier, J. W., Parkinson, D. R., Gould, J. A., Berkman, E. M., and Kaplan, M. M. (1988). Hypothyroidism after treatment with interleukin 2 and lymphokine activated killer cells. *N. Engl. J. Med.* **318**, 1557–1563.
204. Ehrenstein, M. R., McSweeney, E., Swana, M., Worman, C. P., Goldstone, A. H., and Isenberg, D. A. (1993). Appearance of anti-DNA antibodies in patients treated with interferon-α. *Arthritis Rheum.* **36**, 279–280.
205. Gisslinger, H., Gilly, B., Woloszczuk, W., Mayr, W. R., Havelec, L., and Linkesch, W. (1992). Thyroid autoimmunity and hypothyroidism during long-term treatment with recombinant interferon-alpha. *Clin. Exp. Immunol.* **90**, 363–367.
206. Conlon, K. C., Urba, W. J., Smith, J. W., Steis, R. G., Longo, D. L., and Clark, J. (1990). Exacerbation of symptoms of autoimmune disease in patients receiving alpha-interferon therapy. *Cancer* **65**, 2237–2242.
207. Flores, A., Olive, A., Feliu, E., and Tena, X. (1994). Systemic lupus erythematosus following interferon therapy. *Br. J. Rheumatol.* **33**, 787–792.
208. Obermoser, G., Semenitz, B., Thaler, J., and Sepp, N. (2001). Lupus-like syndrome following interferon therapy in a patient with chronic myelogenous leukemia. *Lupus* **10**, S121.
209. Massarotti, E. M., Liu, N. Y., Nier, J., and Atkins, M. B. (1992). Chronic inflammatory arthritis after treatment with high-dose interleukin-2 for malignancy. *Am. J. Med.* **92**, 693–697.
210. Hoekman, K., von Blomberg-van der Flier, B. M., Wagstaff, J., Drexhage, H. A., and Pinedo, H. M. (1991). Reversible thyroid dysfunction during treatment with GM-CSF. *Lancet* **2**, 541–542.
211. Feldman, M. (1997). TNF blockade in rheumatoid arthritis. Prog. AAI, CIS, AAAI Annual Meeting, February.
212. Schaible, T. F. (2000). Long term safety of Infliximab. *Can. J. Gastroenterol.* **14**(Suppl. C), 29C–32C.
213. Markham, A., and Lamb, H. M. (2000). Infliximab: A review of its use in the management of rheumatoid arthritis. *Drugs* **59**, 1341–1359.
214. Reidenberg, M. M., Durant, P. J., Harris, R. A., De Boccardo, G., Lahita, R., and Stenzel, K. H. (1983). Lupus erythematosus-like disease due to hydrazine. *Am. J. Med.* **75**, 365–370.
215. Pereyo, N. (1980). Tartrazine and drug-induced lupus. *Schock Lett.* **30**, 1.
216. Pereyo, N. (1987). Tartrazine, hydrazine, amino compounds and systemic lupus erythematosus. *Science-Ciencia* **14**, 31–35.

217. Hochberg, M. C., and Kaslow, R. A. (1983). Risk factors for the development of systemic lupus erythematosus: A case-control study (abstract). *Clin. Res.* **31**, 732A.
218. Freni-Titulaer, L. W. J., Kelley, D. B., Grow, A. G., McKinley, T. W., Arnett, F. C., and Hochberg, M. C. (1989). Connective tissue disease in southeastern Georgia: A case control study of etiologic factors. *Am. J. Epidemiol.* **130**, 404–409.
219. Petri, M., and Allbritton, J. (1992). Hair product use in systemic lupus erythematosus—a case controlled study. *Arthritis Rheum.* **35**, 625–629.
220. Reidenberg, M. M., Drayer, D. E., Lorenzo, B., Strom, B. L., West, S. L., Snyder, E. S., Freundlich, B., and Stolley, P. D. (1993). Acetylation phenotypes and environmental chemical exposure of people with idiopathic systemic lupus erythematosus. *Arthritis Rheum.* **36**, 971–973.
221. Sanchez-Guerrero, J., Karlson, E. W., Colditz, G. A., *et al*. (1996). Hair dye use and the risk of developing systemic lupus erythematosus. *Arthritis Rheum.* **39**, 657–662.
222. Hardy, C. J., Palmer, B. P., Muir, K. R., *et al*. (1999). Systemic lupus erythematosus (SLE) and hair treatment: A large community based case-control study. *Lupus* **8**, 541–544.
223. Cooper, G. S., Dudley, M. A., Treadwell, E. L., St. Clair, E. W., and Gilkeson, G. S. (2001). Smoking and use of hair treatments in relation to risk of developing systemic lupus erythematosus. *J. Rheumatol.* **28**, 2653–2656.
224. Vojdani, A., Choneum, M., and Brautbar, N. (1992). Immune alteration associated with exposure to toxic chemicals. *Toxicol. Ind. Health* **8**, 239–254.
225. Kilburn, K. H., and Warshaw, R. H. (1992). Prevalence of symptoms of systemic lupus erythematosus (SLE) and of fluorescent antinuclear antibodies associated with chronic exposure to trichloroethylene and other chemicals in well water. *Environ. Res.* **57**, 1–9.
226. Walsh, B. T., Reed, M., Emerson, J., Gall, E. P., and Clark, L. (1993). A large cluster of systemic lupus erythematosus individuals in a Mexican-American border town in Arizona (abstract). *Arthritis Rheum.* **36**, S145, B22.
227. Kardestuncer, T., and Frumkin, H. (1997). Systemic lupus erythematosus in relation to enviornmental pollution: An investigation in an African-American community in north Georgia. *Arch. Environ. Health* **52**, 85–90.
228. Sanchez-Roman, J., Wichmann, I., Salaberri, J., Varela, J. M., and Nunez-Roldan, A. (1993). Multiple clinical and biological autoimmune manifestations in 50 workers after occupational exposure to silica. *Ann. Rheum. Dis.* **52**, 534–538.
229. Conrad, K., Mehlhorn, J., Luthke, K., Dorner, T., and Frank, K. H. (1996). Systemic lupus erythematosus after heavy exposure to quartz dust in uranium mines: Clinical and serological characteristics. *Lupus* **5**, 62–69.
230. Rodnan, G. P., Benedek, T. G., Medsger, T. A., Jr., and Cammarta, R. J. (1967). The association of progressive systemic sclerosis (scleroderma) with coal miners' pneumoconiosis and other forms of silicosis. *Ann. Intern. Med.* **66**, 323–334.
231. Kumagai, Y., Shiokawa, Y., Medsger, T. A., Jr., and Rodnan, G. P. (1984). Clinical spectrum of connective tissue disease after cosmetic surgery. *Arthritis Rheum.* **27**, 1–12.
232. Kaiser, W., Biesenbach, G., *et al.* (1990). Human adjuvant disease: Remission of silicone-induced autoimmune disease after explanation of breast augmentation. *Ann. Rheum. Dis.* **49**, 937–938.
233. Bridges, A. J., Conley, C., Wang, G., Burns, D. E., and Vasey, F. B. (1993). A clinical and immunologic evaluation of women with silicone breast implants and symptoms of rheumatoid disease. *Ann. Intern. Med.* **118**, 929–936.
234. Gabriel, S. E., O'Fallon, W. M., Kurland, L. T., Beard, C. M., Woods, J. E., and Melton III, L. J. (1994). Risk of connective tissue diseases and other disorders after breast implantation. *N. Engl. J. Med.* **330**, 1697–1702.
235. Sanchez-Guerrero, J., Colditz, G. A., Karlson, E. W., Hunter, D. J., Speizer, F. E., and Liang, M. (1995). Silicone breast implants and the risk of connective-tissue diseases and symptoms. *N. Engl. J. Med.* **332**, 1666–1670.
236. Hennekens, C. H., Lee, I.-M., Cook, N. R., Hebert, P. R., Karlson, E. W., La Motte, F., Manson, J. E., and Buring, J. E. (1996). Self reported breast implants and connective tissue diseases in female health professionals. *JAMA* **275**, 616–621.
237. Hochberg, M. C., Perlmutter, D. L., Medsger, T. A., Jr., *et al*. (1996). Lack of association between augmentation mammoplasty and systemic sclerosis (scleroderma). *Arthritis Rheum.* **39**, 125–131.
238. Edworthy, S. M., Martin, L., Barr, S. G., *et al*. (1996). A clinical study of the relationship between silicone breast implants and connective tissue disease. *J. Rheumatol.* **25**, 254–260.
239. Karlson, E. W., Hankinson, S. E., Liang, M. H., *et al*. (1999). Association of silicone breast implants with immunologic abnormalities: A prospective study. *Am. J. Med.* **106**, 11–19.
240. Tugwell, P., Wells, G., Peterson, J., Welch, V., Page, J., Davison, C., McGowan, J., Ramroth, D., and Shea, B. (2001). Do silicone breast implants cause rhematologic disorders? A systematic review for a court-appointed national science panel. *J. Rheumatol.* **44**, 2477–2484.
241. Tabuenca, J. M. (1981). Toxic-allergic syndrome caused by ingestion of rapeseed oil denatured with aniline. *Lancet* **2**, 567–568.
242. Alonso-Ruiz, A., Zea-Mendoza, A. C., Salazar-Vallinas, J. M., Rocamora-Ripoli, A., and Beltran-Gutierrez, J. (1986). Toxic oil syndrome: A syndrome with features overlapping those of various forms of scleroderma. *Semin. Arthritis Rheum.* **15**, 200–212.
243. Alonso-Ruiz, A., Calabazo, M., Perez-Ruiz, F., and Mancebo, L. (1993). Toxic oil syndrome. A long term follow up of a cohort of 332 patients. *Medicine* **72**, 285–295.
244. Edison, M., Philen, R. M., Sewell, C. M., Voorhess, R., and Kilbourne, E. M. (1990). L-Tryptophan and eosinophilic-myalgia syndrome in New Mexico. *Lancet* **335**, 645–648.
245. Varga, J., Heiman-Patterson, T. D., Emery, D. L., Griffin, R., Lally, E. V., Uitto, J. J., and Jimenez, S. A. (1990).

Clinical spectrum of the systemic manifestations of the eosinophilia-myalgia syndrome. *Semin. Arthritis Rheum.* **19**, 313–328.
246. Bigazzi, P. E. (1994). Autoimmunity and heavy metals. *Lupus* **3**, 449–453.
247. Hultman, P., Turley, S. J., Enestrom, S., Lindh, U., and Pollard, K. M. (1996). Murine genotype influences the specificity, magnitude and persistence of murine mercury-induced autoimmunity. *J. Autoimmun.* **9**, 139–149.
248. Pollard, K. M., Lee, D. K., Casiano, C. A., Bluthner, M., Johnston, M. M., and Tan, E. M. (1997). The autoimmunity-inducing xenobiotic mercury interacts with the autoantigen fibrillarin and modifies its molecular and antigenic properties. *J. Immunol*. **158**, 3521–3528.
249. Pollard, K. M., Kono, D. H., Pearson, D. L., *et al*. (1998–1999). Autoimmunity induced by xenobiotics. *Mol. Exp. Med.* 267–269.
250. Pollard, K. M., Pearson, D. L., Hultman, P., *et al*. (1998). Xenobiotic-induced acceleration of Systemic Autoimmune Disease. National Institute of Environmental Health Sciences, Linking Environmental Agents and Autoimmune Diseases, September 1–3, 1998, NIEHS Research Triangle Park, NC. Abstract presented at the Environmental Meeting.
251. Lindqvist, K. J., Makene, W. J., Shaba, J. D., and Nantulya, V. (1974). Immunofluorescence and electron microscopic studies of kidney biopsies from patients with nephrotic syndrome, possibly induced by skin lightening creams containing mercury. *E. Afr. Med.* **51**, 168–169.
252. Bardana, E. J., Jr., Manilow, M. R., Houghton, D. C., McNulty, W. P., Wuepper, K. D., Parker, F., and Pirofsky, B. (1982). Diet-induced systemic lupus erythematosus (SLE) in primates. *Am. J. Kidney Dis.* **1**, 345–352.
253. Manilow, M. R., Bardana, E. J., Jr., Pirofsky, B., Craig, S., and McLaughlin, P. (1982). Systemic lupus erythematosus-like syndrome in monkeys fed alfalfa sprouts: Role of a nonprotein amino acid. *Science* **216**, 415–417.
254. Manilow, M. R., Bardana, E. J., Jr., and Goodnight, S. H. (1981). Pancytopenia during ingestion of alfalfa seeds [letter]. *Lancet* **1**, 615.
255. Roberts, J. L., and Hayashi, J. A. (1983). Exacerbation of SLE associated with alfalfa ingestion [letter]. *N. Engl. J. Med.* **308**, 1361.
256. Hess, E. V. (1995). Environmental lupus syndromes. *Br. J. Rheumatol.* **34**, 597–601.

46

CORTICOSTEROID USE IN SYSTEMIC LUPUS ERYTHEMATOSUS

Joseph C. Shanahan
Robert P. Kimberly

GENERAL BIOCHEMISTRY, PHARMACOLOGY, PHYSIOLOGY

Structure of Glucocorticoids

Adrenal steroids have a basic 17-carbon structure consisting of a cyclopentanoperhydrophenanthrane nucleus. This structure is composed of three 6-carbon hexane rings and a single 5-carbon pentane ring (Fig. 1). Glucocorticoids contain 4 carbons in addition to this basic 17-carbon nucleus. The substituent methyl groups are located at positions C-18 and C-19 with a 2-carbon side chain containing C-20 and C-21 which originates at C-17. Twenty-one carbon steroids may have either primarily glucocorticoid or mineralocorticoid properties.

Steroidogenesis starts with cholesterol derived either from the diet or from endogenous synthesis. Adrenal steroid biosynthesis follows three major pathways which lead to glucocorticoids, mineralocorticoids, or adrenal androgens. Glucocorticoids and mineralocorticoids, both 21-carbon molecules, differ at C-17, where the former have a hydroxyl group, and at C-18 where mineralocorticoids have an aldehyde group instead of a methyl group.

Glucocorticoid activity requires a hydroxyl group at carbon-11. Compounds such as cortisone and prednisone, which have 11-keto groups, require *in vivo* conversion to the corresponding 11-hydroxyl compounds, cortisol and prednisone, in order to exhibit glucocorticoid activity. This conversion occurs predominantly in the liver [1, 2]. Whether the presence of liver disease can cause clinically significant impairment of conversion is not clearly established. Topical preparations of glucocorticoids must be 11-hydroxyl compounds since the conversion of 11-keto groups cannot take place locally [3].

Pharmacodynamics

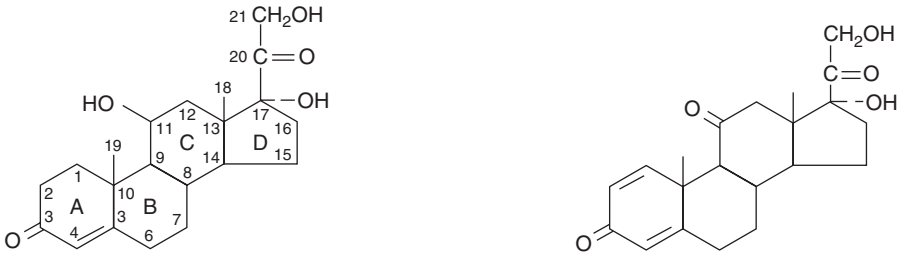
Many factors contribute to the net clinical potency of exogenously administered glucocorticoids. These factors include the adsorption, transport, and metabolism of the steroid compounds.

Bioavailability

In normal subjects receiving equivalent doses of prednisone and prednisolone, comparable plasma levels of prednisolone are achieved [4–6]. Some observations suggest that plasma levels may be more variable after prednisone rather than prednisolone [6]. Bioavailability of both prednisone and prednisolone appears to be equivalent [7–10]. Similarly, studies of prednisolone and dexamethasone in various forms have demonstrated comparable bioavailabilities.

Drug Distribution

Both cortisol and other glucocorticoid derivatives bind primarily to transcortin (corticosteroid-binding



CORTISOL
(HYDROCORTISONE)

PREDNISONE

FIGURE 1 The 21-carbon atom glucocorticoid skeleton consists of a 17-carbon cyclopentanoperhydrophen-anthrane nucleus and four additional carbons. The individual carbon atoms are designated by numbers. The four rings are designated by letters.

globulin; CBG) which occurs normally in plasma. Albumin, functioning as a low-affinity but high-capacity system, binds most of the glucocorticoid not bound to transcortin. Bound steroid is not active, and therefore only a small percentage of administered steroid is able to have physiologic effects. Patients who have had prolonged therapy with a glucocorticoid preparation may demonstrate altered transcortin-binding properties. Both the normal circadian fluctuation in binding capacity and the total capacity are reduced [11]. These alterations represent a second mechanism, in addition to the modulation of endogenous steroid secretion, by which exogenous glucocorticoid administration can influence endogenous glucocorticoid effects.

Changes in the circulating level of transcortin are accompanied by parallel changes in the amount of protein-bound glucocorticoid. Increased CBG and plasma 17-hydroxycorticosteroids are seen in the third trimester of pregnancy and with estrogen-containing oral contraceptive medications. Although plasma levels of glucocorticoids may rise, free levels of endogenously produced steroids remain normal.

Metabolism and Excretion

The principal endogenous glucocorticoids, cortisol and corticosterone, are distributed in a volume which approximates total extracellular fluid space. The plasma concentration is determined by three factors: the rate of secretion, the rate of inactivation, and the rate of excretion of free cortisol. The normal plasma half-life of cortisol is between 60 and 120 min [12].

Glucocorticoids are inactivated by several different biotransformations. 11-Dehydrogenation converts the 11-hydroxyl group, necessary for glucocorticoid activity, to a ketone group. This reversible reaction is a major factor in the regulation of circulating cortisol. Hyperthyroidism markedly accelerates 11-dehydrogenation [13]. Reduction of the double bond in the A-ring leads to dihydro derivatives and reduction of the keto group at C-3 leads to tetrahydro derivatives. These compounds are then conjugated in the liver with glucuronic acid and excreted in the urine. Drugs which activate microsomal enzymes may accelerate steroid inactivation to tetrahydro derivatives [14–21]. Hydroxylation of the ketone group at C-20, cleavage of the C-20 : C-21 side chain, and 6-beta hydroxylation are other transformations leading to inactivation.

Potency and Duration of Action

The net clinical potency of any glucocorticoid preparation reflects both the intrinsic biologic activity and the duration of action. Thus, a long-acting compound has the potential for a greater biologic effect but the correlation between half-life and either potency or duration of action is not precise.

Among the preparations used in clinical settings, the duration of action can be divided into three groups: short-, intermediate-, and long-acting. These classifications are based on the duration of adrenocorticotropin hormone (ACTH) suppression following a single dose as described by Harter [22] (Table 1). Equivalent glucocorticoid dosages are *estimates* based on consolidation of data from several different sources and animal models.

The plasma half-life of various glucocorticoid preparations show a several-fold range [12]. For prednisone, the half-life is approximately 60 min; for prednisolone, 115 to 252 min; for methylprednisolone, from 78 to 180 min; and for dexamethasone, 110 to 282 min [23]. The kinetics of steroid clearance may be dose-dependent in part because of changes in the volume of distribution and because of nonlinear binding of the compound to the transport proteins [24]. The duration of the physiologic effect of glucocorticoids may show even greater

TABLE 1 Therapeutic Glucocorticoid Compounds

Systemic preparations	Potency equivalent
Short-acting	
Hydrocortisone	1
Prednisone	4
Prednisolone	4
Methylprednisolone	5
Intermediate-acting	
Triamcinolone	4–5
Long-acting	
Betamethasone	30
Dexamethasone	25–30

Topical preparations	Brand names
Lowest potency	
Hydrocortisone, 1%, 2.5%	
Low potency	
Fluocinolone acetonide, 0.01%	Synalar
Triamcinolone acetonide, 0.025%	Aristocort
Intermediate potency	
Betamethasone valerate, 0.1%	Beta-Val
Fluocinolone acetonide, 0.025% Cordran	Synalar, Synemol flurandrenolide, 0.05%
Triamcinolone acetonide, 0.1%	Aristocort, Triacet
High potency	
Betamethasone dipropionate, 0.05%	Diprolene, Lotrisone
Fluocinonide, 0.05%	Lidex
Halcinonide, 0.1%	Halog
Triamcinolone acetonide, 0.5%	Aristocort
Very high potency	
Betamethasone dipropionate (with propylene glycol), 0.05%	Diprolene
Clobetasol, 0.05%	Temovate, Cormax
Halobetasol, 0.05%	Ultravate

variability. The duration of action depends on the specific function being monitored, and for any given function, duration may vary with the dose administered [22, 25–27]. Commonly cited durations of action reflect the studies of Harter who used the duration of ACTH secretion suppression following a single dose of glucocorticoid [22]. However, it should be noted that the studies did not examine individual variability between subjects nor did they address any effects of prolonged therapy.

The discrepancy between circulating half-life of given glucocorticoid compounds and the duration of action measured by ACTH suppression demonstrates that the duration of action is not primarily determined by presence of the steroid in the circulation. Steroids pass through the cell membrane and bind to specific receptors in the cytoplasm. The steroid–receptor complexes pass through the cytoplasm into the nucleus where they bind to DNA and alter rates of DNA transcription. Many genes can be affected, both directly and indirectly [reviewed in References 28 and 29]. Thus, the site of action is removed from the circulation per se. Once within the nucleus, the steroid may continue to act or may set into motion a chain of events which can continue for hours or days after the initial stimulus.

Secretion and the Hypothalamic–Pituitary Axis

The secretion of the principal endogenous glucocorticoids, cortisol and corticosterone, is under the control of the hypothalamic–pituitary axis. The hypothalamus secretes corticotropin-releasing factor (CRF) which acts on the anterior pituitary and stimulates adrenocorticotropin hormone (ACTH) secretion. ACTH is a linear polypeptide containing 39 amino acids. The corticotropic actions, however, reside in smaller polypeptide fragments, and the sequence of basic amino acids, Lys-Lys-Arg-Arg, occurring in position 15–18, contain much of the high adrenocorticotropin activity. The biologic half-life of ACTH in the circulation is less than 10 min.

The principal factor controlling the release of CRF and ACTH is the plasma-free cortisol level which acts by a feedback mechanism at the level of both the hypothalamus and the pituitary. This regulation can be modified through the central nervous system (CNS) by a variety of physiologic stresses such as severe emotional trauma, surgery, and pyrogens, each of which elevate ACTH levels and blood glucocorticoid levels. The sleep–wake cycle has an important effect on the pattern of ACTH secretion. Under normal conditions, there is an approximately diurnal pattern in the plasma level of ACTH with a peak just prior to wakening and a nadir just prior to sleep.

Because plasma glucocorticoid levels feed back to the hypothalamic–pituitary–adrenal axis (HPA) to control endogenous steroid secretion, exogenous steroids can alter normal HPA regulation and suppress the ability of the HPA to respond to stress with an increased production of ACTH. The ability of different glucocorticoid preparations to suppress the HPA varies but does not correlate well with the circulation half-life of the compound. The potential for HPA suppression is also dependent on the timing of the exogenous dose in relation to the sleep–wake cycle. Administration synchronized with the normal ACTH peak has less suppressive potential than doses given at the time of the ACTH nadir.

Prednisone given in a dose of more than 15 mg a day suppresses the HPA within about 1 week [12, 30]. Doses between 7.5 and 15 mg a day take approximately 1 month although some patients may not suppress if the

single dose is given only in the morning. Early morning doses of 5 mg of prednisone or less do not suppress the hypothalamic–pituitary axis [31, 32] although the same dose given in the afternoon can alter CRF release and obscure cyclic cortisol secretion [32, 33]. Harter [22] examined the effects of a single dose of different glucocorticoid preparations in a single patient and demonstrated substantially longer suppression after use of long-acting compounds such as dexamethasone. There is, however, substantial variability among the different subjects [34, 35]. Consequently, most physicians emphasize the use of short-acting preparations such as prednisone.

Although the duration of suppression depends in part on the dose and duration of the therapeutic course which lead to suppression [32, 36], there is clear variation among individuals. Streck and Lockwood [37] demonstrated that a 5-day course of 50 mg of prednisone daily affected cortisol production of up to 5 days. Longer courses of high-dose therapy may lead to HPA suppression which does not reverse completely for 6 months. The adrenal component of cortisol production may take as long as 1 year to recover appropriate responsiveness despite adequate ACTH levels [12]. Supplementation with exogenous glucocorticoids during the year after long-term high-dose therapy is usually recommended for physiologically stressful situations such as surgery with general anesthesia, although uncertainty and controversy do exist [38, 39].

Special Situations

There are several clinical situations in which the handling of glucocorticoids may be altered.

Liver Disease

The conversion of prednisone to prednisolone is impaired in patients with active, severe liver disease [5, 6, 40]. However, this appears to be counterbalanced by a decreased rate of clearance of prednisolone from the blood in such patients [6]. The fraction of glucocorticoid bound by plasma proteins may be altered [5, 40] and may lead to variable free plasma levels of prednisolone after oral doses of either prednisone or prednisolone [6]. Low serum albumin levels have been associated with a concomitantly increased frequency of prednisone side effects. Despite these various effects, they appear small and often counterbalancing. Potential adjustments of glucocorticoid dosages in the presence of liver disease are complex and unpredictable. Thus, there is no clear indication to use prednisolone rather than prednisone in patients with liver disease or to reduce the dose of glucocorticoid in patients with marked hypoalbuminemia [41] and normal hepatic function. A modestly lower dose may be appropriate in the setting of marked hypoalbuminemia and active liver disease.

Pregnancy

Glucocorticoids do cross the placenta and may induce subnormal responsiveness to ACTH [42, 43]. Hypothalamic pituitary suppression and Cushing's syndrome are not clinically significant in a neonate born to a mother taking glucocorticoids [44, 45]. Since hydrocortisone, prednisone, and prednisolone are more susceptible to inactivation by placental enzymes than are dexamethasone and betamethasone, prednisone and prednisolone are the preferred drugs for use in pregnancy.

Maternal glucocorticoid therapy is not associated with an increased incidence in congenital anomalies in newborns [45–47]. Reduction in fetal size has been suggested but not differentiated from the effects of the underlying disease per se. However, in comparison to various control interventions, prednisone administration was associated with an increased rate of prematurity in three separate trials of prevention of pregnancy loss in women with anti-phospholipid antibody syndrome [48–50]. Although intrauterine growth retardation was not observed more frequently among those treated with prednisone, preeclampsia and gestational hypertension occurred more commonly in prednisone-treated subjects [48, 50]. Both prednisone and prednisolone occur only in low concentration in breast milk [51] and consequently, glucocorticoid therapy for the nursing mother does not pose a major hazard for the infant.

Selective Steroid Compounds

The ideal therapeutic corticosteroid would possess only the desired beneficial effect with no other accompanying side effects. Such dissociation of actions could result from novel chemical structures with selective agonist properties, selective access to appropriate target tissue sites, or perhaps the development of new properties. Examples include dexamethasone and betamethasone, two synthetic corticosteroids which dissociate glucocorticoid from mineralocorticoid activities through chemical modification of the basic corticosteroid structure. Although glucocorticoid effects are retained, many mineralocorticoid side effects are avoided. A different approach to selectivity has been taken with raloxifene, a nonsteroidal benzothiophene that is classified as a selective estrogen receptor modulator [52, 53]. In human studies, raloxifene appears to have tissue-specific estrogen-agonist or -antagonist

activity which is putatively beneficial for bone mineral density and serum cholesterol.

Other steroid compounds have attempted to achieve a higher glucocorticoid therapeutic ratio by further structural modifications of the basic molecule. Deflazacort, a prednisolone analog with an oxazoline group at carbon 17, has reduced lipid solubility and may have a greater effect on circulating, rather than sessile, cells. Relatively smaller changes in the urinary excretion of calcium and less hyperglycemia with deflazacort compared to equipotent anti-inflammatory doses of prednisone may indicate some sparing of carbohydrate metabolism and calcium balance [54–59]. Modification of drug access to and distribution in tissues may also be achieved by specialized drug delivery systems, such as incorporation into liposomes [60–63]. Neither deflazacort nor liposome delivery, however, has received much attention.

As the actions of corticosteroids at the transcriptional and cell-signaling levels have become more clearly defined, increasingly specific therapeutic targets are available for novel steroids. The concept of "dissociated steroids" now refers to synthetic compounds that retain either the positive or negative effects on gene transcription. Many of the anti-inflammatory effects of corticosteroids stem from inhibition of NF-κB- or activated protein-1 (AP-1)-mediated transcription of inflammatory mediators, including cytokines and cell adhesion molecules. However, some anti-inflammatory and immunosuppressive effects of corticosteroids are mediated by enhancing transcriptional activity. Dissociated steroids retain the ability to repress the transcriptional activities of NK-κB or AP-1, but are no longer capable of interacting with glucocorticoid receptor (GR) response elements to initiate new gene transcription. The steroid antagonist RU486, for example, fails to activate glucocorticoid receptor-mediated gene transcription, yet significantly reduces transcription of AP-1-dependent genes [64]. By retaining certain properties, dissociated steroids exert strong anti-inflammatory effects but avoid the complications associated with transcription of GR-dependent genes. However, some adverse effects of corticosteroid therapy may be due to transrepressive functions. Therefore, further study is required to assess the clinical impact of dissociated corticosteroids in animal models and humans.

MECHANISMS AND ACTIONS

Overview

In inflammatory rheumatic diseases such as systemic lupus erythematosus, glucocorticoids are used both as an anti-inflammatory and as immunosuppressive agents. The exact mechanisms of these general effects are complex and include alteration of both the trafficking of circulating immunocompetent cells and the actions of such cells [65–70] (Table 2). Thus, decreased neutrophil margination and altered peripheral lymphocyte transendothelial cell migration may be important. Similarly, modulation of the synthesis and release of inflammatory mediators and cytokines, reduction of cell surface expression of cytokine receptors and MHC class II molecules, induction of lymphocyte cell death, and modification of intracellular communication among immunocompetent cells may underlie some of the effects of glucocorticoids. These effects may represent both pharmacologic and physiologic actions.

The general schema for the action of glucocorticoids involves the binding of the glucocorticoid to specific intracellular, cytoplasmic receptors in the target cell after passive movement of the steroid through the cell membrane [28, 29]. The glucocorticoid–receptor complex is rapidly translocated to the nucleus where steroid–receptor complexes form homodimers, which subsequently bind to specific regions of DNA (glucocorticoid response elements; GREs). This binding modifies the rate of DNA transcription with resultant effects manifested by alterations in protein synthesis [71]. Thus, many of the effects of glucocorticoids are mediated through transcriptional factors and protein mediators. The role of the intercalation of steroids into cell membranes and lysosomes is less well defined. Glucocorticoids also have negative effects on the promotion of gene transcription by NF-κB and AP-1. These transcriptional complexes are activated by the exposure of cells to inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-1 (IL-1). On activation, NF-κB and AP-1 shed inactivating proteins, permitting translocation to the nucleus where they bind DNA promoters for cytokines, cell adhesion molecules, and other critical mediators of immune and inflammatory processes. Activated GR inhibits NF-κB and AP-1 gene transcription through transcriptional cross talk which likely takes several forms, including direct protein interaction [72–74], upregulation of NF-κB inhibitors, particularly the cytosolic inactivator I-κB [75–77], and competition for limited transcriptional coactivator molecules [78, 79]. Activated GR also inhibits intracellular signaling cascades, including the JAK-STAT system, which contributes to the negative effects of corticosteroids on lymphocyte proliferation and differentiation [80].

The presence of glucocorticoid receptors in many different cell types helps to explain the broad range of biologic effects observed with glucocorticoid administration. Lymphocytes, monocytes and macrophages, osteoblasts, liver cells, muscle cells, fat cells, and

TABLE 2 Anti-inflammatory and Immunosuppressive Mechanisms of Corticosteroid Action

Anti-inflammatory effects	Mechanism of action	Clinical outcome
Antagonism of transcription factors (NF-κB, AP-1, NF-AT)	1. Up-regulates synthesis of transcription factor inhibitors (I-κB, GILZ) 2. Direct interaction between GR and transcription factors, inhibiting protranscriptional activity 3. Competition for essential transcriptional cofactors (CREB-binding protein, SRC-1)	1. Reduction in production of TNF-α, IL-1, GM-CSF, IL-6, IFN-γ, IL-12 2. Reduced expression of IL-8, ICAM-1, ELAM-1, VCAM-1, E-selectin, RANTES, MCP-1
Reduction of cytokine and chemokine expression	1. Synthesis of destabilizing protein reduces mRNA half-life 2. Transcriptional inhibition via direct GR or GRE antagonism and reduction of transcription factor activity (see earlier)	3. Decreased substance P and inducible nitric oxide synthetase expression
Inhibition of arachidonic acid release by enhanced lipocortin activity	1. Rapid translocation of cytosolic lipocortin to cell surface 2. Increased transcription of lipocortin mRNA	Reduced production of prostaglandin mediators of inflammation
Immunosuppression	**Mechanism of action**	**Clinical outcome**
Inhibition of IL-2/IL-2R activity	1. Direct suppression of IL-2R mRNA levels (possibly through transrepression) 2. Up-regulation of GILZ and other mechanisms of NF-κB inhibition) 3. Inhibition of IL-2 signaling by blockade of STAT 5 activation (possibly by inhibition of tyrosine phosphorylation	Modulation of T-cell activation
Inhibition of dendritic cell (DC) function	1. Impaired antigen-presenting function 2. Reduced expression of costimulatory molecules 3. Suppression of IL-12 production 4. Apoptosis of dermal/interstitial DC precursors	
Preferential enhancement of Th2-type cytokine profile	Down-regulation of IL-12 activity 1. Inhibition of STAT 4 phosphorylation (rapid onset but mechanism unknown) 2. Down-regulation of IL-12 receptor	1. Enhanced TGF-β and IL-10 production 2. Reduction of inflammatory cytokines

fibroblasts are among the cell types with such receptors [81, 82]. The presence of intracellular receptors and effects on DNA transcription helps to explain why the biologic effect of glucocorticoids may be so much longer than the plasma half-life. However, quantitative glucocorticoid receptor protein does not necessarily correlate closely with glucocorticoid action since there are many tissue specific effects [67].

A recurrent question in steroid therapy is the equivalence of different types of glucocorticoid compounds. Data regarding this issue depend on the biologic system under evaluation. For example, Rosenberg and Lysz [83] demonstrated that hydrocortisone, methylprednisolone, and dexamethasone, used at equipotent anti-inflammatory doses, did not show equivalent effects on some aspects of *in vitro* cellular immunity. In their systems, methylprednisolone was more effective in suppressing the generation of cytotoxic lymphocytes than dexamethasone. Hofman and colleagues studied the effects of various corticosteroids on cultured CEM-C7 T cells and L929sA fibrosarcoma cells [84]. Triamcinolone, dexamethasone, betamethasone, clobetasol, prednisolone acetate, and hydrocortisone all reduced TNF-α-induced NF-κB activation to a similar extent in L929sA cells. In contrast, hydrocortisone and prednisolone were less effective inducers of apoptosis in CEM T cells in comparison with the other agents. Since "anti-inflammatory" and "immunosuppressive" potency may not be equivalent, identification of the pathophysiologic property underlying the desired clinical effect is

of paramount importance. Recognition of the appropriate steroid-responsive pathophysiologic mechanism allows questions regarding both the specific glucocorticoid compound and optimal dosage range to be investigated directly. It is also important to realize that there are some species differences in glucocorticoid responsiveness [85], that there may be some organ specific differences in drug levels [86], and that some *in vitro* studies may use concentrations of glucocorticoids higher than those achievable in man [67].

Metabolic Effects

By conventional standards, the term *glucocorticoid* describes those corticosteroids which have a dominant effect on intermediary metabolism [87]. The actions of glucocorticoids are anti-insulin-like and include effects on protein, carbohydrate, and nucleic acid metabolism. Glucocorticoids have a catabolic effect on protein metabolism manifested by increased degradation and decreased synthesis of protein in many tissues. During steroid therapy, plasma amino acid levels may rise significantly. Glucocorticoids stimulate glyconeogenesis in part by mobilization of glycogenic amino acid precursors. Increased gluconeogenesis and decreased glucose transport into cells and utilization in peripheral tissues lead to an increase in blood glucose. Hepatic glycogen content is increased by glucocorticoids. Glucocorticoids also promote lipid catabolism by increasing lipolysis in peripheral fat depots and potentiating the activation of cellular lipase by agents such as catecholamines and pituitary peptides. Increased mobilization of free fatty acids and glycerol also promotes glycogenesis.

Glucocorticoids directly stimulate hepatic synthesis of enzymes such as tryptophan pyrrolase and tyrosine aminotransferase. These enzymes may participate in diminished protein metabolism and cytolysis of lymphoid tissue and muscle.

Glucocorticoids may have mineralocorticoid properties. Enhanced renal tubular sodium reabsorption can increase glomerular filtration rate, increase free water clearance, and lead to clinically apparent peripheral edema. Enhanced potassium excretion by renal tubular cells can lead to the low serum potassium. Although these actions on sodium and potassium handling are mediated primarily by the kidneys, they may also be expressed by cells in the intestinal mucosa and sweat glands. The degree of mineralocorticoid activity varies among different glucocorticoid compounds with cortisone and hydrocortisone having the largest and betamethasone and dexamethasone having the least.

Corticosteroids play an important role in the maintenance of adequate vascular tone both by a direct effect on blood vessels and by a synergistic effect with catecholamines. The net effect of corticosteroids may be either vasoconstrictive or vasodilatory depending on the specific vascular bed, the resting tone of that bed, and the nature of any concomitant pathologic process [88, 89]. Interaction with specific receptors on vascular smooth cells [90, 91] and induction of angiotensin converting enzyme activity in vascular endothelial cells [92] are both mechanisms involved in the action of corticosteroids in regulation of local blood flow in vascular beds.

Anti-inflammatory Effects

The effects of glucocorticoids on inflammation reflect the complexity of the inflammatory process itself [70]. No single mechanism underlying the net effects on inflammation has been identified although modification of the synthesis of specific proteins, mediated at the level of transcription, is a common theme [28, 29]. Glucocorticoids exert an anti-inflammatory effect primarily by inhibiting the production of cytokines and cell adhesion molecules. Inhibition of cytokine production is mediated primarily by repressing the transcriptional activity of NF-B and AP-1. Additionally, glucocorticoids exert posttranscriptional effects, directly inhibiting cytokine functions by reducing cytokine mRNA half-life and blocking cytokine-mediated intracellular signaling. Protranscriptional activity of activated glucocorticoid receptor includes the production of inhibitors of NF-κB and AP-1 function, as well as upregulation of anti-inflammatory products, such as the nonfunctioning, decoy IL-1 type II receptor [93]. Gene expression studies of peripheral blood monocytes in normal human subjects treated with dexamethasone have employed DNA microarray technology to evaluate the effects of glucocorticoids [94]. Dexamethasone down-regulated certain proinflammatory cytokines, yet up-regulated expression of important components of innate immunity, including complement factors and toll-like receptors (TLR-2 and TLR-4). In addition, glucocorticoids altered the regulation of certain genes depending on the state of activation of the cell. Therefore, the impact of glucocorticoids may vary depending on cell type, but also on the state of activation. Ultimately, the ability of glucocorticoids to retard the recruitment of neutrophils and monocyte-macrophages to inflammatory sites, by reducing cytokine and chemotaxin activity, represents a major component of the anti-inflammatory potential of steroids [67].

A single dose of glucocorticoid accelerates the release of mature granulocytes from the bone marrow, decreases the migration of neutrophils from the circulating pool, increases the circulating half-life of the neutrophils [66, 95, 96], and leads to a neutrophilic

leukocytosis with a peak level at 4–6 h after administration [66, 97]. The decreased accumulation of neutrophils at an inflammatory site reflects a summation of several different effects. Glucocorticoids decrease the local vasodilatation and capillary permeability which may decrease local exudation, edema formation, and leukocyte migration [67]. Glucocorticoids may modify chemotaxis and decrease the adherence of inflammatory cells to the vascular endothelium [98–103], due in part to changes in the expression and function of various cell adhesion molecules including members of the immunoglobulin supergene family (e.g., ICAMs), various integrins (e.g., LFA1 and CR3) and various LEC-CAMs (e.g., ELAMs) [104–106].

Glucocorticoids cause a monocytopenia peaking 4–6 h after administration [107, 108]. Recovery of the baseline circulating populations occurs within 24 h. Inhibition of the accumulation of monocyte-macrophages at inflammatory sites probably involves similar mechanisms to those which impede the accumulation of neutrophils. Impaired recruitment of stimulated monocytes contributes to the suppression of delayed hypersensitivity responses induced by steroids [109].

In addition to decreasing the recruitment, glucocorticoids exert an anti-inflammatory effect by modifying a number of cell functions. Steroid therapy can depress the bactericidal activity of monocytes [110, 111] and may reduce the phagocytic activity of both neutrophils and monocytes [112, 113]. As applied to IgG-mediated phagocytosis, this latter effect may be related to modulation of Fc receptor expression on the cell surface. However, glucocorticoids do not have a consistent effect on the constitutive expression of Fcγ receptor number or function in several *in vitro* systems [114–120]. Steroids do appear to block the production of gamma-interferon (γ-IFN)-rich lymphokines which can decrease Fcγ receptor density [121]; but given the presence of γ-IFN, steroids may augment the interferon-induced enhancement in Fc receptor expression [122]. The distinction between the action of glucocorticoids on constitutive and on induced functions may explain why *in vivo* studies have not always yielded consistent findings [123, 124].

Glucocorticoids can be effective inhibitors of prostaglandin synthesis [125–128]. Unlike nonsteroidal anti-inflammatory drugs (NSAID) which inhibit the cyclo-oxygenases, steroids decrease the release of the substrate, arachadonic acid, from phospholipid stores. This effect is mediated by the induction of a polypeptide, macrocortin or lipomodulin, which inhibits phospholipase A_2 action [129–132]. Thus, glucocorticoids can inhibit the production of arachidonate metabolites from both the cyclo-oxygenase and lipoxygenase pathways.

Immunosuppressive Effects

The actions of glucocorticoids on immune responses are complex, but the net effect is generally a suppression of immune function. This suppression reflects the summation of altered leukocyte movement, of decreased leukocyte function, and of alterations in a variety of humoral factors.

Administration of glucocorticoids causes a lymphopenia which reaches a nadir at 4–6 h and which resolves within 24–48 h [66, 107, 108, 133–139]. The duration and the nature of the lymphocyte subpopulations affected depend on the dose of steroid given. The $CD4^+$ T inducer/helper subset shows a greater absolute and percentage decrease than the $CD8^+$ T cytotoxic/suppressor subset [107, 108, 137, 139]. As a consequence, the T4/T8 ratio decreases with steroid therapy and shows larger changes as the dose of steroid increases. B lymphocytes show an absolute decrease in number but usually no change in percentage; non-T/non-B lymphocytes show an increase in percentage, especially with doses of prednisone greater than 30 mg [107, 108, 137].

Lymphopenia results from a redistribution of circulating lymphocytes with apparently effective egress from the intravascular space but inefficient return from lymphoid organs [66, 67, 138, 140]. Since splenectomized subjects show the same lymphopenia as those with intact spleens, some redistribution to the bone marrow may occur. Whether the more profound impact of glucocorticoid administration on $CD4^+$ cells relative to $CD8^+$ cells reflects a greater representation of $CD4^+$ cells in the recirculating pool remains for further investigation. This redistribution, however, emphasizes the possibility that changes in peripheral lymphocyte function after *in vivo* administration of steroid may reflect changes in both cell populations and cell function.

Although a short course of glucocorticoids may decrease serum immunoglobulin levels in humans [141–143], the effect on the humoral immune response is variable. Among the factors contributing to this variability may be the dose of glucocorticoid, the nature of the antigenic stimulus, and the type of response (primary or secondary) being elicited. At lower concentrations of glucocorticoids in some experimental systems, stimulation of antibody formation can be seen [144–145]. This effect may require the combined action of both glucocorticoid and other soluble factors on a target cell(s) with glucocorticoids serving as a permissive or as an amplifying component for those factors. Inhibition of antibody production by steroids might result from a number of mechanisms. The circulating traffic of monocytes can be suppressed, the access of monocytes to sites of antigen localization diminished

[107, 108], and perhaps, antigen processing decreased. Glucocorticoids inhibit production of interleukins and other cytokines and may also alter the expression and function of cytokine receptors [145–153]. These cytokines may have direct effects on B cells, or indirect effects through changes in T helper cell function.

Various aspects of cell-mediated immunity are suppressed by glucocorticoids. Steroids decrease proliferation in the mixed leukocyte reaction and in response to mitogens [67, 154, 155] and alter T-lymphocyte-mediated cytotoxicity [156, 157]. Glucocorticoids inhibit interleukin-2 (IL-2) production by lymphocytes, which is important in the maintenance of T-cell proliferation [146], as well as the production of IL-3, granulocyte–macrophage colony stimulating factor (GM-CSF) and γ-IFN [147]. IL-2 and other cytokines critical for T-cell differentiation and proliferation signal through a common pathway, JAK-STAT, which is inhibited *in vivo* by dexamethasone [80]. Some effects of steroids on cell-mediated immunity may reflect effects of changes in circulating monocyte populations, decreases in the production of monokines such as IL-1, or inhibition of the response of macrophages to various lymphokines [158]. Suppression of cutaneous delayed hypersensitivity is an effect of delayed monocyte recruitment to the local site [159].

T-cell activation requires antigen presentation and delivery of a costimulatory signal. Dendritic cells (DC) efficiently activate T cells and appear critical to primary immune responses. Glucocorticoids impair several DC functions and thus reduce the ability of these cells to activate T cells. Dexamethasone has been shown to impair antigen-presenting function *in vivo*, and reduces expression of costimulatory molecules *in vitro* [160]. *In vitro* studies have shown that superantigen-stimulated DCs, pretreated with glucocorticoids, exhibit reduced potential to induce γ-IFN production from $CD4^+$ T cells. This glucocorticoid effect is attributed to a reduction in IL-12 production of activated DCs [161]. Immature DCs exposed to glucocorticoids *in vitro* have decreased T-cell stimulatory capacity and acquire Th2 bias [162, 163]. In addition, glucocorticoid effects may vary among cell types to exert various alterations of immune responses. For example, Woltman and colleagues have shown *in vitro* corticosteroids exposure of $CD34^+$ hemopoeitic dendritic cell precursors specifically inhibits the development of $CD14^+$-derived interstitial/dermal DCs, while no effect was seen on the development of Langerhans cells [164].

The capacity for glucocorticoids to have both stimulatory and inhibitory effects on immune function makes simple predictions about the clinical effects of these agents in autoimmune disease difficult. Such complexity may be less surprising now that the interrelationship between the immune system and the hypophyseal–adrenal axis is increasingly appreciated [70, 165]. Some thymic peptides have corticotropin-releasing factor activity and some cytokines from lymphocytes and monocytes can affect endogenous corticosteroid concentrations [166, 167]. However, despite lack of knowledge about precise mechanisms, glucocorticoid administration in many lupus patients results in reduction of autoantibody titers and circulating immune complexes, an increase in complement levels, and a general amelioration of disease activity.

Immunosuppression-Pulse Therapy

Very large doses of intravenous corticosteroids may have effects which differ either in quality or in quantity (duration and/or magnitude) from conventional oral dosages. Concentration-dependent effects on lymphocyte blastogenesis were suggested in early *in vitro* studies [154]. Similarly, the maximum effect of corticosteroid-induced suppression of mixed lymphocyte cultures (MLC) appeared between methylprednisolone concentrations of 1–10 μg/ml [168]. This plasma level is achieved *in vivo* approximately 2 h after an intravenous dose of 1 g of methylprednisolone in an average adult [169, 170]. Although some studies have suggested a slightly longer duration of depression of lymphocyte responsiveness to mitogens after an intravenous dose of 1 g of methylprednisolone [171] and more prolonged changes in natural killer cell activity [172], other investigators have found no greater alterations in either circulating cell populations or reactivity in assays of cellular immunity with large intravenous doses of glucocorticoids as compared to conventional oral doses [173, 174]. Adequate data on serum IgG levels in normals and patients with rheumatic diseases are not available. However, Trotter and Garvey [175] found the fall in serum IgG in patients with multiple sclerosis was no greater with high-dose intravenous corticosteroids than with alternate-day oral steroids.

Dose-dependent inhibition of granulocyte aggregation, perhaps mediated by the C5a complement fragment, has been suggested by Hammerschmidt and co-workers [176]. Since substantial inhibition was seen at 1 mg/ml of methylprednisolone, such inhibition, or similar effects on other inflammatory processes, may contribute to the clinical impact of very high dose intravenous corticosteroid.

CLINICAL APPLICATIONS

Corticosteroids are central to the management of SLE, but their optimal role often prompts vigorous discussion on clinical rounds and sharp debates in the

literature. The principal issue is the balance of potential risks and potential benefits, magnified by the long duration of therapy which is usually anticipated in the management of lupus. The decision to use systemic glucocorticoids requires knowledge of the natural history of the disease presentation and evidence regarding the impact of treatment on the long-term outcome. Although much is known about the course of lupus and the impact of glucocorticoids, there is room for reasonable debate and disagreement.

Several general guidelines are appropriate. Whenever possible, topical preparations should be used for dermatological manifestations. With systemic glucocorticoid therapy, the smallest dose should be prescribed for the shortest possible period. Preparations with less mineralocorticoid activity should be used whenever possible. Agents which have a prolonged duration of action should be avoided because of the difficulty in regulating dose and duration of action. When the clinical situation permits, alternate-day therapy and adjunctive agents, such as nonsteroidal anti-inflammatory drugs, should be used to minimize the required steroid dose. Finally, the presence of other medical conditions, such as diabetes mellitus, osteoporosis, hypertension, tuberculosis, or psychiatric illness which might predispose to steroid complications, should be considered in monitoring therapy.

An obvious but important caveat applies to corticosteroid treatment in lupus. Many of the steroid-responsive signs and symptoms of SLE can be caused by other processes which may be worsened by steroid use. The pulmonary infiltrate may be bacterial pneumonia, and the abdominal pain may reflect a perforated viscus rather than sterile serositis. The severe joint pain may be aseptic necrosis, not inflammatory arthritis. The list of possibilities is long but the mandate is clear. Careful differential diagnostic evaluation is an important preliminary to the use of glucocorticoids.

Preparations and Routes of Administration

Topical Therapy

Many cutaneous manifestations of systemic lupus can be managed with topical therapy rather than systemic administration of corticosteroids. All glucocorticoid preparations for local use are 11-hydroxyl compounds which eliminate the need for biotransformation in the liver. These preparations are usually grouped into low, intermediate, and high potency categories; the fluorinated compounds as a rule demonstrate greater biologic activity (Table 1). The efficacy of topical steroid preparations in a given clinical setting is related to the potency of the compound, the concentration of the active drug in the vehicle, the nature of the vehicle, and the percutaneous penetration of the compound. In general, ointments facilitate hydration of the skin, enhance permeability, and increase penetration of active drug. The presence of inflammation, adequate hydration of the skin, and occlusive plastic wraps all facilitate penetration of drug into the skin. The presence of calloused skin, as on the feet, decreases penetration.

As a general approach, topical therapy is usually started with a stronger preparation and, once control of the cutaneous manifestations is achieved, less potent preparations are used. Lower potency steroid preparations are preferred for regions with thin skin such as the face, axilla, groin, and genital area. Since generic topical corticosteroids may formulate their vehicles differently from their brand-name counterparts, their efficacy may vary somewhat. Within each grouping in Table 1 efficacy is generally similar [177]. The costs of different compounds may vary greatly.

Intra-articular Therapy

In carefully selected situations, intra-articular corticosteroids may be useful adjunctive therapy for management of inflammatory arthritis [178, 179]. Clinical efficacy is determined by potency, dosage, and chemical formulation. Longer action of steroids prepared for intra-articular injection is achieved by the use of less soluble depot formulations. Usually, a sustained therapeutic effect is preferable for inflammatory arthritis to avoid abrupt symptomatic rebound.

Methylprednisolone acetate, prednisolone tebutate (Hydeltra-TBA), dexamethasone acetate (Decadron-LA, Dalalone DP), betamethasone acetate (Celestone Soluspan), and triamcinolone hexacetonide (Aristocort) are long-acting, repository preparations which can be used for intra-articular injections. The sodium phosphate or succinate salts of these compounds are soluble and have a much shorter effective duration of action. Intra-articular steroids can cause facial flushing [180], suppression of endogenous hydrocortisone levels [181], change in plasma salicylate levels [182], induction of hyperglycemia [183], precipitation of a sickle-cell crisis in susceptible individuals [184], and other systemic effects [185, 186].

Systemic Therapy

Oral and intravenous administration are the primary modes of systemic corticosteroid therapy. Intramuscular injection is usually reserved for special circumstances such as limited vascular access. Some steroid may be absorbed systemically with topical, inhalational, or intra-articular therapy.

Prednisone is the most commonly used compound for oral administration. Prednisolone and methylprednisolone are other short-acting oral preparations with little mineralocorticoid effect. Dexamethasone has the least mineralocorticoid action, but it has all the difficulties of managing a long-acting compound. Methylprednisolone sodium succinate, prednisolone sodium succinate, and dexamethasone sodium phosphate are the soluble forms of glucocorticoids most commonly used in the management of systemic lupus. Hydrocortisone sodium succinate has substantial sodium-retaining properties and is used less frequently.

Therapeutic Situations

Although a great difference of opinion exists as to what constellation of clinical and laboratory findings constitute an indication for steroid treatment [187–198], several situations provoke little debate. The acutely and dangerously ill patient ("lupus crisis") often with high fever, extreme malaise, and other symptoms of active lupus including pleuritis, arthritis, and vasculitic rash deserves systemic steroids in the range of 1 mg/kg of prednisone (for equivalent) per day in divided doses [199]. The hematologic manifestations of severe hemolytic anemia with a hematocrit less than 20 or marked thrombocytopenia with a platelet count less than 40,000–50,000 per mm^3 are also indications for steroid therapy [187, 188, 190, 191]. In the setting of "lupus crisis," the use of steroids is prompted not only by the severity of illness, but also by the reasonable expectation that steroids will ameliorate the flare of disease and that the course of high-dose steroids will be of relatively short duration. Accordingly, the benefit is potentially large and the risk modest in comparison. For the patients with hemolytic anemia or thrombocytopenia, steroids are usually effective in decreasing splenic sequestration. Since the progress can be easily and directly monitored, steroid usage can quickly be adjusted to the smallest possible dose and the side effects kept to a minimum.

Glucocorticoid therapy for major organ involvement including the kidneys and the central nervous system is more problematic.

Renal

Corticosteroids are used commonly in the management of lupus nephritis. The initial clinical observations of Pollak and associates [200, 201], made more than three decades ago, suggested that high-dose prednisone treatment helped to prevent or postpone the onset of renal failure. A similar experience was reported by Boelaert *et al.* [202] in the mid-1970s. Although these data did not result from stratified randomized trials, in combination with a general enthusiasm for steroid therapy in SLE [202–204], they formed the basis for current glucocorticoid use in lupus nephritis.

Unlike the use of steroids in "lupus crisis" or in the context of profound hematologic abnormalities, the immediate clinical consequence of drug administration on lupus nephritis may be inapparent or of uncertain clinical significance. Furthermore, the therapeutic goal is usually a long-term preservation of renal function rather than an immediate reversal of processing clinical manifestations. Thus, the basis for any therapeutic decision in lupus nephritis requires the integration of several disparate bodies of data. These data deal with disease mechanisms, natural history, prognostic indices, drug complications, and outcomes in relation to specific therapeutic interventions.

Pollak reported the effects of high- and low-dose oral corticosteroids on the clinical outcome of patients with lupus glomerulonephritis [200, 201]. The histologic term "glomerulonephritis" indicated a proliferative and/or membranous lesion of the glomeruli associated with tubular and interstitial tissue changes. This was distinguished from "glomerulitis" which did not include significant tubular damage or interstitial changes. Glomerular lesions were more severe in the glomerulonephritis group. Within the group of patients with active glomerulonephritis and a serum urea nitrogen level of 30 mg/dl or less at the start of therapy, administration of high steroid (average 47.5 mg/day prednisone for 6 months) treatment significantly improved survival compared to low steroid (average 10 mg/day prednisone) treatment. No benefit of high-dose therapy in terms of survival was reported for patients with urea nitrogen levels greater than 30 mg/dl at the start of therapy. Using improvement in renal function rather than survival as an outcome measure, Boelaert *et al.* [202] found that high-dose corticosteroid was more likely to lead to improvement in function than low- or no-dose corticosteroid. Not all of Boelaert's patients had urea nitrogen levels less than 30 mg/dl at the start of therapy.

Other authors have also concluded that glucocorticoid therapy is beneficial in lupus nephritis [205–207]. Yet controversy over this point remains. Some investigators could detect no effect overall [208–210] while others remained impressed with the individual variability of both the natural history and the response to therapy [211]. Part of the difficulty with these studies include small patient numbers, variable histologic definitions of the groups being tested, differing (and sometimes unspecified) measures of outcome, and the absence of a treatment comparison group.

Several different therapeutic strategies have evolved as a result of the controversy surrounding the interpretation of data on corticosteroid therapy for lupus nephritis. Since histologic classification of renal biopsy specimens, a measure of end-organ damage, can provide a broad prognostic outline, one general approach emphasizes the use of histologic appearance of glomerular involvement to determine therapeutic strategy. Histologic assessment might emphasize the pattern and extent of glomerular involvement as described by the World Health Organization classification scheme [212, 213]. Alternately, the degree of disease activity and/or chronicity has been evaluated as a useful therapeutic guide [214, 215]. More severe and active lesions are indications for more aggressive therapy. A second approach emphasizes serologic abnormalities as indicators of the degree of activity of the presumed pathophysiologic process. Thus, persistent abnormalities are considered an undesirable circumstance although support for this assumption is not always consistent [216]. According to this therapeutic strategy, sufficient corticosteroids are administered to normalize these serologic values [217, 218]. Unfortunately, neither of these approaches is free of difficulty. The occurrence of clinically silent, but histologically severe nephritis [219, 220] makes selection of patients by clinical criteria for renal biopsy problematic. Histologic transformations from one biopsy class to another are well recognized [212, 221–224]. Serologic abnormalities do not consistently identify patients at high risk for severe disease [216] and may fluctuate spontaneously [225].

Despite the difficulties with the extensive literature on lupus nephritis, several guidelines for the use of glucocorticoids can be formulated. These guidelines recognize that lupus nephritis is often an episodic disease with acute flares which may be managed with limited courses of therapy. Thus, patients with newly documented moderate renal disease such as mesangial glomerulitis and focal glomerulonephritis would receive oral prednisone in the range of 40 mg/day for 6 to 8 weeks. Either an early clinical response in terms of serologic activity, urinary sediment, quantitative urinary protein secretion, and renal function or the early development of substantial drug toxicity would prompt an appropriate gentle taper of the prednisone dose. In the absence of biopsy material, a patient presenting with modest protein excretion and normal renal function might receive a moderate therapeutic course of corticosteroids. The absence of a clinical response over a 6- to 8-week interval would make a biopsy of even greater interest since diffuse proliferative glomerulonephritis would warrant a trial of high-dose therapy.

For patients with diffuse proliferative glomerulonephritis and its accompanying clinical manifestations of proteinuria and possibly renal insufficiency, a course of high-dose oral prednisone in the range of 60–80 mg/day is warranted. As with the more moderate therapeutic strategy, the dose is tapered with the occurrence of significant toxicity, with indications of a clinical response, or with the completion of a predetermined course of 6 to 8 weeks of treatment. In some centers, the lack of response to an adequate course of high-dose therapy would prompt the use of an additional agent [189, 190, 192, 226–228].

Membranous glomerulonephritis in lupus patients is usually treated more conservatively than diffuse proliferative disease. Although there is no unanimity, some experience suggests that the clinical course of patients with membranous nephropathy is not as aggressive [223, 229]. Accordingly, a trial of moderate- to high-dose prednisone is attempted and if quantitative protein excretion responds to therapy, the lowest dose which maintains reasonable protein balance and which is tolerated in terms of toxicity is continued. Alternate-day steroids may be useful in this situation. High-dose alternate prednisone [230] and alternating courses of high-dose intravenous methylprednisolone and chlorambucil [231] have been useful in the management of adult idiopathic membranous glomerulonephritis.

The tempo of renal disease is an important dimension in planning a therapeutic strategy. Marked renal insufficiency as a result of slowly progressive deterioration implies sclerosis of glomeruli and nephron dropout. Such progression may not be on an immunologic basis and usually does not improve with glucocorticoid therapy. Abrupt development of renal insufficiency, however, is an indication for immediate and aggressive corticosteroid therapy. The probability for improvement is much greater and the outcome is usually evident within several weeks [232–233]. This setting may be suitable for high-dose intravenous methylprednisolone therapy.

Central Nervous System

Accurate classification of central nervous system events in patients with systemic lupus is the obvious, but not necessarily straightforward, first step in the management of central nervous system disease [234–236]. Among the many causes of neurologic signs and symptoms, infection, isolated seizure, hypertensive seizure, and hypertensive encephalopathy do not deserve treatment with corticosteroids. Severe headaches, attributed to SLE after all other causes have been excluded, may occasionally be treated with steroids [237]. Episodes of psychosis can be problematic. When attributed to SLE, they may be treated with both steroids and psychotropic agents, but the differentiation of lupus-induced and

steroid-induced psychosis remains difficult [190, 238, 239]. Among systemically and seriously ill patients, CNS events may be complex in origin and reflect an amalgam of metabolic effects, drug influences, and possibly active lupus directly affecting the brain. Corticosteroid therapy in such a situation is often guided primarily by non-CNS considerations with the hope that the neurologic manifestations will resolve as the rest of the medical picture improves.

In the presence of an abnormal cerebrospinal fluid examination and/or focal neurologic findings in a lupus patient, the diagnosis of lupus CNS involvement is made after excluding other possibilities. If the relevant pathophysiologic process is steroid-responsive, then the patient should receive corticosteroids. Although the possibility of underlying vasculitis is often raised, this process has been difficult to document pathologically. Consequently, corticosteroid therapy is approached empirically and is individualized for each patient.

Several guidelines are available [240, 241]. Aseptic meningitis caused by lupus is usually treated with high-dose prednisone, 60–80 mg/day, until clinical manifestations have resolved. Frequent seizures, unresponsive to antiepileptics and to correction of any underlying metabolic abnormalities, deserve treatment with corticosteroids starting at 60–80 mg prednisone or equivalent per day. In status epilepticus, all three management approaches—antiepileptics, metabolic corrections, and steroids—must proceed almost simultaneously. Patients with cerebrovascular accidents in the absence of another explanation such as anti-phospholipid antibodies may receive a trial of steroid therapy. Such treatment is usually directed toward a presumptive underlying vasculitis and the prevention of additional events rather than toward amelioration of current manifestations. Vasculitis is often difficult to document. Movement disorders such as chorea-athetosis and hemiballismus may respond to moderate high-dose corticosteroids with the dose being titrated to the lowest amount able to control symptoms. Other focal neurologic disorders such as transverse myelopathy are treated with glucocorticoids, but the heterogenous experience with these presentations suggests that the dose and duration of therapy need to be individualized. For the patient with coma attributed to SLE, very large doses of steroids are used. The duration of therapy is often determined either by side effects or by the patient's ultimate outcome.

Other System Involvement

Other major organ systems may be involved in systemic lupus and require corticosteroid therapy. Acute lupus pneumonitis, which presents with dyspnea, diffuse acinar infiltrates, and occasional gross hemoptysis [242], can be life-threatening and requires the use of high-dose steroid therapy until improvement is realized. Acute reversible hypoxemia, often with a normal chest X ray, usually responds quickly to high-dose steroids [243]. Other pulmonary manifestations of lesser severity (such as fleeting infiltrates) and of greater chronicity (such as diffuse interstitial lung disease [244]) are often treated with a course of steroids. The limits of clinical experience, however, preclude exact therapeutic guidelines for dose and duration in these situations. Myocardial involvement requiring corticosteroid therapy is uncommon and difficult to diagnose antemortem. Resting tachycardia unexplained by other factors and related features of a new-onset cardiomyopathy may warrant a trial of moderate- to high-dose prednisone to control signs and symptoms. Other features of cardiopulmonary involvement such as symptomatic serositis and small effusions may require low- to modest-dose steroid therapy. However, this is usually instituted only after an adequate trial of nonsteroidal anti-inflammatory drugs and observation.

Vasculitic skin lesions may present in a variety of forms, including tender indurated lesions on the finger tips, palpable purpura with a biopsy picture of leukocytoclastic angiitis, ulcerations on the lower leg, and blotchy erythematous lesions on the palms. All may respond to low- or moderate-dose steroids. High-dose therapy is reserved for vasculitis of major organ systems which is often difficult to document. Myositis associated with lupus usually responds to corticosteroids. Although muscle enzyme abnormalities may vary with muscle mass, exercise, and other factors [245], improvement in strength and enzyme abnormalities are the usual guide to adequate therapy.

Many features of systemic lupus do not require corticosteroid therapy. Arthritis is usually controlled by appropriate nonsteroidal antiinflammatory drugs. Constitutional symptoms may respond to rest and either nonsteroidal anti-inflammatory drugs or antimalarials. While low-dose steroids may play a role on occasion, adequate trials of other management approaches are always warranted.

High-Dose Intravenous Corticosteroids

Large intravenous doses of corticosteroids came into common usage for the management of renal transplant rejection [246, 247]. Such regimens were used as an essential component of immunosuppressive therapy to manage, and perhaps even to prevent, rejection. Because of the similarity of the histologic lesion on renal biopsy specimens from renal transplant rejection to the lesions seen on several renal biopsies from patients with lupus nephritis, Carthcart and colleagues

tried this mode of therapy in a group of seven lupus patients with renal disease [248]. The improvement in some immunologic parameters and in renal function in this group led to the use of short courses of high-dose intravenous corticosteroid therapy in additional patients with SLE and other glomerulonephritides [249]. Interest in "pulse" steroid has focused on attempts to provide an episodic treatment regimen for episodic disease manifestations and on attempts to minimize side effects while providing a therapeutic increment not achieved through other more conventional therapies.

Primary interest regarding high-dose intravenous pulse steroid therapy has centered on lupus nephritis. Glomerular filtration rate and serum creatinine are the most common measures of renal outcome in response to high-dose intravenous methylprednisolone (IV-MP). Rapid improvement in renal function, often within a few days of therapy, has been noted both in lupus nephritis [232, 233, 249–263] and in other glomerulonephritides [231, 264–271]. This dramatic improvement in an *ad hoc* situation, although not observed uniformly in all patients, has stimulated and sustained interest in the clinical value of high-dose intravenous corticosteroids. The lack of recognition of and discussion about such a pattern of rapid spontaneous improvement in renal function in previous reports suggests a direct therapeutic effect of the IV-MP [249]. Although some comparative data are available [233, 260, 261, 263, 271], controlled studies comparing the efficacy of IV-MP to that of conventional-dose oral corticosteroids are unavailable.

Several features may characterize renal disease patients who have an apparent response to high-dose intravenous corticosteroids when used for new onset of or a flare of renal disease. Ofuji *et al.* [256] have suggested the patients with a shorter mean duration of abnormal urinary protein excretion prior to therapy may show greater improvement. Kimberly *et al.* [232] have demonstrated the association between recent deterioration in glomerular filtration rate and the probability of subsequent improvement. The pattern of change in serum creatinine rather than the absolute level of serum creatinine seemed to be the more sensitive predictor of therapeutic responses. In addition to a single course of high-dose intravenous corticosteroids given on an *ad hoc* basis, alternative dosage regimens have been employed including monthly maintenance doses for 1 year [272] and monthly doses until immunologic improvement occurs with the final assessment at 6 months [256]. Each paradigm suggests that IV-MP may provide a therapeutic increment, but controlled trials indicate that monthly maintenance IV-MP is not superior to cyclophosphamide [273, 274]. However, a long-term follow-up study of patients with proliferative lupus glomerulonephritis enrolled in a randomized, controlled trial suggests that intravenous pulse methylprednisolone in addition to pulse cyclophosphamide may further reduce the risk of renal insufficiency over the effect of pulse cyclophosphamide alone [275].

Attempts to treat the nonrenal manifestations of lupus with high-dose intravenous corticosteroids have met with variable success [255, 276–283]. Fessel [255] described three patients with pulmonary manifestations who did well after IV-MP. Reports of several patients with thrombocytopenia suggest that platelet counts may improve in situations refractory to more conventional doses [255, 276, 278]. Other manifestations including fever, vasculitis rash, and polyserositis may also improve with IV-MP [279, 282, 283]. Experience with central nervous system involvement is limited [276, 278, 280, 281]. Case reports suggest, however, that IV-MP is often used for serious disease manifestations, refractory to conventional steroid regimens, before proceeding (if necessary) to other therapeutic regimens.

Dose Reduction and Alternate-Day Therapy

Fulminant systemic lupus requires daily corticosteroid in divided doses. Once the disease is adequately controlled, the therapeutic goals expand to include reduction of dose to minimize side effects while maintaining adequate suppression of disease. Since hypothalamic–pituitary–adrenal axis suppression and many side effects of steroid therapy are less on an alternate-day schedule of medication, many physicians attempt to establish such a regimen. However, others feel that truly active disease requires daily corticosteroids and that an alternate-day schedule is primarily an approach for tapering the total dose of steroid after disease control is achieved.

The first step in tapering is the gradual consolidation of the divided daily doses into a single morning dose to coincide with the normal peak cortisone level. Without changing the total daily dose, four divided doses are consolidated to three, then three to two, and finally two to a single morning dose. The tempo of this process must be individualized and may take from several weeks to several months. When a satisfactory remission of signs and symptoms has been established on a constant single daily dose of steroid, reduction in that dose can proceed. Starting at levels of 60 mg of prednisone per day, the dose can usually be tapered 5 mg per week. However, all patients must be monitored closely for signs of exacerbation and the schedule modified accordingly.

An alternate-day regimen can be approached by various management strategies. The rationale of alternate-day administration is that the anti-inflammatory

effects last longer than the catabolic and ACTH-suppressive effects [284, 285]. Daily steroids can be tapered to approximately one-half of the desired alternate-day dose. Double the daily amount is then given on alternate days and the amount on the "off" day is tapered. Another strategy involves doubling the daily amount for alternate-day use soon after consolidation of the divided daily doses into a single morning dose. This approach may allow a lower "off" day dose to be established more rapidly. The most important aspects of any strategy for tapering corticosteroid therapy and for establishing an alternate-day schedule, however, are to attain adequate disease control before tapering, to maintain adequate control during dose reduction, and to monitor the patient closely so that adjustments in the therapeutic plan can be made should signs of exacerbation become evident.

Supplemental Corticosteroids for Adrenal Suppression with Steroid Therapy

Adrenal suppression during glucocorticoid therapy and subsequent to treatment is highly variable. Some suggest that for acute illness and for elective surgery maintenance of the current dose of steroid is sufficient [286–288]. However, several days of supplemental steroids add essentially no harm and may avoid complications. Salem *et al*. have recommended supplementation based on the previous dose of steroid and the severity of the physiologic stress: (a) for minor stress, 25 mg of hydrocortisone or its equivalent is recommended for 1–3 days; (b) for moderate stress, 50–75 mg is recommended; and (c) for major stress, 100–150 mg is recommended [289]. During the year following long-term high-dose therapy, similar supplementation for physiologically stressful situations would seem prudent.

Dose Response Modifiers: Genetics, Disease States, and Drug Interactions

Genetic factors, various disease states, and environmental influences, including medications and dietary nutrients, can affect drug metabolism in humans. While most attention has been directed toward drug–drug interactions, genetic and dietary factors may also play a role in drug disposition.

Genetics

Genetic effects are evident in the substantial interindividual variability in the rates of drug metabolism [290] and in individual differences in the response of metabolic rates to environmental factors [291]. Differences in phenobarbital metabolism, for example, can in turn influence cortisol metabolism. It is likely that glucocorticoid "sensitivity" and "resistance" also reflect genetic factors [292]. Naturally occurring human glucocorticoid receptor variants do have different biologies although currently recognized ones do not seem to explain "resistance" [293, 294]. Nonetheless it is likely that genetic factors will underlie many of these differences. In occasional patients, glucocorticoid insensitivity leads to unchecked progression of disease. The presence of high levels of GR-β, a splice variant of the classic GR-α, has been associated with corticosteroid-insensitive asthma. Although GR-β does not bind glucocorticoid, it can inhibit the transactivating effects of activated GR [295]. Proinflammatory cytokines may stimulate the production of this receptor [296, 297]. The clinical impact of GR-β induction on the clinical response to corticosteroid therapy for SLE has not been formally investigated.

Diet and Disease States

Dietary protein, carbohydrate, and cruciferous vegetables can alter drug metabolism in humans. Oxidative drug metabolism, studied with the test drugs antipyrine and theophylline, is influenced by high protein intake. Subjects on a high protein diet show shorter plasma half-lives and higher metabolic clearance rates of these test drugs [298]. Similarly, dietary protein can alter the metabolism of the major sex steroids in humans with increased 2-hydroxylation of estradiol and decreased 5α-reduction of testosterone [299]. Glucuronide conjugation and oxidative metabolism of acetaminophen is enhanced by feeding the cruciferous vegetables, brussel sprouts and cabbage [300]. The extent to which these nutrient interactions influence glucocorticoid metabolism and excretion remain to be defined.

The potential for disease states to alter the metabolic handling of and response to glucocorticoids is recognized but often not clearly defined [301]. Hyperthyroidism accelerates deactivation of glucocorticoid activity via 11-dehydrogenation. Liver disease has a variable influence on the hepatic metabolism of drugs [302]. For glucocorticoids the multiple effects are probably off-setting and do not usually require changes in drug dosages. The pharmacokinetics of prednisone in chronic active liver disease do not appear to be significantly altered [303]. Initial studies of the impact of active systemic lupus per se on glucocorticoid metabolism are interesting [301], but the basis for the clinical observation that some patients appear to be steroid resistant and free of the common side effects of high-dose therapy remains an important question for continuing investigation.

Drug Interactions

The drug interactions involving glucocorticoids fall into several major categories: those resulting from the anticipated actions of glucocorticoids and a second drug, those resulting from the induction of hepatic microsomal enzymes, and those for which the mechanism is uncertain. Among the effects in the first grouping, glucocorticoids elevate blood glucose and may increase the dose of insulin or oral hypoglycemics required for optimal control of blood sugar. Glucocorticoids may also cause sodium retention due to a mineralocorticoid effect and increase the optimal dose of antihypertensive agents. A drug-induced increase in intraocular pressure may increase the medications needed for management of glaucoma. Since glucocorticoid administration can lead to hypokalemia from increased urinary excretion of potassium, digitalis toxicity secondary to hypokalemia can occur in a previously stable patient.

Hepatic microsomal enzymes participate in the metabolism of glucocorticoids. Drugs, such as barbiturates, phenytoin and rifampin, which induce these enzymes accelerate metabolism and may increase the steroid requirement [14–21]. Ketoconazole and mitotane can alter cortisol synthesis. However, the basis for some drug interactions is not well understood. Administration of glucocorticoids reduces the serum salicylate level achieved with a fixed dose of salicylate [182, 304]. Accordingly, reduction or withdrawal of steroids in a patient receiving salicylates often leads to an increase in the salicylate level. The neuromuscular blockade induced by pancuronium may also be altered by steroids [305]. An acute paralyzing myopathy may develop in mechanically ventilated, critically ill patients managed with neuromuscular blockade and treated with systemic corticosteroids. This syndrome has been termed critical care myopathy or acute relaxant-steroid myopathy. It appears to develop as a result of the combined effect of corticosteroids and pharmacologic denervation of muscle and usually occurs in patients treated for severe exacerbations of asthma [306, 307]. It is distinguished from chronic steroid myopathy by elevated serum muscle enzyme levels and significant myopathic changes on electromyogram. Troleandomycin, a macrolide antibiotic, reduces methylprednisolone elimination but apparently does not alter prednisolone disposition [308].

Side Effects

Systemic Corticosteroid Therapy

A wide range of adverse reactions to glucocorticoids have been observed during their clinical usage. These include some manifestations of spontaneous Cushing's syndrome, some manifestations more characteristic of iatrogenic Cushing's and hypothalamic–pituitary–adrenal suppression. While some features are shared by both spontaneous and iatrogenic Cushing's syndrome, the clinical manifestations may differ (Table 3). Part of this difference is explained by increased adrenocortical androgens and mineralocorticoids present in spontaneous Cushing's syndrome.

Several complications appear more commonly with exogenous administration of glucocorticoids. Posterior subcapsular cataracts, glaucoma, and benign intracranial hypertension (pseudo-tumor cerebri) can occur with protracted use of glucocorticoids. Aseptic necrosis of bone is often a debilitating complication of glucocorticoid therapy. Studies have failed to consistently show an association with total glucocorticoid dose [309–312] or with pulse corticosteroids [312–314]. However, several investigators have demonstrated an increased risk of aseptic necrosis in SLE patients treated with high-dose steroids [309–317]. In a prospective study of aseptic necrosis in the hip and knee, consecutive SLE patients started on high-dose glucocorticoids underwent magnetic resonance imaging (MRI) scans at the start of therapy and at 1, 3, 6, and 12 months of treatment [318]. A total of 72 patients received the equivalent of 50 mg/day of prednisolone, and after follow-up, 32 patients (44%) developed lesions on MRI consistent with aseptic necrosis. The lesions developed early after initiation of treatment (mean 3.1 months).

TABLE 3 Cushing's Syndrome: Iatrogenic vs Spontaneous

	Iatrogenic	Spontaneous
Hormones involved	Glucocorticoids	Glucocorticoids Adrenocortical androgens Mineralocorticoids
Shared clinical features	Obesity Osteoporosis Psychiatric symptoms Poor wound healing	Obesity Osteoporosis Psychiatric symptoms Poor wound healing
Distinctive clinical features	Glaucoma Osteonecrosis Posterior subcapsular cataracts Benign intracranial hypertension (pseudo-tumor cerebri) Pancreatitis Panniculitis	Hypertension Hirsutism, virilism Altered menses Impotence in men

Differential Diagnostic Problems in SLE

Several complications of exogenous glucocorticoid therapy may be difficult to distinguish from manifestations of active lupus per se. These clinical situations present a special challenge to the clinician.

Central Nervous System Manifestations

Cognitive and emotional changes ranging from irritability and difficulty in concentration on mental tasks to severe depression, confusion, or even frank psychosis may occur with glucocorticoid administration [319–321]. In addition, convulsions have been infrequently associated with such therapy. The central nervous system manifestations of systemic lupus include the same range of clinical symptomatology thus making determination of the etiology of the central nervous system signs and symptoms problematic. The concomitant expression of signs of organicity or of focal neurologic findings favors central nervous system lupus. Similarly, an abnormal cerebrospinal fluid (CSF) examination with either elevated protein or cells supports lupus as the underlying cause. However, an explicit diagnostic conclusion may not be possible and an empiric modification of drug dosage may be necessary. Steroid-induced psychiatric disturbances usually respond to reduction or discontinuation of corticosteroid and phenothiazine treatment [320, 321].

Myopathy

Glucocorticoid administration may lead to muscle wasting and weakness. On biopsy this adverse effect is evidenced by a decrease in both Type 1 and Type 2 fibers without signs of inflammation [322, 323]. In systemic lupus inflammatory muscle disease with weakness and atrophy may also occur. Although this may be associated with tenderness of the muscles and elevations of the creatinine phosphokinase, these additional manifestations are not uniformly present. Muscle biopsies may be entirely normal or show drop-out of Type 1 and Type 2 fibers as in steroid myopathy. The presence of perivascular infiltrates of lymphocytes, plasma cells, monocytes, and neutrophils help to establish a diagnosis of lupus myositis; however, the presence of these findings cannot be relied on. Vacuolar myopathy, which is characterized by swollen sarcolemmal nuclei and prominent central nuclei within a muscle fiber and often within a vacuole, also occurs both with lupus and with steroid therapy [324, 325]. The pattern of serial change in muscle enzymes may be helpful in differential diagnosis [323, 326].

Chronic steroid myopathy has been associated with increased glutamine synthetase activity in skeletal muscle [327–329]. Increased enzyme activity has been attributed to a glucocorticoid response element in the glutamine synthetase gene [330]. Endurance training leads to a reduction in enzyme activity and improvement in skeletal muscle atrophy [331]. Kimura *et al.* [332] have shown that insulin-like growth factor-I decreases glucocorticoid-induced glutamine synthetase activity in rat myocytes while others have demonstrated a similar effect in animal models and humans treated with growth hormone [333, 334]. Pending further study, these agents may be treatment options for patients with steroid myopathy unable to discontinue corticosteroids due to persistent or recurrent lupus activity.

Pancreatitis

Patients with SLE may present with gastrointestinal crises including pancreatitis [335]. The occurrence of pancreatitis in lupus patients either prior to the use of glucocorticoids or during a period without steroid therapy has demonstrated that pancreatitis may result from active lupus. Glucocorticoid therapy has also been implicated as a cause of pancreatitis [336, 337]. Among lupus patients with documented pancreatitis, however, recovery has occurred despite continued glucocorticoid therapy [335]. A series of eight patients treated with glucocorticoids for lupus-associated pancreatitis recovered clinically and biochemically without complications related to the steroids [338].

Specific Problems in Systemic Lupus Erythematosus

Because of the multisystemic and protracted nature of systemic lupus erythematosus, several side effects of glucocorticoid therapy represent specific clinical challenges.

Gastrointestinal Manifestations

The controversy over the association of glucocorticoid therapy in peptic ulcer disease continues [339]). Conn and Blitzer reviewed data from 42 randomized controlled trials and concluded that short-term steroid administration was not ulcerogenic [340]. However, steroid use for more than 30 days for a total dose of 1000 mg of prednisone equivalent appeared to be associated with a small increase in the frequency of peptic ulcer disease. Reexamination of similar data has suggested that corticosteroids do increase the risk of peptic ulcers and gastrointestinal hemorrhage [341]. This analysis, however, excluded trials in which antacids were used since they might serve to prevent or neutralize any ulcerogenic potential.

In systemic lupus the potential concomitant administration of nonsteroidal anti-inflammatory drugs raises the important possibility that NSAID and glucocorticoids together may have a significantly greater risk for

ulcers than steroids alone. Some data, gathered in patients 65 years of age or older, support this possibility [342]. A meta-analysis which reviewed studies of the association between NSAID use and serious adverse gastrointestinal events found an increased the risk among NSAID users taking corticosteroids in comparison to NSAID users not taking steroids (odds ration 1.83) [343].

Infections

Glucocorticoids enhance susceptibility to infection [344]. The basis for this clinical effect is multifactorial and includes alteration of the leukocyte traffic within the circulation, modification of leukocyte function, and decreased inflammatory responses. Patients with systemic lupus also appear to have an intrinsic propensity to infection [345, 346]. Azathioprine does not appear to enhance the rate of infection in SLE patients receiving steroids [345], but other evidence suggests that combinations of steroids and cytotoxic agents may be synergistic in enhancing the risk of infection. For example, the risk of *Pneumocystis carinii* pneumonia may be higher for patients receiving both steroids and cytotoxic agents than either agent alone [347–349]. The true risks, and the applicability of these observations to other organisms, remain to be determined.

Osteonecrosis

Osteonecrosis occurs as a complication of exogenous glucocorticoid therapy in lupus patients [350–352]. Although total steroid dose probably is related to the incidence of osteonecrosis [353], the influence of other factors modifies the potential for any direct relationship. Osteonecrosis can occur with minimal steroid use, but high-dose intravenous methylprednisolone does not seem to increase the risk of its occurrence [354, 355]. It is unclear whether osteonecrosis is part of the natural history of systemic lupus erythematosus in the absence of steroid therapy. The potential for long-term steroid therapy in SLE, however, emphasizes the urgency for use of the smallest amount of steroids consistent with the clinical picture.

Osteoporosis

Glucocorticoids cause severe bone loss both in spontaneous Cushing's syndrome and in many patients receiving exogenous therapy [356–361]. The mechanism of glucocorticoid-induced osteoporosis (GIOP) includes reduced intestinal calcium absorption [362]. Reduction of osteoblastic cell activity including collagen biosynthesis and stimulation of bone resorption by osteoclasts may also contribute. Although lupus patients do not have any known intrinsic predisposition to osteoporosis other than joint inflammation and immobility, the prolonged use of corticosteroids which characterizes most therapeutic regimens probably provides a risk for significant osteoporosis [315]. Of interest, glucocorticoid-induced osteoporosis is reversible [363], but alternate-day steroid treatment may not offer an advantage over daily therapy in avoidance of osteopenia [364].

Dietary calcium and vitamin D have received substantial attention as prophylactic and therapeutic agents for glucocorticoid-induced osteoporosis (GIOP) [365, 366]. Fluoride, sex steroids, calcitonin, and bisphosphonates have also been studied. The bisphosphonates etidronate, alendronate, and risedronate have been shown to effectively prevent and treat GIOP in randomized, controlled trials [367–372]. Both risedronate and alendronate reduce the rate of incident vertebral fractures in patients with GIOP [369, 372, 373]. Bisphosphonate efficacy relates, in part, to a potent reduction in glucocorticoid-induced osteoblast apoptosis [374]. Current recommendations from the American College of Rheumatology suggest treatment with calcium and vitamin D in all patients receiving glucocorticoids. In addition, prevention of bone loss with a bisphosphonate is recommended for all postmenopausal women and men being started on long-term glucocorticoid therapy (≥5 mg/day prednisone or equivalent). Consideration should be given to bisphosphonate treatment for premenopausal women initiating prolonged corticosteroid therapy, with appropriate counseling regarding contraception [375].

Arteriosclerosis

Patients with systemic lupus erythematosus are susceptible to premature atherosclerosis, which is now recognized as a leading cause of mortality in SLE [376]. Epidemiologic studies have shown that young women with SLE have a marked increased risk of myocardial ischemia in comparison to age-matched controls [377]. Ultrasound studies demonstrate that SLE patients with higher damage scores and prolonged use of corticosteroids are at greater risk of developing carotid plaque and intimal thickening [378]. Cumulative corticosteroid dose has been associated with an increased risk of symptomatic coronary atherosclerotic disease and exposure to high-dose prednisone may increase the risk of cerebrovascular ischemia [312, 379]. Any association between corticosteroid use and arteriosclerosis in SLE is likely to be due in part to disease severity, for which increased steroid exposure is an intermediate variable. However, corticosteroids may adversely impact CAD risk factors by elevating serum levels of atherogenic lipids including LDL, apolipoprotein B, and triglycerides, particularly in patients with SLE [380–385]. An

aggressive approach to monitoring and managing hyperlipidemia in addition to other atherosclerosis is an integral component of medical care for SLE patients.

Pulse Corticosteroid Therapy

Since one goal of intravenous methylprednisolone pulse therapy is minimization of side effects attributable to long-term high-dose oral steroids, substantial attention has been focused on side effects of IV-MP. Minor transient events including facial flushing, metallic taste, acute arthralgias, hyperglycemia, irritability, and elevation of blood pressure have been recorded. More important are case reports of anaphylactic reactions [386, 387], seizures [388], arrhythmias [389–392], and sudden death [389, 390, 393]. The occurrence of such reactions must be rare but the true incidence cannot be determined from available data. Among renal transplant patients fewer infections have been found in the IV-MP treatment groups [394–397] but nonetheless serious infection may occur [396, 398], especially, if more than 5–6 g of methylprednisolone are given per course [399, 400]. Among patients receiving oral corticosteroids, IV-MP does not appear to increase the risk of aseptic necrosis [351, 352, 401, 402] although definitive data are lacking.

Topical Corticosteroid Therapy

Topical preparations may cause side effects which are related to both the potency and the absorption of the given preparation. Local atrophy, telangiectasia, purpura, and striae can occur especially with extended use of occlusive dressings or with natural occlusion in the groin or axilla. These effects are attributed to local catabolic effects leading to degeneration of collagen. Atrophy may occur on the face even without occlusion [403]. An eruption similar to rosacea, as well as steroid acne characterized by papules and comedones, may also occur on the face with the use of potent topical preparations.

Unusual side effects of topical corticosteroids include the precipitation of latent glaucoma and production of cataracts when the preparation is applied around the eye. The clinical appearance of superficial infections caused by pathogens such as *Candida* and dermatophytes may be altered. Bacterial superinfection may be expressed as a folliculitis. Intradermal injection at the proximal nail fold may cause a dystrophic nail. Systemic side effects may result from systemic absorption of topically applied corticosteroids. This is not usually a clinically significant problem unless there are extensive eruptions treated with occlusive dressings.

Intra-articular Therapy

Significant untoward effects of intra-articular corticosteroids are uncommon. From a systemic perspective, intra-articular steroids can have a variety of effects [185, 186]: facial flushing [180], suppression endogenous hydrocortisone levels [181], change in plasma salicylate levels [182], induction of hyperglycemia [183], and precipitation of a sickle-cell crisis in susceptible individuals [184]. Local problems usually reflect the cumulative effects of recurrent injections. Altered cartilage metabolism, decreased tensile strength of tendons, and soft tissue atrophy may occur with repeated use. Uncommonly, a single injection may result in an immediate flare of symptoms probably due to a crystal-induced arthritis. Rarely arthrocentesis and injection of corticosteroids may be associated with iatrogenic infection.

Steroid Withdrawal Syndrome

Reduction and withdrawal of corticosteroid therapy can lead to an array of symptomatology ranging from frank adrenal insufficiency to pseudo-rheumatic complaints to flares of the underlying disease [404, 405]. The primary challenge to the clinician is to determine the nature and etiology of the symptoms so that an appropriate adjustment in medication can be made [404–408]. Patients usually respond to reinstitution of the lowest previous dose of steroids.

References

1. Jenkins, J. S. (1966). The metabolism of cortisol by human extrahepatic tissues. *J. Endocrinol.* **34**, 51.
2. Schalm, S. W., Summerskill, W. H. J., and Go, V. L. W. (1976). Development of radioimmunoassays for prednisone and prednisolone: Application to studies of hepatic metabolism of prednisone. *Mayo Clin. Proc.* **51**, 761.
3. Robinson, R. C. V., and Robinson, H. M., Jr. (1956). Topical treatment of dermatoses with steroids. *South. Med. J.* **49**, 260.
4. Jenkins, J. S., and Sampson, P. A. (1967). Conversion of cortisone to cortisol and prednisone to prednisolone. *Br. Med. J.* **2**, 205.
5. Powell, L. W., and Axelsen, E. (1972). Corticosteroids in liver disease: Studies on the biological conversion of prednisone to prednisolone and plasma binding protein. *Gut* **13**, 690.
6. Davis, M., Williams, R., Chakraborty, J., *et al.* (1978). Prednisone or prednisolone for the treatment of chronic active hepatitis? A comparison of plasma availability. *Br. J. Clin. Pharmacol.* **5**, 501.
7. Disanto, A. R., and Desante, K. A. (1975). Bioavailability and pharmacokinetics of prednisone in humans. *J. Pharm Sci.* **64**, 109.

8. Thiessen, J. J. (1976). Prednisolone. *J. Am. Pharm. Assoc.* **16**, 143.
9. Twabo, A. V., Hallmark, M. R., Sakmar, E., *et al.* (1977). Bioavailability of prednisolone tablets. *J. Pharmacokin. Biopharm.* **5**, 257.
10. Sugita, E. T., and Niebergal, P. J. (1975). Prednisone. *J. Am. Pharm. Assoc.* **15**, 529.
11. Angelli, A., Frajria, R., DePaoli, R., *et al.* (1978). Diurnal variation of prednisolone binding to serum. Corticosteroid-binding globulin in man. *Clin. Pharmacol. Ther.* **23**, 47.
12. Axelrod, L. (1976). Glucocorticoid therapy. *Medicine* **55**, 39.
13. Baxter, J. D. (1976). Glucocorticoid hormone action. *Pharmacol. Ther. (B)* **2**, 605.
14. Choi, Y., Thrasher, K., Werk, E. E. Jr., *et al.* (1971). Effect of diphenylhydantoin on cortisol kinetics on humans. *J. Pharmacol. Exp. Ther.* **176**, 27.
15. Haque, N., Thrasher, K., Werk, E. E. Jr., *et al.* (1972). Studies on dexamethasone metabolism in man: Effect of diphenylhydantoin. *J. Clin. Endocrinol. Metab.* **34**, 44.
16. Stjernholm, M. R., and Katz, F. H. (1975). Effects of diphenylhydantoin, phenobarbital, and diazepam on the metabolism of methylprednisolone and its sodium succinate. *J. Clin. Endocrinol. Metab.* **41**, 877.
17. Petereit, L. D., and Meikle, A. W. (1977). Effectiveness of prednisolone during phenytoin therapy. *Clin. Pharmacol. Ther.* **22**, 912.
18. Brooks, S. M., Werk, E. E., Ackerman, S. J., *et al.* (1972). Adverse effects of phenobarbital on corticosteroid metabolism in patients with bronchial asthma. *N. Engl. J. Med.* **286**, 1125.
19. Brooks, P. M., Buchanan, W. W., Grove, M., and Downie, W. W. (1976). Effects of enzyme induction on metabolism of prednisolone. Clinical and laboratory study. *Ann. Rheum. Dis.* **35**, 339.
20. Edwards, O. M., Courtenay-Evans, R. J., Galley, J. M., *et al.* (1974). Changes in cortisol metabolism during rifampin therapy. *Lancet* **2**, 549.
21. Buffington, G. A., Dominguez, J. H., Piering, W. F., *et al.* (1976). Interaction of rifampin and glucocorticoids. Adverse effects on renal allograft function. *J. Am. Med. Assoc.* **236**, 1958.
22. Harter, J. G. (1966). Corticosteroids: Their physiologic use in allergic diseases. *N. Y. State J. Med.* **66**, 827.
23. Meikle, A. W., and Tyler, F. H. (1977). Potency and duration of action of glucocorticoids. Effects of hydrocortisone, prednisone and dexamethasone on human pituitary–adrenal function. *Am. J. Med.* **63**, 200.
24. Pickup, M. E., Lowe, J. R., Leatham, P. A., *et al.* (1977). Dose-dependent pharmacokinetics of prednisolone. *Eur. J. Clin. Pharmacol.* **12**, 213.
25. Walton, J., Watson, B. S., and Ney, R. L. (1970). Alternate day versus shorter interval steroid administration. *Arch. Intern. Med.* **126**, 601.
26. Ellul-Micallef, R., Rorthwick, R. C., and McHardy, J. G. R. (1974). The time course of response to prednisolone in chronic bronchial asthma. *Clin. Sci.* **47**, 105.
27. Grant, S. D., Forsham, P. H. D., and DiRaimondo, V. C. (1965). Suppression of 17-hydroxycorticosteroids in plasma and urine by single and divided doses of triamcinolone. *N. Engl. J. Med.* **273**, 1115.
28. Evans, R. M. (1988). The steroid and thyroid hormone receptor superfamily. *Science* **240**, 889.
29. Beato, M. (1989). Gene regulation by steroid hormones. *Cell* **56**, 335.
30. Paris, J. (1961). Pituitary adrenal suppression after protracted administration of adrenal cortical hormones. *Mayo Clin. Proc.* **36**, 305.
31. Danowski, T. S., Bonessi, J. V., Sabeh, G., *et al.* (1964). Probabilities of pituitary adrenal responsiveness after steroid therapy. *Ann. Intern. Med.* **61**, 11.
32. Nichols, T., Nugent, C. A., and Tyler, F. H. (1965). Diurnal variation in suppression of adrenal function by glucocorticoids. *J. Clin. Endocrinol. Metab.* **25**, 343.
33. Chamberlain, M. A., and Keenan, J. (1976). The effect of low doses of prednisolone compared with placebo on function and on the hypothalamic pituitary axis in patients with rheumatoid arthritis. *Rheumatol. Rehab.* **15**, 17.
34. Christy, N. P., Wallace, E. Z., and Jailer, J. W. (1956). Comparative effects of prednisone and of cortisone in suppression the response of the adrenal cortex to exogenous adrenocorticotropin. *J. Clin. Endocrinol. Metab.* **16**, 1059.
35. Westerhof, L., Vanditmars, M. J., DerKinderen, P. J., *et al.* (1970). Recovery of adrenocorticol function during long-term treatment with corticosteroids. *Br. Med. J.* **4**, 534.
36. Westerhof, L., Vanditmars, M. J., DerKinderen, P. J., *et al.* (1972). Recovery of adrenocortical function during long-term treatment with corticosteroids. *Br. Med. J.* **2**, 195.
37. Streck, W. F., and Lockwood, D. H. (1979). Pituitary adrenal recovery following short-term suppression with corticosteroids. *Am. J. Med.* **66**, 910.
38. Christy, N. P. (1992). Pituitary–adrenal function during corticosteroid therapy: Learning to live with uncertainty. *N. Engl. J. Med.* **326**, 266.
39. Lamberts, S. W. J., Bruining, H. A., and de Jong, F. H. (1997). Corticosteroid therapy in severe illness. *N. Engl. J. Med.* **337**, 1285.
40. Schalm, S. W., Summerskill, W. H. J., and Go, V. L. W. (1977). Prednisone for chronic active liver disease: Pharmacokinetics including conversion to prednisolone. *Gastroenterology* **72**, 910.
41. Lewis, G. P., Jusko, W. J., Burke, C. W., and Graves, L. (1971). Boston Collaborative Drug Surveillance Program: Prednisone side effects and serum protein levels, a collaborative study. *Lancet* **2**, 778.
42. Ohrlander, S., Gennser, G., Nielsson, K. O., and Eneroth, P. (1977). ACTH test to neonate after administration of corticosteroids during gestation. *Obstet. Gynecol.* **49**, 691.
43. Grajwer, L. A., Lillien, L. D., and Pildes, R. S. (1977). Neonatal subclinical adrenal insufficiency: Result of maternal steroid therapy. *J. Am. Med. Assoc.* **238**, 1279.

44. Anderson, G. G., Rotchell, Y., and Kaiser, D. G. (1981). Placental transfer of methylprednisolone following maternal intravenous administration. *Am. J. Obstet. Gynecol.* **140**, 699.
45. Schatz, M., Patterson, R., Zeitz, S., *et al.* (1975). Corticosteroid therapy for the pregnant asthmatic patient. *J. Am. Med. Assoc.* **233**, 804.
46. David, D. S., Grieco, M. H., and Cushman, P., Jr. (1970). Adrenal glucocorticoids after 20 years: A review of their clinically relevant consequences. *J. Chron. Dis.* **22**, 637.
47. Fine, L. J., Barnett, E. V., Danovitch, C. M., *et al.* (1981). Systemic lupus in pregnancy. *Ann. Intern. Med.* **94**, 667.
48. Cowchock, F. S., Reece, E. A., Balaban, D., *et al.* (1992). Repeated fetal losses associated with antiphospholipid antibodies: A collaborative randomized trial comparing prednisone with low-dose heparin treatment. *Am. J. Obstet. Gynecol.* **166**, 1318.
49. Silver, R. K., MacGregor, S. N., Sholl, J. S., *et al.* (1993). Comparative trial of prednisone plus aspirin versus aspirin alone in the treatment of anticardiolipin antibody-positive obstetric patients. *Am. J. Obstet. Gynecol.* **169**, 1411.
50. Laskin, C. A., Bombardier, C., Hannah, M. E., *et al.* (1997). Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. *N. Engl. J. Med.* **337**, 148.
51. McKenzie, S. A., Selley, J. A., Agnew, J. E. (1975). Secretion of prednisolone into breast milk. *Arch. Dis. Child.* **50**, 894.
52. Black, L. J., Sato, M., and Rowley, E. R. (1994). Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats. *J. Clin. Invest.* **93**, 63.
53. Delmas, P. D., Bjarnason, N. H., Mitlak, B. H., Ravoux, A.-C., Shah, A. S., Huster, W. J., Braper, M., and Christiansen, C. (1997). Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women. *N. Engl. J. Med.* **337**, 1641.
54. Hahn, B. H., Pletscher, L. S., and Muniain, M. (1981). Immunosuppressive effects of deflazacort—a new glucocorticoid with bone-sparing and carbohydrate-sparing properties: Comparison with prednisone. *J. Rheumatol.* **8**, 783.
55. Caniggia, A., Nuti, R., Lore, F., *et al.* (1981). Pathophysiology of the adverse effects of glucoactive corticosteroids on calcium metabolism in man. *J. Steroid Biochem.* **15**, 153.
56. Gennari, C., Imbimbo, B., Montagnani, M., *et al.* (1984). *Calcif. Tissue Int.* **36**, 245.
57. Imbimbo, B., Tuzi, T., Porzio, F., *et al.* (1984). *Adv. Exp. Med. Biol.* **171**, 241.
58. Gray, R. E. S., Doherty, S. M., Galloway, J., *et al.* (1991). A double-blind study of deflazacort and prednisone in patients with chronic inflammatory disorders. *Arthritis Rheum.* **34**, 287.
59. Elli, A., Rivolta, R., Palazzi, P., *et al.* (1990). Deflazacort versus 6-methylprednisolone in renal transplantation: Immunosuppressive activity and side effects. *Transplant. Proc.* **22**, 1689.
60. Dingle, J. T. (1978). Novel treatment for joint inflammation. *Nature* **271**, 372.
61. Phillips, N. C., Page-Thomas, D. P., Knight, C. G., *et al.* (1979). Liposome-incorporated corticosteroids. II. Therapeutic activity in experimental arthritis. *Ann. Rheum. Dis.* **38**, 553.
62. DeSilva, M., Hazleman, B., Page-Thomas, D. P., *et al.* (1979). Liposomes in arthritis. A new approach. *Lancet* **1**, 1320.
63. Mizushima, Y., Hamano, T., and Yokoyama, K. (1982). Tissue distribution and anti-inflammatory activity of corticosteroids incorporated in lipid emulsion. *Ann. Rheum. Dis.* **41**, 263.
64. Vayssiere, B. M., Dupont, S., Choquart, A., *et al.* (1997). Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity in vivo. *Mol. Endocrinol.* **11**, 1245.
65. Boumpas, D. T., Chrousos, G. P., Wilder, R. L., Cupps, T. R., and Balow, J. E. (1993). Glucocorticoid therapy for immune-mediated diseases: Basic and clinical correlates. *Ann. Intern. Med.* **119**, 1198–1208.
66. Chan, L., and O'Malley, B. W. (1978). Steroid hormone action: Recent advances. *Ann. Intern. Med.* **89**, 964.
67. Parrillo, J. E., and Fauci, A. S. (1979). Mechanisms of glucocorticoid action on immune processes. *Annu. Rev. Pharmacol. Toxicol.* **19**, 179.
68. Yamamoto, K. R. (1985). Steroid receptor regulated transcription of specific genes and gene networks. *Annu. Rev. Genet.* **19**, 209.
69. Guyre, P. M., Girard, M. T., Morganelli, P. M., *et al.* (1988). Glucocorticoid effects on the production and actions of immune cytokines. *J. Steroid Biochem.* **30**, 89.
70. Chrousos, G. P. (1995). The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. *N. Engl. J. Med.* **332**, 1351–1362.
71. Burnstein, K. L., and Cidlowski, J. A. (1989). Regulation of gene expression by glucocorticoids. *Annu. Rev. Physiol.* **51**, 683.
72. Ray, A., and Prefontaine, K. E. (1994). Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 752.
73. De Bosscher, K., Schmitz, M. L., Vanden Berghe, W, *et al.* (1997). Glucocorticoid-mediated repression of nuclear factor-κB-dependent transcription involves direct interference with transactivation. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 13504.
74. De Bosscher, K., Vanden Berghe, W., Vermeulen, L., *et al.* (2000). Glucocorticoids repress NF-κB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3919.
75. Auphan, N., DiDonato, J. A., Rosette, C., *et al.* (1995). Immunosuppression by glucocorticoids: Inhibition of NF-κB activity through induction of Iκ B synthesis. *Science* **270**, 286.

76. Scheinman, R. I., Cogswell, P. C., Lofquist, A. K., and Baldwin, A. S., Jr. (1995). Role of transcriptional activation of IκB-α in mediation of immunosuppression by glucocorticoids. *Science* **270**, 283.
77. Ayroldi, E., Migliorati, G., Bruscoli, S., *et al.* (2001). Modulation of T-cell activation by the glucocorticoid-induced leucine zipper factor via inhibition of nuclear factor κB. *Blood* **98**, 743.
78. Sheppard, K. A., Phelps, K. M., Williams, A. J., *et al.* (1998). Nuclear integration of glucocorticoid receptor and nuclear factor-κB signaling by CREB-binding protein and steroid receptor coactivator-1. *J. Biol. Chem.* **273**, 29291.
79. McKay, L. I., and Cidlowski, J. A. (2000). CBP (CREB binding protein) integrates NF-κB (nuclear factor-κB) and glucocorticoid receptor physical interactions and antagonism. *Mol. Endocrinol.* **14**, 1222.
80. Bianchi, M., Meng, C., and Ivashkiv, L. B. (2000). Inhibition of IL-2-induced Jak-STAT signaling by glucocorticoids. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 9573.
81. Neifeld, J. P., Lippman, M. E., and Tormey, D. C. (1977). Steroid hormone receptors in normal human lymphocytes: Induction of glucocorticoid receptor activity by phytohemagglutinin stimulation. *J. Biol. Chem.* **252**, 2972.
82. Lippman, M., and Barr, R. (1977). Glucocorticoid receptor in purified subpopulations of human peripheral blood lymphocytes. *J. Immunol.* **118**, 1977.
83. Rosenberg, J. C., and Lysz, K. (1980). Suppression of the immune response by steroids comparative efficacy of hydrocortisone, methylprednisolone and dexamethasone. *Transplantation* **29**, 425.
84. Hofmann, T. G., Hehner, S. P., Bacher, S., *et al.* (1998). Various glucocorticoids differ in their ability to induce gene expression, apoptosis and to repress NF-κB-dependent transcription. *FEBS Lett.* **441**, 441.
85. Claman, H. N. (1975). How corticosteroids work. *J. Allergy Clin. Immunol.* **55**, 145.
86. Vichyanond, P., Irvin, C. G., Larsen, G. L., *et al.* (1989). Penetration of corticosteroids into the lung: Evidence for a difference between methylprednisolone and prednisolone. *J. Allergy Clin. Immunol.* **84**, 867.
87. Haynes, R. C., Jr. (1990). Adrenocorticotropic hormones, adrenocorticosteroids and their synthetic analogues; inhibitors of the synthesis and actions of adrenocorticol hormones. In "The Pharmacologic Basis of Therapeutics" (A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor Eds.), 8th ed., p. 1431. Pergamon, New York.
88. Altura, B. M., and Altura, T. (1974). Peripheral vascular actions of glucocorticoids and their relationship to protection in circulatory shock. *J. Pharmacol. Exp. Ther.* **190**, 300.
89. Bengtsson, B. (1981). Methylprednisolone and adrenergic mechanisms in vascular smooth muscle. *Acta Physiol. Scand.* **112**, 129.
90. Horwitz, K. B., and Horwitz, L. D. (1982). Canine vascular tissues are targets for androgens, estrogens, progestins and glucocorticoids. *J. Clin. Invest.* **69**, 750.
91. Kornel, L., Kanamarlapudi, N., Travers, T., *et al.* (1982). Studies on high affinity binding of mineralo- and glucocorticoids in rabbit aorta cytosol. *J. Steroid Biochem.* **16**, 245.
92. Mendelsohn, F. A. O., Lloyd, C. J., Kachel, C., *et al.* (1982). Induction by glucocorticoids of angiotensin converting enzyme production from bovine endothelial cells in culture and rat lung in vivo. *J. Clin. Invest.* **70**, 684.
93. Re, F., Muzio, M., De Rossi, M., *et al.* (1994). The type II "receptor" as a decoy target for interleukin 1 in polymorphonuclear leukocytes: Characterization of induction by dexamethasone and ligand binding properties of the released decoy receptor. *J. Exp. Med.* **179**, 739.
94. Galon, J., Franchimont, D., Hiroi, N., *et al.* (2002). Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. *FASEB J.* **16**, 61.
95. Bishop, C. R., Athens, J. W., Boggs, D. R., *et al.* (1968). Leukokinetic studies. XIII. A non-steady state kinetic evaluation of the mechanism of cortisone-induced granulocytosis. *J. Clin. Invest.* **47**, 249.
96. Athens, J. W., Haab, O. P., Raab, S. O., *et al.* (1961). Leukokinetic studies. IV. The total blood, circulating and marginal granulocyte pools and the granulocyte turnover rate in normal subjects. *J. Clin. Invest.* **40**, 989.
97. Dale, D. C., Fauci, A. S., Guerry, G. I. V., *et al.* (1975). Comparison of agents producing neutrophilic leukocytosis in man: Hydrocortisone, prednisone, endotoxin, and etiocholanolone. *J. Clin. Invest.* **56**, 808.
98. Wiener, S. L., Wiener, R., Urivetzky, M., *et al.* (1975). The mechanism of action of a single dose of methylprednisolone on acute inflammation in vivo. *J. Clin. Invest.* **56**, 679.
99. Shea, C., and Morse, E. D. (1978). Inhibition of human neutrophil chemotaxis by corticosteroids. *Ann. Clin. Lab. Sci.* **8**, 30.
100. MacGregor, R. R., Spanguolo, P. J., and Lentnek, A. L. (1975). Inhibition of granulocyte adherence by ethanol, prednisone and aspirin measured with an assay system. *N. Engl. J. Med.* **291**, 642.
101. MacGregor, R. R. (1976). The effect of anti-inflammatory agents and inflammation on granulocyte adherence: Evidence for regulation by plasma factors. *Am. J. Med.* **61**, 597.
102. MacGregor, R. R. (1977). Granulocyte adherence changes induced by hemodialysis, endotoxin, epinephrine and glucocorticoids. *Ann. Intern. Med.* **86**, 35.
103. Clark, R. A. F., Gallin, J. I., and Fauci, A. S. (1979). Effects of in vivo prednisone on *in vitro* eosinophil and neutrophil adherence and chemotaxis. *Blood* **53**, 633.
104. Kishimoto, T. K., Larson, R. S., Corbi, A. L., *et al.* (1989). The leukocyte integrins. *Adv. Immunol.* **46**, 149.
105. Stoolman, L. M. (1989). Adhesion molecules controlling lymphocyte migration. *Cell* **56**, 907.
106. Springer, T. A. (1990). Adhesion receptors of the immune system. *Nature* **346**, 425.
107. Bast, R. C., Jr., Reinherz, E. L., Maver, C., *et al.* (1983). Contrasting effects of cyclophosphamide and prednisolone on the phenotype of human peripheral blood leukocytes. *Clin. Immunol. Immunopathol.* **28**, 101.

108. Ten Berge, R. J. M., Sauerwein, H. P., Yong, S. L., *et al.* (1984). Administration of prednisolone in vivo affects the ratio of OKT4/OKT8 and the LDH isoenzyme pattern of human T-lymphocytes. *Clin. Immunol. Immunopathol.* **30**, 91.
109. Weston, W. L., Mandel, M. J., Yeckley, J. A., *et al.* (1973). Mechanism of cortisol inhibition of adoptive transfer of tuberculin sensitivity. *J. Lab. Clin. Med.* **82**, 366.
110. Rinehart, J. J., Balcerzak, S. P., Sagone, A. L., *et al.* (1974). Effects of corticosteroids on human monocyte function. *J. Clin. Invest.* **54**, 1337.
111. Rinehart, J. J., Sagone, A. L., Balcerzak, S. P., *et al.* (1975). Effects of corticosteroid therapy on human monocyte function. *N. Engl. J. Med.* **292**, 236.
112. Jones, C. J. P., Morris, K. J., and Jayson, M. T. V. (1983). Prednisolone inhibits phagocytosis by polymorphonuclear leukocytes via steroid receptor-mediated events. *Ann. Rheum. Dis.* **42**, 56.
113. Atkinson, J. P., and Frank, M. M. (1974). Complement-independent clearance of IgG-sensitized erythrocytes: Inhibition by cortisone. *Blood* **44**, 629.
114. Handin, R. I., and Stossel, T. P. (1978). Effect of corticosteroid therapy on the phagocytosis of antibody-coated platelets by human leukocytes. *Blood* **51**, 771.
115. Crabtree, G. R., Munck, A., and Smith, K. A. (1979). Glucocorticoids inhibit expression of Fc receptors on the human granulocytic cell line HL6O. *Nature* **279**, 338.
116. Crabtree, G. R., Gillis, S., Smith, K. A., *et al.* (1979). Glucocorticoids and immune responses. *Arthritis Rheum.* **22**, 1246.
117. Crabtree, G. R., Gillis, S., Smith, K. A., *et al.* (1980). Mechanisms of glucocorticoid-induced immunosuppression: Inhibitory effects on expression of Fc receptors and production of T-cell growth factor. *J. Steroid Biochem.* **12**, 445.
118. Kurlander, R. J. (1981). The effects of corticosteroids on IgG Fc receptor and complement receptor-mediated interaction of monocytes with red cells. *Clin. Immunol. Immunopathol.* **20**, 325.
119. Schreiber, A. D., Parsons, J., McDermott, V., *et al.* (1975). Effect of corticosteroids of human monocyte IgG and complement receptors. *J. Clin. Invest.* **56**, 1189.
120. Tolone, G., Bonascera, L., and Sajeva, R. (1979). Effects of hydrocortisone on binding of IgG or C3b coded erythrocytes to human monocytes or polymorphonuclear leukocytes. *J. Pharm. Pharmacol.* **31**, 563.
121. Guyre, P. M., Bodwell, J. E., and Munck, A. (1981). Glucocorticoid actions on the immune system: Inhibition of production of an Fc receptor augmenting factor. *J. Steroid Biochem.* **15**, 35.
122. Girard, M. T., Hjaltadottir, S., Fejes-Toth, A. N., *et al.* (1987). Glucocorticoids enhance the gamma interferon augmentation of human monocyte IgG Fc receptor expression. *J. Immunol.* **138**, 3235.
123. Ralph, P., Ito, M., Broxmeyer, H. E., *et al.* (1978). Corticosteroids block newly induced but not constitutive functions of macrophage cell lines: Myeloid colony stimulating activity production, latex phagocytosis, and antibody-dependent lysis of RBC and tumor targets. *J. Immunol.* **121**, 300.
124. Norton, J. M., and Munck, A. (1980). *In vitro* actions of glucocorticoids on murine macrophages: Effects on glucose transport and metabolism, growth in culture, and protein synthesis. *J. Immunol.* **125**, 259.
125. Lewis, G. P., and Piper, P. J. (1975). Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids. *Nature* **254**, 308.
126. Tashjian, A. H., Jr., Voelkel, E. F., McDonough, J., *et al.* (1975). Hydrocortisone inhibits prostaglandin production by mouse fibrosarcoma cells. *Nature* **258**, 739.
127. Hong, S. L., and Levine, L. (1976). Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1730.
128. Tam, S., Hong, S. L., and Levine, L. (1977). Relationships among the steroids of anti-inflammatory properties in inhibition of prostaglandin production and arachidonic acid release by transformed mouse fibroblasts. *J. Pharmacol. Exp. Ther.* **203**, 162.
129. Flower, R. J., and Blackwell, G. U. (1979). Anti-inflammatory steroids induce biosynthesis of a phospholipase A2 inhibitor which prevents prostaglandin generation. *Nature* **278**, 456.
130. Hirata, F., Schiffman, E., Venkatasubramanian, K., *et al.* (1980). A phospholipase A2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2533.
131. Blackwell, G. J., Carnuccio, R., DiRosa, M., *et al.* (1980). Macrocortin: A polypeptide causing anti-phospholipase effect of glucocorticoids. *Nature* **287**, 147.
132. Needleman, P., Turk, J., Jakschik, B. A., *et al.* (1985). Arachidonic acid metabolism. *Annu. Rev. Biochem.* **55**, 69.
133. Yu, D. T. Y., Clements, P. J., Paulus, H. E., *et al.* (1974). Human lymphocyte subpopulations: Effect of corticosteroids. *J. Clin. Invest.* **53**, 565.
134. Clarke, J. R., Gagnon, R. F., Gotch, F. M., *et al.* (1977). The effect of prednisolone on leukocyte function in man: A double-blind controlled study. *Clin. Exp. Immunol.* **28**, 292.
135. Cooper, D. A., Petts, V., Luckhurst, E., *et al.* (1977). The effect of acute and prolonged administration of prednisolone and ACTH on lymphocyte subpopulations. *Clin. Exp. Immunol.* **28**, 467.
136. Craddock, C. J. (1978). Corticosteroid induced lymphopenia, immunosuppression, and body defense. *Ann. Intern. Med.* **88**, 564.
137. Slade, J. D., and Hepburn, B. (1983). Prednisone induced alterations of circulating human lymphocyte subsets. *J. Lab. Clin. Med.* **101**, 479.
138. Bloemena, E., Weinreich, S., and Schellenkens, P. T. A. (1990). The influence of prednisolone on the recirculation of peripheral blood lymphocytes in vivo. *Clin. Exp. Immunol.* **80**, 460.
139. Raziuddin, S., Nur, M. A., and Al-Wabel, A. A. (1990). Increased circulating HLA-DR$^+$CD4$^+$ T cells in systemic

lupus erythematosus: Alterations associated with prednisolone therapy. *Scand. J. Immunol.* **31**, 139.
140. Fauci, A. S., and Dale, D. C. (1975). The effect of hydrocortisone on the kinetics of normal human lymphocytes. *Blood* **46**, 235.
141. Butler, W. T., and Rossen, R. D. (1973). Effects of corticosteroids on immunity in man. I. Decreased serum Ig concentration caused by three or five days of high dose methylprednisolone. *J. Clin. Invest.* **52**, 2629.
142. Markham, R. B., Stashak, P. W., Prescott, B., *et al.* (1978). Selective sensitivity to hydrocortisone of regulatory functions that determine the magnitude of the antibody response to type III pneumococcal polysaccharide. *J. Immunol.* **121**, 829.
143. Settipane, G. A., Pudupakkam, R. K., and McGowan, J. H. (1978). Corticosteroid effect on immunoglobulins. *J. Allergy Clin. Immunol.* **62**, 162.
144. Grayson, J., Dooley, N. J., Kosski, I. R., *et al.* (1981). Immunoglobulin production induced *in vitro* by glucocorticosteroid hormones. *J. Clin. Invest.* **68**, 1539.
145. Orson, F. M., Grayson, J., Pike, S., *et al.* (1983). T-cell-replacing factor for glucocorticosteroid-induced immunoglobulin production. A unique steroid-dependent cytokine. *J. Exp. Med.* **158**, 1473.
146. Gillis, S., Crabtree, G. R., and Smith, K. A. (1979). Glucocorticosteroid-induced inhibition of T-cell growth factor production. I. The effect on mitogen-induced lymphocyte proliferation. *J. Immunol.* **123**, 1624.
147. Culpepper, J., and Lee, F. (1987). Glucocorticoid regulation of lymphokine production by murine T lymphocytes. In "Lymphokines" (D. R. Webb and D. Goeddel, Eds.), Vol. 13, p. 275. Academic Press, New York.
148. Gillis, S., Crabtree, G. R., and Smith, K. A. (1979). Glucocorticosteroid-induced inhibition of T-cell growth factor production. II. The effect on the *in vitro* generation of cytolytic T-cells. *J. Immunol.* **123**, 1632.
149. Snyder, D. S., and Unanue, E. R. (1982). Corticosteroids inhibit macrophage Ia expression and interleukin 1 production. *J. Immunol.* **129**, 1803.
150. Gessani, S., McCandless, S., and Baglioni, C. (1988). The glucocorticoid dexamethasone inhibits synthesis of interferon by decreasing the level of its mRNA. *J. Biol. Chem.* **263**, 7454.
151. Lee, S. W., Tsou, A. P., Chan, H., Thomas, J., Petrie, K., Eugui, E. M., and Allison, A. C. (1988). Glucocorticoids selectively inhibit the transcription of the interleukin 1B gene and decrease the stability of interleukin 1B mRNA. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1204.
152. Kull, F. C., Jr. (1988). Reduction in tumor necrosis factor receptor affinity and cytotoxicity by glucocorticoids. *Biochem. Biophys. Res. Commun.* **153**, 402.
153. Akahoshi, T., Oppenheim, J. J., and Matsushima, K. (1988). Induction of high-affinity interleukin 1 receptor on human peripheral blood lymphocytes by glucocorticoid hormones. *J. Exp. Med.* **167**, 924.
154. Webel, M. L., and Aritts, R. E., Jr. (1977). The effects of corticosteroid concentrations on lymphocyte blastogenesis. *Cell. Immunol.* **32**, 287.
155. Mendelsohn, J., Multer, M. M., and Bernheim, J. L. (1977). Inhibition of human lymphocyte stimulation by steroid hormones: Cytokinetic mechanisms. *Clin. Exp. Immunol.* **27**, 127.
156. Fauci, A. S. (1976). Mechanisms of corticosteroid action on lymphocyte subpopulations. II. Differential aspects of in vivo hydrocortisone, prednisone and dexamethasone on *in vitro* expression of lymphocytc function. *Clin. Exp. Immunol.* **24**, 54.
157. Katz, P., Zaytoun, A. M., and Lee, J. H., Jr. (1984). The effects of in vivo hydrocortisone on lymphocyte-mediated cytotoxicity. *Arthritis Rheum.* **27**, 72.
158. Balow, J. E., and Rosenthal, A. S. (1973). Glucocorticoid suppression of macrophage migration inhibitory factor. *J. Exp. Med.* **137**, 1031.
159. Weston, W. L., Mandel, M. J., Yeckley, J. A., *et al.* (1973). Mechanism of cortisol inhibition of adoptive transfer of tuberculin sensitivity. *J. Lab. Clin. Med.* **82**, 366.
160. Moser, M., De Smedt, T., Sornasse, T., *et al.* (1995). Glucocorticoids down-regulate dendritic cell function *in vitro* and in vivo. *Eur. J. Immunol.* **25**, 2818.
161. Vieira, P. L., Kalinski, P., Wierenga, E. A., *et al.* (1998). Glucocorticoids inhibit bioactive IL-12p70 production by *in vitro*-generated human dendritic cells without affecting their T cell stimulatory potential. *J. Immunol.* **161**, 5245.
162. Rea, D., van Kooten, C., van Meijgaarden, K. E., *et al.* (2000). Glucocorticoids transform CD40-triggering of dendritic cells into an alternative activation pathway resulting in antigen-presenting cells that secrete IL-10. *Blood* **95**, 3162.
163. de Jong, E. C., Vieira, P. L., Kalinski, P., and Kapsenberg, M. L. (1999). Corticosteroids inhibit the production of inflammatory mediators in immature monocyte-derived DC and induce the development of tolerogenic DC3. *J. Leuk. Biol.* **66**, 201.
164. Woltman, A. M., Massacrier, C., de Fijter, J. W., *et al.* (2002). Corticosteroids prevent generation of $CD34^+$-derived dermal dendritic cells but do not inhibit Langerhans cell development. *J. Immunol.* **168**, 6181.
165. Healy, D. L., Hodgen, G. D., Schulte, H. M., *et al.* (1983). The thymus–adrenal connection: Thymosin has corticotropin releasing activity in primates. *Science* **222**, 1353.
166. Sivas, A., Uysal, M., and Oz, H. (1982). The hyperglycemic effect of thymosin F5 a thymic hormone. *Horm. Metab. Res.* **14**, 330.
167. Mattison, J. C., Schreiber, R. D., LaForest, A. C., *et al.* (1983). Suppression of ACTH-induced steroidogenesis by supernatants from LPS-treated peritoneal exudate macrophages. *J. Immunol.* **130**, 2757.
168. Hirschberg, T., Randazzo, B., and Hirschberg, H. (1980). Effects of methylprednisolone on the in vivo induction and function of suppressor cells in man. *Scand. J. Immunol.* **12**, 33.
169. Perez, H. D., Kimberly, R. P., Kaplan, H. B., *et al.* (1981). Effect of high-dose methylprednisolone infusion on polymorphonuclear leukocyte function in patients with systemic lupus erythematosus. *Arthritis Rheum.* **24**, 641.

170. Baylis, E. M., Williams, I. A., English, J., *et al.* (1982). High dose intravenous methylprednislone "pulse" therapy in patients with rheumatoid disease. Plasma methylprednisolone levels and adrenal function. *Eur. J. Clin. Pharmacol.* **21**, 385.
171. Webel, M. L., Ritz, R. E., Jr., Toswell, H. F., *et al.* (1984). Cellular immunity after intravenous administration of methylprednisolone. *J. Lab. Clin. Med.* **83**, 383.
172. Pedersen, B. K., Beyer, J. M., Rasmussen, A., *et al.* (1984). Methylprednisolone pulse therapy induced fall in natural killer cell activity in rheumatoid arthritis. *Acta Pathol. Microbiol. Immunol. Scand. (C)* **92**, 319.
173. Fan, P. T., Yu, D. T. Y., Clements, P. J., *et al.* (1978). Effects of corticosteroids on the human immune response: Comparison of one and three daily 1 gm intravenous pulses of methylprednisolone. *J. Lab. Clin. Med.* **91**, 625.
174. Fan, P. T., Yu, D. T. Y., Targoff, C., *et al.* (1978). Effects of corticosteroids on the human immune response: Suppression of mitogen induced lymphocyte proliferation by pulse methylprednisolone. *Transplantation* **26**, 266.
175. Trotter, J. L., and Garvey, W. F. (1980). Prolonged effects of large dose methylprednisolone infusion on multiple sclerosis. *Neurology* **30**, 702.
176. Hammerschmidt, U. E., White, J. G., Craddock, P. R., *et al.* (1979). Corticosteroids inhibit complement-induced granulocyte aggregation. *J. Clin. Invest.* **63**, 789.
177. The Medical Letter (1988). Generic topical corticosteroids. *Med. Lett. Drugs Ther.* **30**, 49.
178. Fitzgerald, R. H. (1976). Intrasynovial injection of steroids. Uses and abuses. *Mayo Clin. Proc.* **51**, 655.
179. Gray, R. G., Tenenbaum, J., and Gottlieb, N. L. (1981). Local corticosteroid injection treatment in rheumatic disorders. *Semin. Arthritis Rheum.* **10**, 231.
180. Pattrick, M., and Doherty, M. (1987). Facial flushing after intra-articular injection of steroid. *Br. Med. J.* **295**, 1380.
181. Derendorf, H., Mollmann, H., Gruner, A., *et al.* (1986). Pharmacokinetics and pharmacodynamics of glucocorticoid suspensions after intra-articular administration. *Clin. Pharmacol. Ther.* **39**, 313.
182. Edelman, J., Potter, J. M., and Hackett, L. P. (1986). The effect of intra-articular steroids on plasma salicylate concentrations. *Br. J. Clin. Pharmacol.* **21**, 301.
183. Black, D. M., and Filak, A. T. (1989). Hyperglycemia with non-insulin-dependent diabetes following intra-articular steroid injection. *J. Family Pract.* **28**, 462.
184. Gladman, D. D., and Bombardier, C. (1987). Sickle cell crisis following intra-articular steroid therapy for rheumatoid arthritis. *Arthritis Rheum.* **30**, 1065.
185. Armstrong, R. D., English, J., Gibson, T., *et al.* (1981). Serum methylprednisolone levels following intraarticular injection of methylprednisolone acetate. *Ann. Rheum. Dis.* **40**, 571.
186. Koehler, B. E., Urowitz, M. B., and Killinger, D. W. (1974). The systemic effects of intra-articular corticosteroid. *J. Rheumatol.* **1**, 117.
187. Ropes, M. W. (1976). Treatment. In "Systemic Lupus Erythematosus" (M. W. Ropes, Ed.), p. 96. Harvard Univ. Press, Cambridge.
188. Ginzler, E. M., Bollet, A. J., and Friedman, E. A. (1980). The natural history and response to therapy of lupus nephritis. *Ann. Rev. Med.* **31**, 463.
189. Friedman, E. A. (1980). Double-think in lupus nephritis. *J. Am. Med. Assoc.* **244**, 68.
190. Decker, J. L. (1982). The management of systemic lupus erythematosus. *Arthritis Rheum.* **25**, 891.
191. Wong, K.-F., Hui, P.-K., Chan, J. K. C., *et al.* (1991). The acute lupus hemophagocytic syndrome. *Ann. Intern. Med.* **114**, 387.
192. Stevens, M. B., and Hahn, B. H. (1982). Management of systemic lupus erythematosus. *Bull. Rheum. Dis.* **32**, 35.
193. Donadio, J. V., Jr. (1982). Renal involvement in SLE: The argument for aggressive treatment. In "The Kidney and the Rheumatic Disease" (P. A. Bacon and N. M. Hadler, Eds.), p. 45. Butterworth Scientific, London.
194. Klippel, J. H. (1982). Renal involvement in SLE: The argument for conservative treatment. In "The Kidney and the Rheumatic Disease" (P. A. Bacon and N. M. Hadler, Eds.), p. 61. Butterworth Scientific, London.
195. Pollak, V. E., and Dosekun, A. K. (1982). Evaluation of treatment in lupus nephritis: Effects of prednisone. *Am. J. Kidney Dis.* **1**(Suppl. 1), 170.
196. Coggins, C. H. (1982). Overview of treatment of lupus nephropathy. *Am. J. Kidney Dis.* **1**(Suppl. 1), 197.
197. Hughes, G. R. V. (1982). The treatment of SLE: The case for conservative management. In "Systemic Lupus Erythematosus" (G. R. V. Hughes, Ed.), Vol. 14, p. 299. *Clin. Rheum. Dis.* Saunders, London.
198. Kimberly, R. P. (1988). Treatment: Corticosteroids and anti-inflammatory drugs. In "Systemic Lupus Erythematosus" (J. H. Klippel, Ed.), Vol. 14, p. 203. *Rheum. Dis. Clin. N. Am.* Saunders, Philadelphia.
199. Albert, D. A., Hadler, N. M., and Ropes, M. W. (1979). Does corticosteroid therapy affect the survival of patients with systemic lupus erythematosus? *Arthritis Rheum.* **22**, 945.
200. Pollak, V. E., Pirani, C. L., and Kark, R. M. (1961). The effect of large doses of prednisone on the renal lesions and life span of patients with lupus glomerulonephritis. *J. Lab. Clin. Med.* **57**, 495.
201. Pollak, V. E., Pirani, C. L., and Schwartz, F. D. (1964). The natural history of the renal manifestations of systemic lupus erythematosus. *J. Lab. Clin. Med.* **63**, 537.
202. Boelaert, J., Morel-Maroger, L., and Mery, J.-P. (1974). Renal insufficiency in lupus nephritis. *Adv. Nephrol.* **4**, 249.
203. Medical Research Council (1961). Treatment of systemic lupus erythematosus with steroids. *Br. Med. J.* **2**, 915.
204. Lange, K., Ores, R., Strauss, W., *et al.* (1965). Steroid therapy of systemic lupus erythematosus based on immunologic considerations. *Arthritis Rheum.* **8**, 244.
205. Baldwin, D. S., Lowenstein, J., Rothfield, N. F., *et al.* (1970). The clinical course of the proliferative and membranous forms of lupus nephritis. *Ann. Intern. Med.* **73**, 929.
206. Ackerman, G. L. (1970). Alternate day steroid therapy in lupus nephritis. *Ann. Intern. Med.* **72**, 511.

207. Mackay, I. R. (1973). Chronic lupus erythematosus: Observation on long-term survivors of "suppressive" therapy of lupus nephritis with prednisolone. In "Glomerulonephritis. Morphology, Natural History, and Treatment" (P. Kincaid-Smith, T. H. Mathew, and E. L. Becker, Eds.), Vol. 2, p. 1211. Wiley, New York.
208. Comerford, F. R., and Cohen, A. S. (1967). The nephropathy of systemic lupus erythematosus. *Medicine* **46**, 425.
209. Striker, G. E., Kelly, M. R., Quadracci, L. J., *et al.* (1973). The course of lupus nephritis: Relationship to renal histologic findings. In "Glomerulonephritis. Morphology, Natural History and Treatment" (P. Kincaid-Smith, T. H. Mathew, and L. E. Becker, Eds.), Vol 2, p. 1141. Wiley, New York.
210. Kagen, L. J., and Christian, C. L. (1966). Clinicopathologic studies of SLE nephritis (abstract). *Arthritis Rheum.* **9**, 516.
211. Zweiman, B., Kornblum, J., Cornog, J., *et al.* (1968). The prognosis of lupus nephritis. Role of clinical–pathologic correlations. *Ann. Intern. Med.* **69**, 441.
212. Appel, G. B., Silva, F. G., Pirani, C. L., *et al.* (1978). Renal involvement in systemic lupus erythematosus: A study of 56 patients emphasizing histologic classification. *Medicine* **57**, 371.
213. Appel, G. B., Cohen, D. J., Pirani, C. L., *et al.* (1987). Long-term follow-up of patients with lupus nephritis. A study based on the classification of the World Health Organization. *Am. J. Med.* **83**, 877.
214. Austin III, H. A., Muenz, L. R., and Joyce, K. M. (1983). Prognostic factors in lupus nephritis. Contribution of renal histologic data. *Am. J. Med.* **75**, 382.
215. Carette, S., Klippel, J. H., Decker, J. L., *et al.* (1983). Controlled studies of oral immunosuppressive drugs in lupus nephritis: A long-term follow-up. *Ann. Intern. Med.* **99**, 1.
216. Gladman, D. D., Urowitz, M. B., and Keystone, E. C. (1979). Serologically active, clinically quiescent systemic lupus erythematosus. A discordance between clinical and serologic features. *Am. J. Med.* **66**, 210.
217. Appel, A. E., Sablay, L. B., Golden, R. A., *et al.* (1978). The effect of normalization of serum complement and anti-DNA antibody on the course of lupus nephritis. *Am. J. Med.* **64**, 274.
218. Urman, J. D., and Rothfield, N. F. (1977). Corticosteroid treatment on systemic lupus erythematosus. *J. Am. Med. Assoc.* **238**, 227.
219. Bennett, W. M., Bardana, E. J., Houghton, D. C., *et al.* (1977). Silent renal involvement in systemic lupus erythematosus. *Int. Arch. Allergy Appl. Immunol.* **55**, 420.
220. Mahajan, S. K., Ordonez, N. G., Feitelson, P. J., *et al.* (1977). Lupus nephropathy without clinical renal involvement. *Medicine* **256**, 493.
221. Ginzler, E. M., Nicastri, A. D., Chen, C. K., *et al.* (1974). Progression of mesangial and focal to diffuse lupus nephritis. *N. Engl. J. Med.* **291**, 693.
222. Zimmerman, S. W., Jenkins, P. G., Schelp, W. D., *et al.* (1975). Progression from minimal or focal to diffuse proliferative glomerulonephritis. *Lab. Invest.* **32**, 665.
223. Baldwin, D. S., Gluck, M. C., Lowenstein, J., *et al.* (1977). Lupus nephritis. Course as related to morphologic forms and their transitions. *Am. J. Med.* **62**, 12.
224. Mahajan, S. K., Ordonez, N. G., Spargo, B. H., *et al.* (1978). Changing histology patterns in lupus nephropathy. *Clin. Nephrol.* **10**, 1.
225. Lightfoot, R. W., Jr., and Hughes, G. R. V. (1976). Significance of persisting serologic abnormalities in SLE. *Arthritis Rheum.* **19**, 837.
226. Balow, J. E., Austin, H. S., Muenz, L. R., *et al.* (1984). Effect of treatment on the evolution of renal abnormalities in lupus nephritis. *N. Engl. J. Med.* **311**, 491.
227. Austin, H. A., Klippel, J. H., Balow, J. E., *et al.* (1986). Therapy of lupus nephritis: Controlled trial of prednisone and cytotoxic drugs. *N. Engl. J. Med.* **314**, 614.
228. Klippel, J. H. (1987). Morbidity and mortality. Lupus nephritis. (J. E. Balow, moderator). *Ann. Intern. Med.* **106**, 89.
229. Donadio, J. V., Jr., Burgess, J. H., and Polley, K. E. (1977). Membranous lupus nephropathy: A clinical pathologic study. *Medicine* **56**, 527.
230. Collaborative Study of the Adult Idiopathic Nephrotic Syndrome (1979). A controlled study of short-term prednisone treatment in adults with membranous nephropathy. *N. Engl. J. Med.* **301**, 1301.
231. Ponticelli, C., Zucchelli, P., Passerini, P., *et al.* (1989). A randomized trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N. Engl. J. Med.* **320**, 8.
232. Kimberly, R. P., Lockshin, M. D., Sherman, R. L., *et al.* (1981). High-dose intravenous methylprednisolone pulse therapy in systemic lupus erythematosus. *Am. J. Med.* **70**, 817.
233. Kimberly, R. P., Lockshin, M. D., Sherman, R. L., *et al.* (1983). Reversible "endstage" lupus nephritis. Analysis of patients able to discontinue dialysis. *Am. J. Med.* **74**, 361.
234. Feinglass, E. J., Arnett, F. C., Dorsch, C. A., *et al.* (1976). Neuropsychiatric manifestations of systemic lupus erythematosus: Diagnosis, clinical spectrum and relationship to other features of the disease. *Medicine* **55**, 323.
235. Bennahum, D. A., and Messner, R. P. (1975). Recent observations on central nervous system systemic lupus erythematosus. *Semin. Arthritis Rheum.* **4**, 253.
236. Kassan, S. S., and Lockshin, M. D. (1979). Central nervous system lupus erythematosus: The need for classification. *Arthritis Rheum.* **22**, 1382.
237. Brandt, K. D., and Lessell, S. (1978). Migrainous phenomena in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 7.
238. Sergent, J. S., Lockshin, M. D., Klempner, M. S., *et al.* (1975). Central nervous system disease in systemic lupus erythematosus. *Am. J. Med.* **58**, 644.
239. Clinical Conferences at the Johns Hopkins Hospital (1981). *Johns Hopkins Med. J.* **149**, 142.
240. Klippel, J. H., and Zvaifler, N. J. (1975). Neuropsychiatric abnormalities in systemic lupus erythematosus. In "Systemic Lupus Erythematosus" (N. F. Rothfield, Ed.), Vol. 1, p. 621. *Clin. Rheum. Dis.* Saunders, London.

241. McCune, W. J., and Golbus, J. (1988). Neuropsychiatric lupus. In "Systemic Lupus Erythematosus" (J. H. Klippel, Ed.), Vol. 14, p. 149. *Rheum. Dis. Clin. North Am.* Saunders, Philadelphia.
242. Matthay, R. A., Schwarz, M. I., Petty, T. L., *et al.* (1974). Pulmonary manifestations of systemic lupus erythematosus: Review of 12 cases of acute lupus pneumonitis. *Medicine* **54**, 397.
243. Abramson, S. B., Dobro, J., Eberle, M. A., *et al.* (1991). Acute reversible hypoxemia in systemic lupus erythematosus. *Ann. Intern. Med.* **114**, 941.
244. Eisenberg, H., Dubois, E. L., Sherwin, R. P., *et al.* (1973). Diffuse interstitial lung disease in systemic lupus erythematosus. *Ann. Intern. Med.* **79**, 37.
245. Munsat, T. L., and Bradley, W. G. (1977). Serum creatine phosphokinase levels and prednisone treated muscle weakness. *Neurology* **27**, 96.
246. Bell, P. R. F., Briggs, J. D., Calman, K. G., *et al.* (1971). Reversal of acute clinical and experimental organ rejection using large doses of intravenous prednisolone. *Lancet* **1**, 876.
247. Turcotte, J., Feduska, N., Carpenter, E., *et al.* (1972). Rejection crises in human renal transplant recipients. *Arch. Surg.* **105**, 230.
248. Cathcart, E. S., Idelson, B. A., Scheinberg, M. A., *et al.* (1976). Beneficial effects of methylprednisolone "pulse" therapy in diffuse proliferative lupus nephritis. *Lancet* **1**, 163.
249. Kimberly, R. P. (1982). Pulse methylprednisolone in SLE. In "Systemic Lupus Erythematosus" (G. R. V. Hughes, Ed.), Vol. 8, p. 261. *Clin. Rheum. Dis*. Saunders, London.
250. Levinsky, R. J., Cameron, J. S., and Soothill, J. F. (1977). Serum immune complexes and disease activity in lupus nephritis. *Lancet* **1**, 564.
251. Nebout, T., Sobel, A., and Lagrue, G. (1977). Intravenous methylprednisolone pulses in diffuse proliferative lupus nephritis (letter). *Lancet* **1**, 909.
252. Ponticelli, C., Tarantino, A., Pioltelli, P., *et al.* (1977). High-dose methylprednisolone pulses in active nephritis (letter). *Lancet* **1**, 1063.
253. Dosa, S., Cairns, S. A., Lawler, W., *et al.* (1979). The treatment of lupus nephritis by methylprednisolone pulse therapy. *Postgrad. Med. J.* **54**, 628.
254. Cameron, J. S., Turner, D. R., Ogg, C. S., *et al.* (1979). Systemic lupus with nephritis: A long-term study. *Q. J. Med.* **189**, 1.
255. Fessel, W. J. (1980). Megadose corticosteroid therapy in systemic lupus erythematosus. *J. Rheumatol.* **7**, 486.
256. Ofuji, T., Kurata, N., and Kinashi, M. (1980). Pulse therapy for lupus nephritis. In "Systemic Lupus Erythematosus" (M. Fukase, Ed.), p. 361. Univ. Park Press, Baltimore.
257. Schlansky, R., DeHoratius, R. J., Pincus, T., *et al.* (1981). Plasmapheresis in systemic lupus erythematosus: A cautionary note. *Arthritis Rheum.* **24**, 49.
258. Hoch, S., and Schur, P. H. (1984). Methylprednisolone pulse therapy for lupus nephritis: A follow-up study. *Clin. Exp. Rheumatol.* **2**, 315.
259. Ponticelli, C., Zucchelli, P., Banfi, G., *et al.* (1982). Treatment of diffuse proliferative lupus nephritis by intravenous high-dose methylprednisolone. *Q. J. Med. New Ser.* **51**, 16.
260. Barron, K. S., Person, D. A., Brewer, E. J., Jr., *et al.* (1982). Pulse methylprednisolone therapy in diffuse proliferative lupus nephritis. *J. Pediatr.* **101**, 137.
261. Kinashi, M., Kurata, N., Hara, I., *et al.* (1981). Effects of pulse therapy on renal insufficiency of systemic lupus erythematosus. *Nippon-Jinzo-Gakkao-Shi* **23**, 35.
262. Garin, E. H., Sleasman, J. W., Richard, G. A., *et al.* (1986). Pulsed methylprednisolone therapy compared to high dose prednisone in systemic lupus erythematosus nephritis. *Eur. J. Pediatr.* **145**, 380.
263. Yeung, C. K., Ng, W. L., Wong, W. S., *et al.* (1985). Acute deterioration in renal function in systemic lupus erythematosus. *Q. J. Med.* **56**, 393.
264. Cole, B. R., Brocklebank, J. T., Kienstra, R. A., *et al.* (1976). "Pulse" methylprednisolone therapy in the treatment of severe glomerulolonephritis. *J. Pediatr.* **88**, 307.
265. Bolton, W. K., and Couser, W. G. (1979). Intravenous pulse methylprednisolone therapy of acute crescentic rapidly progressive glomerulonephritis. *Am. J. Med.* **66**, 495.
266. O'Neill, W. M., Etheridge, W. B., and Bloomer, H. A. (1979). High-dose corticosteroids. Their use in treating idiopathic rapidly progressive glomerulonephritis. *Arch. Intern. Med.* **139**, 514.
267. Oredugba, O., Mazumdar, D. C., Meyer, J. S., *et al.* (1980). Pulse methylprednisolone therapy in idiopathic rapidly progressive glomerulonephritis. *Ann. Intern. Med.* **92**, 504.
268. Bolton, W. K., and Sturgill, B. C. (1989). Methylprednisolone therapy of idiopathic acute crescentic, rapidly progressive glomerulonephritis. *Am. J. Nephrol.* **9**, 368.
269. Rose, G. M., Cole, B. R., and Robson, A. M. (1981). The treatment of severe glomerulopathies in children using high-dose intravenous methylprednisolone pulses. *Am. J. Kidney Dis.* **1**, 148.
270. Friedman, G., Granot, H., Koplovic, Y., *et al.* (1981). Pulse methylprednisolone and cyclophosphamide therapy in idiopathic rapidly progressive glomerulonephritis. *Postgrad. Med. J.* **57**, 54.
271. Said, R., Hamzeh, Y., Tarawneh, M., *et al.* (1989). Rapid progressive glomerulonephritis in patients with familial mediterranean fever. *Am. J. Kidney Dis.* **14**, 412.
272. Liebling, M. R., McLaughlin, K., Boonsue, S., *et al.* (1982). Monthly pulses of methylprednisolone in SLE nephritis. *J. Rheumatol.* **9**, 543.
273. Gourley, M. F., Austin, H. A., Scott, D., Yarboro, C. H., Vaughan, E. M., Muir, J., Boumpas, D. T., Klippel, J. H., Balow, J. E., and Steinberg, A. D. (1996). Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis: A randomized, controlled trial. *Ann. Intern. Med.* **125**, 549.
274. Boumpas, D. T., Austin, H. A., Vaughn, E. M., Klippel, J. H., and Steinberg, A. D. (1992). Controlled trials of pulse methylprednisolone versus two regimens of pulse

cyclophosphamide in severe lupus nephritis. *Lancet* **340**, 741.
275. Illei, G. G., Austin, H. A., Crane, M., *et al.* (2001). Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. *Ann. Intern. Med.* **135**, 248.
276. Eyanson, S., Passo, M. H., Aldo-Benson, M. A., *et al.* (1980). Methylprednisolone pulse therapy for non-renal lupus erythematosus. *Ann. Rheum. Dis.* **39**, 377.
277. Akashi, K., Nagasawa, K., Mayumi, T., *et al.* (1990). Successful treatment of refractory systemic lupus erythematosus with intravenous immunoglobulins. *J. Rheumatol.* **17**, 375.
278. Lurie, D. P., and Kahaleh, M. B. (1982). Pulse corticosteroid therapy for refractory thrombocytopenia in systemic lupus erythematosus. *J. Rheumatol.* **9**, 311.
279. Mackworth-Young, C. G., David, J., Morgan, S. H., *et al.* (1988). A double blind, placebo controlled trial of intravenous methylprednisolone in systemic lupus erythematosus. *Ann. Rheum. Dis.* **47**, 496.
280. Boumpas, D. T., Patronas, N. J., Dalakas, M. C., *et al.* (1990). Acute transverse myelitis in systemic lupus erythematosus: Magnetic resonance imaging and review of the literature. *J. Rheumatol.* **17**, 89.
281. Davies, U. M., and Ansell, B. M. (1988). Central nervous system manifestations in juvenile systemic lupus erythematosus: A problem of management. *J. Rheumatol.* **15**, 1720–1721.
282. Oto, A., Sozen, T., and Boyacioglu, S. (1981). Pulsed methylprednisolone (letter). *Ann. Rheum. Dis.* **40**, 630.
283. Isenberg, D. A., Morro, W. J. W., and Snaith, M. L. (1982). Methylprednisolone pulse therapy in the treatment of systemic lupus erythematosus. *Ann. Rheum. Dis.* **41**, 347.
284. Fauci, A. S. (1978). Alternate day corticosteroid therapy. *Am. J. Med.* **64**, 729.
285. Dale, D. C., Fauci, A. S., and Wolff, S. M. (1974). Alternate-day prednisone. Leukocyte kinetics and susceptibility to infections. *N. Engl. J. Med.* **291**, 1154.
286. Bromberg, J. S., Alfrey, E. J., and Barker, C. F. (1991). Adrenal suppression and steroid supplementation in renal transplant recipients. *Transplantation* **51**, 385.
287. Friedman, R. J., Schiff, C. F., and Bromberg, J. S. (1995). Use of supplemental steroids in patients having orthopaedic operations. *J. Bone Joint Surg. Am.* **77**, 1801.
288. Glowniak, J. V., and Loriaux, D. L. (1997). A double blind study of perioperative steroid requirements in secondary adrenal insufficiency. *Surgery* **121**, 123.
289. Salem, M., Tainsh, R. E., Jr., Bromberg, J., Loriaux, D. L., and Chernow B. (1994). Perioperative glucocorticoid coverage: A reassessment 42 years after emergence of a problem. *Ann. Surg.* **219**, 416.
290. Vesell, E. S. (1979). Intraspecies differences in frequency of genes directly affecting drug disposition: The individual factor in drug response. *Pharmacol. Rev.* **30**, 555.
291. Vesell, E. S., and Page, J. G. (1969). Genetic control of the phenobarbital-induced shortening of plasma antipyrine half-lives in man. *J. Clin. Invest.* **48**, 2202.
292. Lamberts, S. W., Huizenga, A. T., de Lange, P., de Jong, F. H., and Koper, J. W. (1996). Clinical aspects of glucocorticoid sensitivity. *Steroids* **61**, 157.
293. de Lange, P., Koper, J. W., Huizenga, N. A., Brinkmann, A. O., de Jong, F. H., Karl, M., Chrousos, G. P., and Lamberts, S. W. (1997). Differential hormone-dependent transcriptional activation and -repression by naturally occurring human glucoccorticoid receptor variants. *Mol. Endocrinol.* **11**, 1156.
294. Koper, J. W., Stolk, R. P., de Lange, P., Huizenga, N. A., Molijn, G. J., Pols, H. A., Grobbee, D. E., Karl, M., de Jong, F. H., Brinkmann, A. O., and Lamberts, S. W. (1997). Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. *Hum. Genet.* **99**, 663.
295. Leung, D. Y., Hamid, Q., Vottero, A., *et al.* (1997). Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *J. Exp. Med.* **186**, 1567.
296. Strickland, I., Kisich, K., Hauk, P. J., *et al.* (2001). High constitutive glucocorticoid receptor beta in human neutrophils enables them to reduce their spontaneous rate of cell death in response to corticosteroids. *J. Exp. Med.* **193**, 585.
297. Webster, J. C., Oakley, R. H., Jewell, C. M., and Cidlowski, J. A. (2001). Proinflammatory cytokines regulate human glucocorticoid receptor gene expression and lead to the accumulation of the dominant negative beta isoform: A mechanism for the generation of glucocorticoid resistance. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6865.
298. Kappas, A., Anderson, K. E., Conney, A. H., *et al.* (1976). Influence of dietary protein in carbohydrate on antipyrine and theophylline. Metabolism in man. *Clin. Pharmacol. Ther.* **20**, 643.
299. Anderson, K. E., Kappas, A., Conney, A. M., *et al.* (1984). The influence of dietary protein and carbohydrate on the principle oxidative biotransformations of estradiol in normal subjects. *J. Clin. Endocrinol. Metab.* **59**, 103.
300. Pantuck, E. J., Pantuck, C. B., Garland, W. A., *et al.* (1978). Effect of dietary brussels sprouts and cabbage on human drug metabolism. *Clin. Pharmacol. Ther.* **25**, 88.
301. Homma, M., Ichikawa, Y., Abe, Y., *et al.* (1981). Prednisolone metabolism in SLE patients resistant to steroid therapy. In "Systemic Lupus Erythematosus" (M. Fukase, Ed.), p. 367. Univ. Park Press, Baltimore.
302. Farrell, G. C., Cooksley, W. G. E., Hart, P., *et al.* (1978). Drug metabolism in liver disease, identification of patients with impaired hepatic drug metabolism. *Gastroenterology* **75**, 580.
303. Williams, R. L. (1983). Drug therapy: Drug administration in hepatic disease. *N. Engl. J. Med.* **309**, 1616.
304. Klinenberg, J. R., and Miller, F. (1965). Effect of corticosteroids on blood salicylate concentration. *J. Am. Med. Assoc.* **194**, 131.
305. Laflin, M. J. (1977). Interaction of pancuronium and corticosteroids. *Anesthesiology* **47**, 471.
306. Lacomis, D., Giuliani, M. J., Van Cott, A., and Kramer, D. J. (1996). Acute myopathy of intensive care: Clinical,

electromyographic, and pathological aspects. *Ann. Neurol.* **40**, 645.
307. Behbehani, N. A., Al-Mane, F., D'Yachkova, Y., *et al.* (1999). Myopathy following mechanical ventilation for acute severe asthma: The role of muscle relaxants and corticosteroids. *Chest* **115**, 1627.
308. Szefler, S. J., Brenner, M., Jusko, W. J., *et al.* (1982). Dose and time related effect of troleandomycin on methylprednisolone elimination. *Clin. Pharmacol. Ther.* **32**, 166.
309. Mok, C. C., Lau, C. S., and Wong, R. W. (1998). Risk factors for avascular bone necrosis in systemic lupus erythematosus. *Br. J. Rheumatol.* **37**, 895.
310. Gladman, D. D., Urowitz, M. B., Chaudhry-Ahluwalia, V., *et al.* (2001). Predictive factors for symptomatic osteonecrosis in patients with systemic lupus erythematosus. *J. Rheumatol.* **28**, 761.
311. Mont, M. A., Glueck, C. J., Pacheco, I. H., *et al.* (1997). Risk factors for osteonecrosis in systemic lupus erythematosus. *J. Rheumatol.* **24**, 654.
312. Zonana-Nacach, A., Barr, S. G., Magder, L. S., and Petri, M. (2000). Damage in systemic lupus erythematosus and its association with corticosteroids. *Arthritis Rheum.* **43**, 1801.
313. Migliaresi, S., Picillo, U., Ambrosone, L., *et al.* (1994). Avascular osteonecrosis in patients with SLE: Relation to corticosteroid therapy and anticardiolipin antibodies. *Lupus* **3**, 37.
314. Felson, D. T., and Anderson, J. J. (1987). Across-study evaluation of association between steroid dose and bolus steroids and avascular necrosis of bone. *Lancet* **1**, 902.
315. Abeles, M., Urman, J. D., and Rothfield, N. F. (1978). Aseptic necrosis of bone in systemic lupus erythematosus. Relationship to corticosteroid therapy. *Arch. Intern. Med.* **138**, 750.
316. Massardo, L., Jacobelli, S., Leissner, M., *et al.* (1992). High-dose intravenous methylprednisolone therapy associated with osteonecrosis in patients with systemic lupus erythematosus. *Lupus* **1**, 401.
317. Zizic, T. M., Marcoux, C., Hungerford, D. S., *et al.* (1985). Corticosteroid therapy associated with ischemic necrosis of bone in systemic lupus erythematosus. *Am. J. Med.* **79**, 596.
318. Oinuma, K., Harada, Y., Nawata, Y., *et al.* (2001). Osteonecrosis in patients with systemic lupus erythematosus develops very early after starting high dose corticosteroid treatment. *Ann. Rheum. Dis.* **60**, 1145.
319. The Boston Collaborative Drug Surveillance Program (1972). Acute adverse reactions to prednisone in relation to dosage. *Clin. Pharmacol. Ther.* **13**, 694.
320. Hall, R. W. C., Popkin, M. K., Stickney, S. K., *et al.* (1979). Presentation of the steroid psychoses. *J. Nervous Mental Dis.* **167**, 229.
321. Ling, M. H. M., Perry, P. J., and Tsaung, M. T. (1981). Side effects of corticosteroid therapy. Psychiatric aspects. *Arch. Gen. Psychiatr.* **38**, 471.
322. Afifi, A. K., Bergman, K. A., and Harvey, J. C. (1968). Steroid myopathy: Clinical, histologic and cytologic observations. *Johns Hopkins Med. J.* **123**, 158.
323. Askari, A., Vignos, P. J., Jr., and Moskowitz, R. W. (1976). Steroid myopathy in connective tissue disease. *Am. J. Med.* **61**, 485.
324. Perkoff, G. T., Silber, R., Tyler, F. H., *et al.* (1959). Studies in disorders of the muscle. XII. Myopathy due to the administration of therapeutic amounts of 17 hydroxy corticosteroids. *Am. J. Med.* **26**, 891.
325. Sibrans, D. F., and Holley, H. L. (1967). Vacuolar myopathy in a patient with a positive LE cell preparation. *Arthritis Rheum.* **10**, 141.
326. Kanayama, Y., Shiota, K., Horiguchi, T., *et al.* (1981). Correlation between steroid myopathy and serum lactate dehydrogenase in systemic lupus erythematosus. *Arch. Intern. Med.* **141**, 1176.
327. Smith, R. J., Larson, S., Stred, S. E., and Durschlag, R. P. (1984). Regulation of glutamine synthetase and glutaminase activities in cultured skeletal muscle cells. *J. Cell. Physiol.* **120**, 197.
328. Max, S. R., Mill, J., Mearow, K., *et al.* (1988). Dexamethasone regulates glutamine synthetase expression in rat skeletal muscles. *Am. J. Physiol.* **255**, E397.
329. Hickson, R. C., Czerwinski, S. M., and Wegrzyn, L. E. (1995). Glutamine prevents downregulation of myosin heavy chain synthesis and muscle atrophy from glucocorticoids. *Am. J. Physiol.* **268**, E730.
330. Chandrasekhar, S., Souba, W. W., and Abcouwer, S. F. (1999). Identification of glucocorticoid-responsive elements that control transcription of rat glutamine synthetase. *Am. J. Physiol.* **276**, L319.
331. Horber, F. F., Scheidegger, J. R., Grunig, B. E., and Frey, F. J. (1985). Evidence that prednisone-induced myopathy is reversed by physical training. *J. Clin. Endocrinol. Metabol.* 83.
332. Kimura, K., Kanda, F., Okuda, S., and Chihara, K. (2001). Insulin-like growth factor 1 inhibits glucocorticoid-induced glutamine synthetase activity in cultured L6 rat skeletal muscle cells. *Neurosci. Lett.* **302**, 154.
333. Kovacs, G., Fine, R. N., Worgall, S., *et al.* (1991). Growth hormone prevents steroid-induced growth depression in health and uremia. *Kidney Int.* **40**, 1032.
334. Horber, F. F., and Haymond, M. W. (1990). Human growth hormone prevents the protein catabolic side effects of prednisone in humans. *J. Clin. Invest.* **86**, 265.
335. Reynolds, J. C., Inman, R. D., Kimberly, R. P., *et al.* (1982). Acute pancreatitis in SLE: Report of 20 cases and a review of the literature. *Medicine* **61**, 25.
336. Mallory, A., and Kern, F. (1980). Drug-induced pancreatitis: A critical review. *Gastroenterology* **78**, 813.
337. Levine, R. A., and McGuire, R. F. (1988). Corticosteroid-induced pancreatitis: A case report demonstrating recurrence with rechallenge. *Am. J. Gastroenterol.* **83**, 1161.
338. Saab, S., Corr, M. P., and Weisman, M. H. (1998). Corticosteroids and systemic lupus erythematosus pancreatitis: A case series. *J. Rheumatol.* **25**, 801.
339. Spiro, H. (1983). Is the steroid ulcer a myth? *N. Engl. J. Med.* **309**, 45.

340. Conn, H. O., and Blitzer, B. L. (1976). Non-association of adrenocorticosteroid therapy in peptic ulcer. *N. Engl. J. Med.* **294**, 473.
341. Messer, J., Reitman, D., Sacks, H. S., *et al.* (1983). Association of adrenocorticoid steroid therapy and peptic ulcer disease. *N. Engl. J. Med.* **309**, 21.
342. Piper, J. M., Ray, W. A., Daugherty, J. R., *et al.* (1991). Corticosteroid use and peptic ulcer disease: Role of nonsteroidal anti-inflammatory drugs. *Ann. Intern. Med.* **114**, 735.
343. Gabriel, S. E., Jaakkimainen, L., and Bombardier, C. (1991). Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis. *Ann. Intern. Med.* **115**, 787.
344. Ginzler, E., Diamond, H. S., Kaplan, D., *et al.* (1978). Computer analysis of factors influencing frequency of infection in systemic lupus erythematosus. *Arthritis Rheum.* **21**, 37.
345. Staples, P. J., Gerding, D. N., Decker, J. L., *et al.* (1974). Incidence of infection in systemic lupus erythematosus. *Arthritis Rheum.* **17**, 110.
346. Perez, D. H., Goldstein, I. M., Chernoff, D., *et al.* (1980). Chemotactic activity of C5a des Arg: Evidence of a requirement for an anionic peptide "helper factor" and inhibition by a cationic protein in serum from patients with systemic lupus. *Mol. Immunol.* **17**, 163.
347. Kovacs, J. A., Hiemenz, J. W., Macker, A. M., *et al.* (1984). *Pneumocystis carinii* pneumonia: A comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. *Ann. Intern. Med.* **100**, 663.
348. Pohl, M. A., Lan, S. P., Berl, T., *et al.* (1991). Plasmapheresis does not increase the risk for infection in immunosuppressed patients with severe lupus nephritis. *Ann. Intern. Med.* **114**, 924.
349. Porges, A. J., Beattie, S. L., Ritchlin, C., Kimberly, R. P., and Christian, C. L. (1992). Patients with systemic lupus erythematosus at risk for *Pneumocystis carinii* pneumonia. *J. Rheumatol.* **19**, 1191.
350. Klipper, A. R., Stevens, M. R., Zizic, T., *et al.* (1976). Ischemic necrosis of bone in systemic lupus erythematosus. *Medicine* **55**, 251.
351. Abeles, M., Urman, J. D., and Rothfield, A. F. (1978). Aseptic necrosis of bone in systemic lupus erythematosus. *Arch. Intern. Med.* **138**, 750.
352. Zizic, T., Hungerford, D., and Stevens, M. B. (1980). Ischemic bone necrosis in SLE. I. The early diagnosis of ischemic necrosis of bone. *Medicine* **259**, 134.
353. Weiner, E. S., and Abeles, M. (1990). More on aseptic necrosis and glucocorticoids in systemic lupus erythematosus (letter). *J. Rheumatol.* **17**, 119.
354. Shipley, M. E., Bacon, P. A., Berry, H., *et al.* (1988). Pulsed methylprednisolone in active early rheumatoid disease: A dose-ranging study. *Br. J. Rheum.* **27**, 211.
355. Williams, I. A., Mitchell, A. D., Rothman, W., *et al.* (1988). Survey of the long term incidence of osteonecrosis of the hip and adverse medical events in rheumatoid arthritis after high dose intravenous methylprednisolone. *Ann. Rheum. Dis.* **47**, 930.
356. Nordin, R. E. C., Marshall, D. H., Francis, R. M., *et al.* (1981). The effect of sex steroid in corticosteroid hormones on bone. *J. Steroid Biochem.* **15**, 171.
357. Adinoff, A. D., and Hollister, J. R. (1983). Steroid-induced fractures and bone loss in patients with asthma. *N. Engl. J. Med.* **309**, 265.
358. Baylink, D. J. (1983). Glucocorticoid-induced osteoporosis. *N. Engl. J. Med.* **309**, 306.
359. Bockman, R. S., and Weinerman, S. A. (1990). Steroid-induced osteoporosis. *Orthop. Clin. North Am.* **21**, 97.
360. LoCasscio, V., Bonucci, E., Imbimbo, B., *et al.* (1990). Bone loss in response to long-term glucocorticoid therapy. *Bone Miner.* **8**, 39.
361. Dhillon, V. B., Davies, M. C., Hall, M. L., *et al.* (1990). Assessment of the effect of oral corticosteroids on bone mineral density in systemic lupus erythematosus: A prelimary study with dual energy x ray absorptiometry. *Ann. Rheum. Dis.* **49**, 624.
362. Morris, H. A., Need, A. G., O'Loughlin, P. D., *et al.* (1990). Malabsorption of calcium in corticosteroid-induced osteoporosis. *Calcif. Tissue Int.* **46**, 305.
363. Lufkin, E. G., Wahner, H. W., and Bergstralh, E. J. (1988). Reversibility of steroid-induced osteoporosis. *Am. J. Med.* **85**, 887.
364. Gluck, O. S., Murphy, W. A., Hahn, T. J., *et al.* (1981). Bone loss in adults receiving alternate day glucocorticoid therapy: A comparison with daily therapy. *Arthritis Rheum.* **24**, 892.
365. Adachi, J. D., Bensen, W. G., Bianchi, F., Cividino, A., Pillersdorf, S., Sebalt, R. J., Tugwell, P., Gordon, M., Steele, M., and Goldsmith, C. (1996). Vitamin D and calcium in the prevention of corticosteroid induced osteoporosis. *J. Rheumatol.* **23**, 995.
366. Sambrook, P. N. (1996). Calcium and vitamin D therapy in corticosteroid bone loss—what is the evidence? *J. Rheumatol.* **23**, 963.
367. Adachi, J. D., Bensen, W. G., Brown, J., *et al.* (1997). Intermittent etidronate therapy to prevent corticosteroid-induced osteoporosis. *N. Engl. J. Med.* **337**, 382.
368. Roux, C., Oriente, P., Laan, R., *et al.* (1998). Randomized trial of effect of cyclical etidronate in the prevention of corticosteroid-induced bone loss. Ciblos Study Group. *J. Clin. Endocrinol. Metab.* **83**, 1128.
369. Adachi, J. D., Saag, K. G., Delmas, P. D., *et al.* (2001). Two-year effects of alendronate on bone mineral density and vertebral fracture in patients receiving glucocorticoids: A randomized, double-blind, placebo-controlled extension trial. *Arthritis Rheum.* **44**, 202.
370. Cohen, S., Levy, R. M., Keller, M., *et al.* (1999). Risedronate therapy prevents corticosteroid-induced bone loss: A twelve-month, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Arthritis Rheum.* **42**, 2309.
371. Reid, D. M., Hughes, R. A., Laan, R. F., *et al.* (2000). Efficacy and safety of daily risedronate in the treatment of corticosteroid-induced osteoporosis in men and women: A randomized trial. European Corticosteroid-Induced Osteoporosis Treatment Study. *J. Bone Miner. Res.* **15**, 1006.

372. Wallach, S., Cohen, S., Reid, D. M., *et al.* (2000). Effects of risedronate treatment on bone density and vertebral fracture in patients on corticosteroid therapy. *Calcif. Tissue Int.* **67**, 277.
373. Reid, D. M., Adami, S., Devogelaer, J. P., and Chines, A. A. (2001). Risedronate increases bone density and reduces vertebral fracture risk within one year in men on corticosteroid therapy. *Calcif. Tissue Int.* **69**, 242.
374. Plotkin, L. I., Weinstein, R. S., Parfitt, A. M., *et al.* (1999). Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *J. Clin. Invest.* **104**, 1363.
375. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis (2001). 2001 update. American College of Rheumatology Ad Hoc Committee on Glucocoritcoid-Induced Osteoporosis. *Arthritis Rheum.* **44**, 1496.
376. Urowitz, M., Gladman, D., and Bruce, I. (2000). Atherosclerosis and systemic lupus erythematosus. *Curr. Rheumatol. Rep.* **2**, 19.
377. Manzi, S., Meilahn, E. N., Rairie, J. E., *et al.* (1997). Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: Comparison with the Framingham Study. *Am. J. Epidemiol.* **145**, 408.
378. Manzi, S., Selzer, F., Sutton-Tyrrell, K., *et al.* (1999). Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum.* **42**, 51.
379. Svenungsson, E., Jensen-Urstad, K., Heimburger, M., *et al.* (2001). Risk factors for cardiovascular disease in systemic lupus erythematosus. *Circulation* **104**, 1887.
380. Becker, D. M., Chamberlain, B., Swank, R., *et al.* (1988). Relationship between corticosteroid exposure and plasma lipid levels in heart transplant recipients. *Am. J. Med.* **85**, 632.
381. Ettinger, W. H., Goldberg, A. P., Applebaum-Bowden, D., and Hazzard, W. R. (1987). Dyslipoproteinemia in systemic lupus erythematosus. Effect of corticosteroids. *Am. J. Med.* **83**, 503.
382. Ettinger, W. H., Klinefelter, H. F., and Kwiterovitch, P. O. (1987). Effect of short-term, low-dose corticosteroids on plasma lipoprotein lipids. *Atherosclerosis* **63**, 167.
383. Ettinger, W. H., Jr., and Hazzard, W. R. (1988). Prednisone increases very low density lipoprotein and high density lipoprotein in healthy men. *Metab. Clin. Exp.* **37**, 1055.
384. Ettinger, W. H., Jr., and Hazzard, W. R. (1988). Elevated apolipoprotein-B levels in corticosteroid-treated patients with systemic lupus erythematosus. *J. Clin. Endocrinol. Metab.* **67**, 425.
385. Formiga, F., Meco, J. F., Pinto, X., *et al.* (2001). Lipid and lipoprotein levels in premenopausal systemic lupus erythematosus patients. *Lupus* **10**, 359.
386. Mendelson, L. M., Meltzer, E. O., and Hamburger, R. N. (1974). Anaphylaxis-like reactions to corticosteroid therapy. *J. Allergy Clin. Immunol.* **54**, 125.
387. Freedman, M. D., Schocket, A. L., Chapel, N., *et al.* (1981). Anaphylaxis after intravenous methylprednisolone therapy. *J. Am. Med. Assoc.* **245**, 607.
388. Suchman, A. L., Condemi, J. J., and Leddy, J. P. (1983). Seizure after pulse therapy with methylprednisolone. *Arthritis Rheum.* **26**, 117.
389. Schmidt, G. B., Meyer, M. A., and Sadove, M. S. (1972). Sudden appearance of cardiac arrhythmias after dexamethasone. *J. Am. Med. Assoc.* **221**, 1402.
390. Moses, R. E., McCormick, A., and Nickey, W. (1981). Fatal arrhythmia after pulse methylprednisolone therapy. *Ann. Intern. Med.* **95**, 781.
391. Warren, D. J., and Swith, R. S. (1983). High-dose prednisolone (letter). *Lancet* **1**, 594.
392. Barrett, D. F. (1983). Pulse methylprednisolone therapy (letter). *Lancet* **2**, 800.
393. Bocanegra, T. S., Castaneda, M. O., Espinoza, L. R., *et al.* (1981). Sudden death after methylprednisolone pulse therapy (letter). *Ann. Intern. Med.* **95**, 122.
394. Clarke, A. G., and Salaman, J. R. (1974). Methylprednisolone in the treatment of renal transplant rejection. *Clin. Nephrol.* **2**, 230.
395. Mussche, M. M., Ringoir, S. M. G., and Lameire, N. N. (1976). High intravenous doses of methylprednisolone for acute cadaveric renal allograft rejection. *Nephron* **16**, 287.
396. Vineyard, G. C., Fadem, S. Z., Dmochowski, J., *et al.* (1974). Evaluation of corticosteroid therapy for acute renal allograft rejection. *Surg. Obstet. Gynecol.* **138**, 225.
397. Gray, D., Shepherd, H., Daar, A., *et al.* (1978). Oral versus intravenous high-dose steroid treatment of renal allograft rejection. The big shot or not? *Lancet* **1**, 117.
398. Liebling, M. R., Lieb, E., McLaughlin, K., *et al.* (1981). Pulse methylprednisolone in rheumatoid arthritis: A double-blind cross-over trial. *Ann. Intern. Med.* **94**, 21.
399. Vincente, F., Amend, W., Feduska, N. J., *et al.* (1980). Improved outcome following renal transplantation with reduction in the immunosuppression therapy for rejection episodes. *Am. J. Med.* **69**, 107.
400. Nakajima, N., Rao, S., Sakai, A., *et al.* (1977). Effects of intravenous bolus dosages of methylprednisolone and local radiation on renal allograft rejection and patient mortality. *Surg. Obstet. Gynecol.* **144**, 63.
401. Susan, L. P., Braun, W. E., Banowsky, L. H., *et al.* (1978). Avascular necrosis following renal transplantation. Experience with 449 allographs with and without high-dose steroid therapy. *Urology* **11**, 225.
402. Massardo, L., Jacobelli, S., Leissner, M., Gonzalez, M., Villarroel, L., and Rivero, S. (1992). High-dose intravenous methylprednisolone therapy associated with osteonecrosis in patients with systemic lupus erythematosus. *Lupus* **1**, 401.
403. McMeekin, T. O., and Moschella, S. L. (1979). Iatrogenic complications of dermatologic therapy. *Med Clin North Am.* **63**, 441.
404. Dickson, R. B., and Christy, N. P. (1980). On the various forms of corticosteroid withdrawal syndrome. *Am. J. Med.* **68**, 224.

405. Newmark, K. J., Mitra, S., and Berman, L. B. (1974). Acute arthralgia following high-dose intravenous methylprednisolone therapy. *Lancet* **2**, 229.
406. Morgan, H. G., Boulnois, J., and Burns-Cox, C. (1973). Addiction to prednisone. *Br. Med. J.* **2**, 93.
407. Flavin, D. K., Frederickson, P. A., Richardson, J. W., *et al.* (1983). Corticosteroid abuse—an unusual manifestation of drug dependence. *Mayo Clin. Proc.* **58**, 764.
408. Semel, J. D. (1984). Fever on a drug-free day of alternate day steroid therapy. *Am. J. Med.* **76**, 315.

47

ANTIMALARIALS AND IMMUNOSUPPRESSIVE THERAPIES

John C. Davis
John H. Klippel

Drug therapies currently used in the treatment of systemic lupus have not been specifically developed for lupus. Most of the agents have been adopted from transplant medicine or oncology and used based on the basis of: (1) known immune modulating properties, (2) efficacy in murine models of lupus, or (3) reported clinical observations. The principal noncorticosteroid agents currently used in lupus management derive from empiric experiences or small studies (antimalarials, methotrexate, and leflunomide), the observed effects of cancer chemotherapies on immune function (cyclophosphamide), and immunosuppressive drugs developed for the prevention of rejections of allogenic transplanted organs (azathioprine, cyclosporine, and mycophenolate mofetil). Although the exact mechanisms by which these drugs mediate improvement in lupus have not been defined with certainty, it is generally accepted that modulation or suppression of immune functions contribute to their modes of actions.

Antimalarials and immunosuppressive agents have a therapeutic role that spans the clinical spectrum of lupus (Table 1). Practical use of the agents is based on evidence from clinical trails, and efforts to match the risks of toxicities from the drugs with the potential for disease morbidity.

ANTIMALARIALS

The earliest clinical reports of antimalarials described improvements in discoid skin lesions following empirical treatment with quinine[1] and quinacrine.[2] Of the antimalarials, hydroxychloroquine is most commonly used (Table 2). There are few contraindications to these agents, although they should be used with caution in patients with G6PD deficiency, underlying hepatic disease, or porphyria.

Antimalarials are rapidly and completely absorbed in the gastrointestinal tract with peak plasma concentrations 2–8 hours following a single dose. A small percentage of antimalarials undergo deamination in the liver to inactive metabolites. The majority of the drug is bound to plasma proteins and is avidly concentrated within certain tissues particularly the liver, pituitary, skin, and adrenal glands. Metabolites and unchanged drug are renally excreted which may be enhanced by acidification of the urine. Equilibrium plasma levels are achieved at 4 weeks for chloroquine and quinacrine, and at 6 months for hydroxychloroquine. Therefore, the onset of action in lupus may require up to 4 to 6 weeks of therapy.

Both immunomodulatory and anti-inflammatory properties have been ascribed to antimalarials.[3] The high affinity of antimalarials for DNA with intercalation of the drug between adjacent base pairs inhibits cellular division as well as RNA transcription and translation.[4] In vitro drug effects on both macrophage and B-cell function have been described.[5,6] There is conflicting data in humans, however, as to whether antimalarials actually suppress antibody responses.[7,8] Antimalarials are highly concentrated within mononuclear cells and inhibit phagocytosis, migratory properties,

TABLE 1 Principal Indications for Disease-Modifying Drugs in Lupus

Drug	Principal Indications
Antimalarials	
Hydroxychloroquine	Rashes, Constitutional Symptoms, Arthritis, Arthralgias, Serositis
Chloroquine	
Quinacrine	
Alkylating Agents	
Cyclophosphamide	Major Organ Involvement (particularly nephritis and neurologic disorders)
Antimetabolites	
Azathioprine	Major Organ Involvement (particularly nephritis)
Mycophenolate mofetil	Nephritis
Methotrexate	Arthritis, Serositis
Leflunomide	Arthritis
Macrolide Immunosuppressants	
Cyclosporine	Nephritis (particularly membranous nephropathy
Tacrolimus	

and the metabolism of membrane phospholipids.[9–11] Hydroxychoroquine has been shown to induce apoptosis in peripheral blood lymphocytes.[12] Inhibition of the secretion of cytokines including interleukin-1 (IL-1), intereukin-6 (IL-6), and tumor necrosis factor alpha (TNF α) by mononuclear cells is thought to occur as a result of interference with posttranslational events. A number of effects of antimalarials on platelets have been described including cyclic gmP interactions and antiphospholipase inhibition. These combine to inhibit fibrinogen binding, thrombin-induced platelet responses, and platelet aggregation.[13]

Quinacrine and hydroxychloroquine are the best studied of the antimalarials; the clinical experience with chloroquine is substantially less.[14–20] It is generally believed that antimalarials have little role in the management of lupus involving major organs, although this has not been specifically studied. Antimalarials have a prominent role in the management of chronic cutaneous lupus, especially discoid lupus.[22] Combinations of antimalarials have been shown to be useful in patients with treatment-resistant subacute or chronic cutaneous lupus.[23,24] Hydroxychloroquine has been shown to provide subjective pain relief of musculoskeletal symptoms in lupus patients.[25] A 12-month double-blind, placebo-controlled trial of chloroquine diphosphate (250 mg daily) revealed that chloroquine led to improvements in non-major organ manifestations, reduced corticosteroid requirements, and prevented disease exacerbations.[26] Additional clinical benefits from hydroxychloroquine therapy in SLE may include the prevention of thromboemboloic events in patients with anti-phospholipid antibodies[27] and as a cholesterol-lowering agent. [28]

The efficacy of hydoxychloroquine as a disease-modifying agent in lupus has been most convincingly demonstrated in a prospective, double-blind trial in which patients on stable doses of hydroxychloroquine were randomized to continue drug therapy or be changed to a placebo.[29] The discontinuation of low-dose hydroxychloroquine in patients with clinically stable lupus was associated with significant increases in the risks of both clinical flares as well as severe exacerbations of the disease including vasculitis, transverse myelitis, and nephropathy. The study has led to reluctance to completely discontinue antimalarials in stable lupus patients who have clearly benefited from the drug.

The use of quinicrine remains less than that of hydroxycholorquine, but has been advocated as a second line substitution for hydroxychloroquine in patients with preexisting macular degeneration or those unable to tolerate hydroxychloroquine. [1] It also may be used in combination with hydroxychloroquine in patients with refractory skin manifestations of lupus. [1]

In general, antimalarials are well tolerated and rarely need to be discontinued for an adverse reaction (Table 2). Of the various toxicities associated with low-dose antimalarial therapy, gastrointestinal intolerance, cutaneous eruptions, dizziness, and nonspecific constitutional complaints are most frequent. Although hematologic toxicities are distinctly uncommon, complete blood counts should be obtained periodically.

Much of the concern regarding antimalarials has focused on potential ocular toxicities. Deposition of the drug in the cornea may be associated with complaints of blurred vision, photophobia, focusing difficulties, and visual halos. This side effect is most commonly seen within the first several weeks of starting the drug and typically resolves with the continuation of therapy. In the retina, antimalarials bind to the melanin of the pigmented epithelial layer and may damage rods and cones. Early retinal changes are typically first detected in the macula with findings of macular edema, increased pigmentation and granularity, and loss of the foveal reflex. Although patients with early macular disease (so called premaculopathy) generally have no visual complaints, on testing a paracentral scotomata to a red, but not white, test object may be detected. These types of retinal changes are entirely reversible upon discontinuation of the antimalarial drug. Advance macular disease is characterized by a central area of patchy depigmentation of the macula surrounded by a concentric ring of pigmentation ("bull's eye" lesion). Narrowing of the

TABLE 2 Dosage Guidelines and Potential Toxicities of Antimalarial Therapy in SLE

Agent	Brand Name(s)	Recommended Dose*
4-Aminoquinolones		
Hydroxychloroquine	Plaquenil	200–400 mg/day
Chloroquine	Aralen/Avloclor	250 mg/day
Chloroqune9-Aminocridines		
Quinacrine	Atabrine	100 mg/day
Potential Toxicities		
Constitutional	Malaise, irritability, weight loss	
Gastrointestinal	Anorexia, nausea, vomiting, diarrhea, abdominal pain, bloating flatulence	
Dermatologic	Pruritis, urticaria, multiple forms of rash, skin discoloration (particularly quinacrine), alopecia, bleaching of hair	
Opthalmologic	Keratopathy, preretinopathy, retinopathy, cycloplegia, extraocular muscle palsy	
Neuromuscular	Headache, dizziness, insomnia, tinnitus, psychosis, seizures, peripheral neuropathy, myopathy of proximal muscles, cardiomyopathy	
Hematologic	Leukopenia, agranulocytosis, aplastic anemia	

* Dose reduction required for patients less than 45 kg; 5–7 mg/kg/day for hydroxychloroquine, 4 mg/kg/day for chloroquine, and 1–2 mg/kg/day for quinacrine.

retinal vessels, optic atrophy, and diffuse depigmentation of the peripheral retina are very late changes. Patients with retinal pathology from antimalarials may be entirely asymptomatic or may complain of nyctalopia and scotomatous vision with field defects of paracentral, pericentral ring types and typical temporal scotomas.

The risks of retinal damage from antimalarials are increased with doses that exceed 6 mg/kg daily, cumulative doses greater than 800 g, and patient age, the latter presumably due to the increased prevalence of macular disease with age.[30–32] In addition, retinal toxicity may be more common with chloroquine as opposed to hydroxychloroquine.[33] Although the risks of retinal toxicity in patients with lupus treated with low does of antimalarials are extremely low, rare cases of retinal damage in lupus patients have been described.[34] Routine ophthalmologic examinations including slit lamp, funduscopic, and visual field testing should be performed at baseline in patients over the age of 40 and every 6–12 months during drug treatment. Patients with impaired renal function or those who have received antimalarials for more than 10 years may require more frequent monitoring.[32] An Amsler grid test, which can be self-administered, is a reliable and useful screening instrument for antimalarial retinal toxicity.[35]

Antimalarials cross the placenta and rare instances of congenital defects including cleft palate, sensorineural hearing loss, and posterior column defects have been reported with the use of chloroquine given for malarial prophylaxis. This poses a dilemma in lupus patients on antimalarial therapy in which there is the clear potential for disease exacerbation if the antimalarial is discontinued. It is generally recommended that antimalarials be avoided in pregnancy and ideally, because of the long drug half-life; they should be stopped several months in advance of an anticipated pregnancy (FDA Use in Pregnancy Rating Category C, Table 3), On the other hand, the safety of antimalarials in pregnancy has been noted in several small series of patients with lupus.[36–38] Although opinions vary widely, there appears to be increasing acceptance that the risks of flaring lupus exceed the risks of fetal congenital

TABLE 3 FDA-Use in Pregnancy Rating for Agents Used in SLE

Category	Evidence and Risks
A	Controlled clinical trials demonstrate no risk to the fetus
B	No evidence of risk to fetus in humans, either (1) human studies are lacking but animal studies show no risk, or (2) animal studies show risk but human studies do not
C	Human studies are lacking and animal studies are either positive for risk or lacking, potential benefits may justify potential risk
D	Evidence of risk to the fetus, however potential benefits may outweigh the risk
X	Definitely contraindicated in pregnancy—either human or animal studies have shown clear risk to the fetus

abnormalities and that antimalarials should be continued throughout the lupus pregnancy.

ALKYLATING AGENTS

There is a clinical experience with three nitrogen mustard alkylating agents in lupus—mechlorethamine, chlorambucil, and cyclophosphamide. For all practical purposes, mechlorethamine and chlorambucil are of historical interest only and infrequently used to treat lupus. Cyclophosphamide has become the standard of care for the drug management of most severe forms of lupus, particularly lupus nephritis.

Mechlorethamine

Mechlorethamine hydrochloride (mustargen, nitrogen mustard) is highly unstable and must be reconstituted from a powder immediately before use. A dose of 0.2–0.4 mg/kg body weight is infused rapidly through an indwelling venous catheter. Typically, the dose is repeated on a monthly basis. The drug is highly irritating, and great caution must be taken to avoid extravasation or drug contact with skin or mucous membranes. Following infusion, the drug rapidly undergoes transformation and is essentially undetectable within the bloodstream within several minutes. In comparison with other nitrogen mustard alkylating agents, mechlorethamine is considered to have relatively weak effects on immune factions.

A number of studies have documented benefits of mechlorethamine combined with corticosteroids in lupus, particularly in patients with lupus nephritis[39–42] For all practical purposes, nitrogen mustard has been replaced by the alkylating agent cyclophosphamide, which is easier to use. However, rare case reports of diffuse proliferative lupus nephritis with nephritic syndrome resistant to treatment with intravenous cyclophosphamide, but responsive to nitrogen mustard, have been noted to suggest that there may be a continued limited role for the drug.[42]

Cyclophosphamide

Substitution of a cyclic phosphamide for the N-methyl group of mechlorethamine yields an inert, stable alkylating agent, cyclophosphamide (Cytoxan, Endoxan). Activation of the drug required oxidation by hepatic microsomes to yield a number of metabolites. The key intermediate metabolite, aldophosphamide, is nonenzymatically cleaved to carboxyphosphamide and the potent alkylating compound phosphoramide mustard. These metabolites, in addition to alkylation, also have the capacity to phosphorylate molecular structures of the cell.

Cyclophosphamide is well absorbed following oral administration with peak plasma levels occurring one hour following the dose. Pharmacokinetics of oral and intravenous routes are comparable with a serum half-life of approximately 6 hours. Drug metabolism may be theoretically affected by drugs that enhance (corticosteroids, sex hormones, barbiturates) or inhibit (allopurinol, chloroquine, phenothiazine) hepatic mixed-function oxidase microsomal enzymes. However, the practical consequences of these influences in the clinical use of the drug are minimal. Alkylating metabolites circulate bound to plasma proteins and are renally excreted. Thus, plasma levels may be significantly increased in patients with compromised renal function and appropriate reductions of the drug dose are necessary.

Cyclophosphamide may be given by oral or intravenous routes (Table 4—not typed but is Table 4 from previous edition of book). Although there is no clear evidence that oral and intravenous regimens differ in clinical efficacy, in clinical practice oral cyclophosphamide has largely been abandoned in lupus in favor of intermittent, intravenous bolus therapy.[43,44] Drug schedules are empiric and largely based on acceptable levels of bone marrow suppression. The most commonly used intravenous regimen consists of monthly infusions (0.5–1.0 gm^2 body surface area) for a course of 6 months. Studies in patients with lupus nephritis suggest that continuation of bolus cyclophosphamide infusions every 3 months reduces the likelihood of disease relapse (see below). Studies of intravenous doses of 500 mg given

TABLE 4 Dose Guidelines and Monitoring of Cyclophosphamide Therapy

Dose	Routine Monitoring Studies
Oral: 1–3 mg/kg/day[a]	Hematocrit, white blood cell count/differential, and platelet count every 1 to 2 weeks with change in dosage and every 1 to 3 months thereafter. Urinalysis and urine cytology every 6 to 12 months[b]
IV: 0.5–1.0 mg/kg in 150 ml saline given over 60 minutes	Hematocrit, white blood cell count/differential days 7, 10, and 14 postdrug. Urinalysis and urine cytology every 6–12 months[b]
IV fluids (2–3 liters)/24 hr following dose	

[a] Drug should be taken in the morning in a single dose. Dose needs to be decreased in patients with impaired renal function.

[b] Urine cytology/cystoscopy for unexplained hematuria.

weekly have been reported to show comparable clinical improvements with fewer adverse effects.[45–48] In addition, high-dose oral administration has been shown to have a pharmacokinetic profile that is similar to that observed following intravenous administration.[49] Although the clinical experience with high dose bolus oral cyclophosphamide is limited, it appears to be well tolerated and most likely has an important role in outpatient management.[49,50]

Randomized controlled trials in lupus nephritis have demonstrated that cyclophosphamide retards progressive scarring within the kidney, preserves renal function, and reduces the risk for the development of end-stage renal failure requiring dialysis or renal transplantation.[51–56] Most recent studies have emphasized an important role for intermittent monthly boluses of intravenous cyclophosphamide in the preservation of renal function in lupus nephritis.[57–59] Studies suggest a similar important role in children with lupus nephritis.[60–64]

Variables such as drug dose, interval between boluses, and duration of intravenous cyclophosphamide therapy in lupus nephritis have not been well studied so as to define the optimal regimen for treatment. The relapse rate after 6 months of continuous monthly therapy is approximately 50% with some evidence to suggest that chronic maintenance therapy with less frequent infusions of cylophosphamide[53,54] reduces the likelihood of relapse.

There are no good studies that provide guidelines to indicate when cyclophosphamide should be discontinued. It is commonly recommended that cyclophosphamide be continued for a period of one year after the nephritis has been judged to be in remission; however, there are no data to support the recommendation. The relapse rate following even chronic therapy appears to be substantial,[65–67] and a further course of intravenous cyclophosphamide may be needed for patients who relapse.[68] In addition, a number of patients with severe nephritis fail to show any evidence of improvement with cyclophosphamide therapy.[69] Recent studies suggest that African-American lupus patients,[70] as well as patients with an elevated serum creatinine and advanced interstitial fibrosis[71] respond less well to drug treatment.

Although less rigorously studied, there is convincing evidence from case reports that intravenous cyclophosphamide has an important role in the management of most other forms of serious, major organ lupus. Improvements in a variety of neurologic or psychiatric manifestations have been reported following cyclophosphamide including transverse myelitis,[72–75] mononeuritis multiplex,[76] organic brain syndrome,[37] and optic neuropathy.[78] Other major organ lupus manifestations that have been reported to have responded to treatment with intravenous cyclophosphamide include hematologic abnormalities,[79–82] vasculitis,[83–85] myositis,[86] interstitial lung disease[87], pulmonary hemorrhage,[88] pulmonary hypertension,[89] and cardiomyopathy.[90]

There has been limited experience with combining cyclophosphamide with other therapeutic approaches. Combination regimens of oral cyclophosphamide and azathioprine have been studied in lupus nephritis.[51,91] A metaanalysis performed on a small number of patients reported that the drug combination was more effective than prednisone alone in reducing the incidence of end-stage renal disease.[92] Studies have shown that the sequential use of oral cyclophosphamide followed by maintenance oral azathioprine may preserve renal function and prevent disease relapses.[93,94] The use of combination methotrexate and cyclophosphamide has not been studied in adult SLE; however, there have been reports of its use in pediatric populations with beneficial results.[95] The combination of intravenous methylprednisolone and cyclophosphamide has been reported to increase the likelihood of remission in patients with lupus nephritis.[54,56] Recent evidence suggests that the combination of intravenous cyclophosphamide with plasmapheresis has little role in the management of severe, major organ lupus.[96,97]

Recent interest has focused on the use of higher-doses of cyclophosphamide as 'immunoablative' therapy followed by the infusion of autologous stem cells[98–102] or bone marrow growth factors.[103,104] The clinical experience with this approach is limited, although marked improvements in patients with severe forms of lupus have been well documented. Whether the improvements are a result of the cyclophosphamide alone or occur as a result of a readjustment of the immune system due to the replacement or growth of autologous stem cells is unknown. Studies are currently in progress to determine how the therapy compares with conventional monthly intravenous therapy in patients with nephritis and treatment—resistance cutaneous lupus. The risks of toxicities increases with high-dose cyclophosphamide.

The potential toxicities of cyclophosphamide administration are substantial (Table 5), and serve as a major deciding factor in patient selection of immunosuppressive therapy in lupus.[105] Nausea and vomiting from stimulation of central nervous system vomiting receptors are common, particularly after intravenous infusions, and occur approximately 12 hours after drug administration. In most patients, premedication with antiemetics such as oral ondansetron combined with dexamethasone[106] effectively minimizes gastrointestinal side effects. Hair loss is common with cyclophosphamide therapy and may occasionally be severe so as

TABLE 5 Potential Toxicities of Cyclophosphamide

Constitutional	Malaise, irritability, weight loss
Gastrointestinal	Anorexia, nausea, vomiting, diarrhea, abdominal cramps
Dermatologic	Alopecia, nail changes
Cardiopulmonary	Myocardial necrosis, pulmonary fibrosis (high dose only)
Genitourinary	Hemorrhagic cystitis, bladder fibrosis, bladder carcinoma, gonadal failure
Metabolic	Inappropriate antidiuretic hormone syndrome
Hematologic	Leukopenia (dose dependent), aplastic anemia with chronic therapy
Malignancy	Gladder, skin, cervical (?), and vulvar (?) carcinoma

to require a wig for cosmetic purposes. Patients need to be reassured that the hair will regrow even with continued therapy. Cyclophosphamide is a well established teratogen,[107,108] and effective birth control during therapy is essential.

The cytotoxic action of cyclophosphamide is most commonly expressed by its effects on bone marrow function, particularly while blood cells.[109] Peripheral leukopenia is dose dependent with the nadir of the depression occurring from 8 to 12 days after intravenous drug administration or increase in oral drug dose. During this period, serial white counts should be obtained several times to document the effect and to alter the drug does accordingly. As a general rule, the absolute white blood count should not be depressed below 2000 cells per cubic milliliter and the drug dose should be adjusted accordingly to maintain counts above this level.

Cyclophosphamide increases patient susceptibility to bacterial, fungal, and viral infections.[110–114] Several drug-induced effects such as leukopenia, depressed antibody synthesis, and altered cellular immune function likely contribute to this increased susceptibility. Risk factors shown to be associated with serious infection following cyclophosphamide include multiple organ involvement, concomitant high-dose corticosteroid administration, and white blood cell count nadirs below 3000 cells/mm^3.[111,115] Prophylaxis with low-dose trimethoprim/sulfamethoxazole to prevent *Pneumocystis carinii* has been recommended in non-sulfa-allergic patients with vasculitis treated with the combination of cyclophosphamide and high-dose daily corticosteroids.[114]

Damage to gonadal tissue follows prolonged cyclophosphamide administration and leads to ovarian failure and oligo/azoospermia.[116–124] During cyclophosphamide treatment serum levels of follicle-stimulating and luteinzing hormones are increased, and estradiol levels are reduced. The deficiency of estrogens produces signs and symptoms of menopause with amenorrhea, hot flashes, endometrial hypoplasisa and atrophy of vaginal epithelium. Pathologic examination of ovaries following prolonged cyclophosphamide treatment reveals destruction of follicles and fibrosis of the interstitial areas. In men, the primary damage is to the germinal epithelial lining layer of the seminiferous tubules. Clinically, there is a decrease in testicular volume and oligo or azoospermia leading to infertility. This associated with marked elevations of serum follicle-stimulating hormone, a measurement that might be useful in monitoring cyclophosphamide effects on the testes. Libido and sexual function are generally unaffected. Recovery of spermatogenesis or ovarian function following cyclophosphamide is unpredictable, although has been observed even months or years after the drug has been discontinued. Several studies have shown that patient age and cumulative drug dose are the two major factors that determine the risk of ovarian failure.[121–125] Cumulative drug dose also appears to be a factor in menstrual disorders that occur in adolescent girls with lupus treated with cyclophosphamide.[123] Recent evidence suggests that testosterone may prevent azoospermia in men treated with oral cyclophosphamide.[126] Theoretically estrogen supplementation or the use of gonadotropin-releasing hormone agonists[127] should help prevent ovarian failure, however neither strategy has been well studied. Ova or sperm banking prior to cyclophosphamide administration might be considered in selected patients.

Several forms of bladder toxicity, a complication unique to cyclophosphamide and not apparent from other alkylating agents, are evident including hemorrhagic cystitis,[128] fibrosis,[129] and transitional and squamous cell carcinoma.[130–133] Bladder complications appear to be associated with oral drug administration with no evidence, at least to date, that intermittent, intravenous therapy causes bladder problems. The drug metabolite acrolein has been shown to be the irritant responsible for acute cystitis.[134] Daily oral cyclophosphamide should be taken in the morning with attention to generous fluid intake throughout the day to reduce concentrations of acrolein in the bladder to minimize the risk of bladder toxicities. In patients treated with intravenous bolus cyclophosphamide, mesna (Mesnex) which binds acrolein is increasingly being used in conjunction with intravenous bolus cyclophosphamide therapy as a way to further protect the bladder, particularly in patients with know cyclophosphamide-induced bladder damage in whom continued drug administration is required.[135,136] The dose of mesna used is 20% of the total cyclophosphamide dose given either intravenously or orally start-

ing immediately before cyclophosphamide administration and every 3 hours thereafter for a total of 4 doses. In lupus patients with urinary retention, for example a neurogenic bladder secondary to transverse myelitis, catheterization with bladder irrigation using a three-way Foley catheter should be used for 24 hours following the cyclophosphamide infusion.

Patients treated with prolonged courses of cyclophosphamide probably should be screened indefinitely for malignant changes in the bladder. Urine cytology is useful if abnormal but has a poor sensitivity to detect low-grade cancers. Nonglomerular hematuria has been found to be the most useful marker to identify patients at risk for developing bladder cancer and urinalysis should be done every 3–6 months even after cyclophosphamide has been discontinued.[128] The development of gross or microscopic hematuria, particularly of new onset and after prolonged drug administration, should be assessed by cystoscopy for possible malignant changes of the bladder. Patients treated with cyclophosphamide who develop evidence of reduced bladder capacity, such as frequency and small urinarv volumes, should undergo cystometric evaluation.

Cyclophosphamide is a well-documented carcinogen in animals and a number of case reports have documented the development of malignancies in patients treated with cyclophosphamide.[133] A United Kingdom-Australasian collaborative study of immunosuppression in nontransplant patients suggests an increased overall risk of malignancy following cyclophosphamide (13 observed versus 5.86 expected, $P < 0.03$).[137,138] By comparision, the relative risk of bladder cancer was found to be 10.3 ($P < 0.01$). Malignancies of hematopoietic or lymphoreticular origin have been of particular concern. Although the quantitative risks of these malignancies in lupus patients treated with cyclophosphamide are unknown, case reports of reticulum cell sarcoma,[139] lymphoma,[140] and leukemias[140–145] have been observed. It is encouraging that follow-up studies of large series of lupus patients treated with cyclophosphamide have failed to detect evidence of increases in malignancies with the possible exception of cervical and vulvar malignancies.[146,147]

Unusual rare complications of cyclophosphamide therapy that have been reported include interstitial pneumonitis,[148] myocardial necrosis with doses,[149] liver damage,[150] inappropriate antidiuretic hormone syndrome, [151,152] and various hypersensitivity reactions.[153,154]

Chlorambucil

Chlorambucil (Leukeran) is an aromatic bifunctional alkylating agent with substitution of the N-methyl group of mechlorethamine with phenylbutyric acid. The drug is given orally (0.1–0.20 mg/kg body weight daily) with good absorption. Serum half-life is approximately 90 minutes with drug metabolism involving both methylation and beta-oxidation. Drug effects on immune functions are comparable to those described from cyclophosphamide.

There are few clinical studies of chlorambucil in systemic lupus, but improvements in nephritis as well as manifestations such as central nervous system signs, vasculitis, and multisystem involvement have been reported.[155–157] Recent studies in idiopathic membranous nephropathy have shown that combined methylprednisolone and chlorambucil significantly alters the long-term course of the renal disease.[158–159]

Side effects of chlorambucil are similar to those observed with cyclophosphamide with the exception that there is no bladder toxicity associated with chlorambucil. Drug monitoring is the same as that described for cyclophosphamide (see Table 4). Bone marrow suppression, including irreversible marrow failure, may be more severe and less predictable that with cyclophosphamide. There is a markedly increased risk of leukemia, in particular acute myeloblastic leukemia, with chlorambucil.[160,161]

ANTIMETABOLITE IMMUNOSUPPRESANTS

Azathioprine

Early clinical experience with purine analogs in the treatment of lupus included studies of 6-thioguanine and 6-mercaptopurine.[162–165] The only purine analog that continues to be widely used in lupus management is azathioprine, an involves enzymatic conversion by sulfhydral compounds into 6-mercaptopurine. Metabolism of azathioprine involves two major pathways. Direct oxidation by the enzyme xanthine oxidase yields 6-thiouric acid that is eventually converted to uric acid. This pathway can be blocked by the xanthine oxidase inhibitor allopurinol. Metabolism is then shifted to the second major pathway which involves methylation of the sulfhydral group and subsequent oxidation. Mono-, di-, and triphosphate nucleosides of the methylthiopurine products accumulate, which leads to alterations of cellular purine biosynthesis as well as DNA function. Because metabolites from this latter pathway produce the immunosuppressive and toxic effects of 6-mercaptopurine, the shift of drug metabolism produced by allopurinol significantly enhances drug effects.[166] Therefore, it is important that the dose of azathioprine be decreased in patients receiving concomitant treatment with allopurinol. Generally, a reduction of the standard

TABLE 6 Dosage and Potential Toxicities of Azathioprine

Dose	Monitoring Recommendations
Oral 1–3 mg/kg/day*	Complete blood count every 1–2 weeks with changes in dose and every 1–3 months thereafter. Liver enzymes every 4 months.
Toxicities	
Constitutional	Malaise, weight loss, fever
Gastrointestinal	Anorexia, nausea, vomiting, diarrhea, abdominal pain, hepatotoxicity
Dermatologic	Hypersensitivity skin eruptions
Hematologic	Leukopenia, macrocytic anemia
Neurologic	Aseptic meningitis
Maligancy	Cervical atypia, non-Hodgkin's lymphoma, leukemia

* Reduce dose in patients concomitantly treated with allopurinol

dose to approximately one-third is sufficient to produce comparable effects achieved by full dose in most patients.

Initial oral doses of between 25 and 50 mg/day of azathioprine are suggested to determine acute toxicity/sensitivity; in addition drug metabolism varies widely in individual patients and may account for both efficacy and toxicity. (Table 6) The dose can be increased by 0.5 mg/kg/day every 4–6 weeks with the goal of achieving a range between 2 and 3 mg/kg/day. Monitoring should include full hematologic testing every 1–2 weeks while the dose is being adjusted and every 1–3 months thereafter.[167] Liver function tests and serum creatinine should be performed every 4–6 months.

There have been multiple clinical studies of azathioprine in patients with lupus that have documented improvements in various nonrenal manifestations, stabilization or improvement of impaired renal function, reduction of proteinuria, and corticosteroid-sparing properties of the drug.[168–179] A serial histologic study in lupus nephritis reported a decrease in cellular proliferation and electron dense deposits as well as a transition from proliferative to membranous disease following azathioprine treatment.[173] Exacerbations of disease observed in the several month period following azathioprine withdrawal have been regarded as further evidence of efficacy.[174]

A number of controlled trials have evaluated the effects of azathioprine in patients with lupus nephritis.[180–187] Although the studies themselves reached different conclusions as to whether azathioprine modified the course of lupus nephritis, a meta-analysis combining the data from these trials concluded that azathioprine was a valuable drug in the management of lupus nephritis.

Other approaches using azathioprine in the treatment of lupus have included combination with low-dose cyclophosphamide,[182,188] heparin,[184] bolus methylprednisolone,[189] and antilymphocyte globulin.[179] The efficacy of any of these combinations remains to be proven.

The most common side effects of azathioprine that limit therapy involve various forms of gastrointestinal intolerance and bone marrow toxicity (Table 6).[190] Azathioprine affects both erythroid and myeloid elements of the bone marrow. Macrocytosis often with megaloblastic erythroid changes of the marrow develops in the absence of vitamin B_{12} or folic acid deficiency. Rarely, patients with severe macrocytosis have progressed to selective erythroid hypoplasia. The onset of leukopenia may be abrupt and blood counts should be monitored regularly to detect any changes early.[167,191] Bone marrow toxicity is generally reversible with reduction of the dose or discontinuation of the drug.

Azathioprine increases patient susceptibility to infections from common bacterial and opportunistic organisms. Azathioprine can produce elevations of liver enzymes, particularly the pyruvic and glutamic oxaloacetic transaminases. On liver biopsy hepatocellular necrosis and mild biliary stasis have been seen. Hepatotoxicity is thought to result from drug hypersensitivity and may be accompanied by clinical features such as fever, diffuse abdominal pain, diarrhea, and maculopapular skin rash.[192] Hepatic abnormalities are typically reversible upon stopping the drug. There are a number of well-documented reports of azathioprine-induced pancreatitis.[193–197] In addition, a severe hypersensitivity reaction with fever, hypotension, and oliguria has been described.[198,199]

Although uneventful pregnancies have been documented in transplant recipients, lupus patients, and patients with other inflammatory conditions treated with azathioprine,[200–205] it is generally recommended that birth control be used during azathioprine therapy and that the drug not be given during pregnancy or periods of breastfeeding (FDA Use in Pregnancy Rating D, Table 3).[206] Both congenital defects and evidence of severe immune deficiency have been described in infants born to mothers treated with azathioprine during pregnancy.[207,208] Azathioprine is known to induce chromosome abnormalities as well as increases in sister chromatic exchanges. The relationship of chromosomal defects to teratogenicity is unknown.[209]

There is strong evidence to suggest that azathioprine is carcinogen in humans. Case reports of the development of solid tumors, lymphoma, and leukemia[210–215] and a fourfold increase in cervical atypia[216] in lupus patients treated with azathioprine have been documented. An epidemiologic survey identified statistically increased frequencies of non-Hodgkin's lymphoma and reticlum

cell sarcomas in patients treated with azathioprine for indications other that organ transplantation.[217,218]

Mycophenolate Mofetil

Mycophenolate mofetil has been used in the prevention of renal allograft rejection. It is a potent inhibitor of inosine monophosphate dehydrogenase, an enzyme critical for *de novo* synthesis of guanine nucleosides. Therefore, it has similar functional consequences as the purine analogs. Mycophenolate mofetil is rapidly hydrolyzed to its active form mycophenolic acid. The functional selectivity of mycophenolic acid for T and B-lymphocytes derives from the reliance of these cells on the *de novo* purine pathway rather than the hypoxanthine-guanine phosphoribosyl transferase salvage pathway for purine biosynthesis. Its effects on immune function include suppression of T and B lymphocyte proliferation, monocyte activation, antibody formation, and recruitment of leukocytes into inflammatory sites.

Both oral and parenteral preparations are available. The drug has excellent bioavailability and has a half-life of seventeen hours. It is metabolized to its active form which is excreted in urine and bile. Dosage adjustment is recommended for patients with impaired renal function. There have been no studies performed in pregnant women, but the drug has shown teratogenic effects in animals. Therefore, it is recommended that pregnancy be avoided in patients taking mycophenolate mofetil (FDA Category C Table 3).

Mycophenolate mofetil has been used extensively in the prophylaxis of organ rejection concomitantly with cyclosporine and corticosteroids in patients receiving allogenic solid organ transplantation. It has been shown to be more effective than azathioprine in the prevention of acute rejection of transplanted organs.[219] The drug is given orally (1–2 gm twice daily) on an empty stomach with rapid absorption and hydrolysis to free mycophenolic acid in the liver and gastrointestinal tract. The most common adverse reactions reported are gastrointestinal intolerance, leukopenia, and infection which appear to improve with lower doses.

Recent studies have begun to assess its role as an immunosuppressive agent in immunologic disorders, including lupus. In murine models, mycophenolate mofetil has been shown to inhibit the development of nephritis and increase survival.[220,221]

Mycophenolate mofetil has been used with some success in rheumatoid arthritis and psoriasis. Studies in patients with lupus have reported benefit in patients with cyclophosphamide refractory nephritis.[222,223] A more recent study reported that treatment with mycophenolate mofetil in combination with corticosteroids was as effective as an oral regimen of cyclophosphamide for six months followed by azathioprine for six months.[224] Relapse rates were similar in both groups as well. Mycophenolate mofetil was generally well tolerated with infections being the most common adverse event.[225] Common side effects are leukopenia and diarrhea. A randomized controlled trial is underway comparing monthly intravenous cyclophosphamide with mycophenolate mofetil in lupus nephritis.

Methotrexate

Clinical experience with methotrexate (MTX) in SLE has been until recently limited to case series and open-label trials.[225–235] Most dose schedules have involved low-dose, weekly oral or parenteral doses with escalations and dose ranges (7.5–15 mg/week) similar to those used in rheumatoid arthritis. Because MTX is eliminated by both glomerular filtration and active tubular secretion, reductions in dose are necessary in patients with impaired renal function. Absorption appears to be dose dependent and peak serum levels are reached within 1–2 hours. In general, doses of 30 mg/m^2 or less are well absorbed of which 60% is bioavailable.

After oral or parenteral administration, MTX is present either in its native state or an active metabolite 7-hydroxy-MTX for only a few hours. These compounds are taken up by Kupfer cells and converted intracellularly to their polyglutamate forms, which are both biologically active and have longer half-lives than the parent compound. It is thought that it is the accumulation of MTX polyglutamates that is, in part, responsible for both the therapeutic and toxic effects of the drug. Although the mechanism of action is unknown, the therapeutic effects of MTX have been attributed to properties: (1) the inhibition of methylation reactions and (2) the promotion of adenosine release.[236]

Analogs of folic acid inhibit cellular single-carbon transfer reactions necessary for the biosynthesis of purine nucleotides, thymidilate, and ultimately DNA and RNA. Normally, folic acid is actively transported in the cell, converted to dihydrofolate, and reduced to tetrahydrofolic acid by the enzyme dihydrofolate reductase. The tetraphydrofolate serves as a coenzyme onto which single carbon fragments are added and then transferred as part of multiple specific cellular biosynthetic reactions. Folic acid analogs, all of which are 4-amino folate derivatives, have an affinity for the dihydrofolate reductase, which is much greater than the natural substrate and prevents the formation of tetrahydrofolate. Folate metabolism is thus blocked at the level of dihydrofolate, a metabolically inactive product, a cellar synthetic reactions involving single carbon transfer are prevented.

Methotrexate polyglutamates inhibit the enzyme 5-aminoimidazole-4-carbonxamide ribonucleotide (AICAR) transformylase leading to the intracellular accumulation of AICAR. Intracellular accumulation of AICAR inhibits AMP deaminase leading to the accumulation of adenosine and its subsequent release of adenosine into the extracellular space. Interaction of adenosine with it's A2 receptor on cell surfaces has been reported to inhibit release of toxic oxygen metabolites, inhibit lymphocyte proliferation to mitogens, and induce suppresser phenotype and function. Adenosine has also been demonstrated to inhibit the production of TNF_{α} IL-8, and increases in IL-10 production. Although these studies have largely involved animal models of rheumatoid arthritis, MTX has also been shown to produce the same effects in murine models of SLE.[237] This has let to the hypothesis that promotion of adenosine release mediates at least in some part the anti-inflammatory effects of low-dose MTX therapy in several autoimmune diseases.

Clinical studies have emphasized improvements in polyarthritis, skin rashes, serositis, fever, and leukocytoclastic vasculitis in lupus patients treated with MTX. In addition, MTX has also been reported to have a steroid-sparing effect and to lower overall measures of disease activity such as the SLEDAI score.[233] Reports of improvement in renal manifestations have been conflicting and most series have excluded patients with either renal or central nervous system manifestations.[231] MTX in low, weekly doses has been shown to be useful as adjunctive therapy for selected cases of childhood SLE.[225] Prompt recurrences of disease activity with discontinuation of MTX have been documented in most reports, while others have described sustained benefit over a period of months when used for severe, refractory cases of lupus.

One controlled trial reported suppression of disease activity with doses ranging from fifteen to twenty milligrams per week over a six month period.[238] As with the open label studies, articular and cutaneous manifestations appear to be the most responsive to therapy.

Toxicities of MTX have consisted mainly of gastrointestinal intolerance, malaise, transient elevations of transaminases, mucositis; no instances of pulmonary toxicities have been described in SLE. Administration of folic acid may reduce some of these adverse events without decreasing efficacy. MTX can cause teratogenic effects and fetal death and should not be used in women considering pregnancy. Pregnancy should be avoided if either partner is receiving therapy with MTX during treatment and for a minimum of 3 months after discontinuation for males and one ovulatory cycle for females (FDA Category D Table 3).

Overall, studies of the use of MTX in SLE are encouraging and suggest that MTX may be used as an alternative to antimalarials or low-dose corticosteroids for the management of musculoskeletal manifestations, serositis, and constitutional symptoms. Furthermore, in view of its relatively less severe long-term side effects when compared to cyclophosphamide therapy, further evaluation of the use of MTX in lupus involving major organs appears to be warranted.

Leflunomide

Leflunomide is an isoxazole derivative that has been approved for the treatment of rheumatoid arthritis to reduce symptoms and reduce structural damage.[239–241] The drug is rapidly absorbed after oral dosing and converted into the active metabolite A77–1726 (a malononitrilamide). The half life of the metabolite is approximately 15 days. There is extensive enterohepatic recirculation of the drug and it is strongly bound to plasma proteins. The mechanism of action is by inhibition of pyrimidine synthesis thereby resulting in anti-inflammatory effects. This occurs through inhibition of the enzyme dihydroorrotate dehydrogenase leading to decreased production of rUMP in lymphocytes. Low levels of rUMP in lymphocytes prevent further cellular activation. This leads also to decreased production of inflammatory cytokines.

Because of its long half life, leflunomide requires a loading dose of 100 mg/day for three days followed by 20 mg/day thereafter. The drug is eliminated through the feces and urine. Leflunomide is teratogenic and is contraindicated in patients trying to become or are pregnant. To accelerate the elimination of the drug, cholestyramine (8 gm orally for 8–11 days) is recommended.

Animal models have shown that leflunomide is effective in preventing (1) the development of autoantibodies in the MRL/lpr mouse, and (2) development of tubulointerstitial nephritis in rats.[242,243]

Leflunomide has been reported to be safe and efficacious in patients with rheumatoid arthritis.[239–241] Results from a pilot study of eighteen patients with lupus treated with leflunomide would seem to suggest efficacy and safety.[244] Patients were treated with standard doses and evaluated after two to three months of therapy. Two thirds of the patients treated had reported improvement despite failing other conventional therapy. Large placebo controlled trials will be needed to confirm the role of this agent in the treatment of lupus.

MACROLIDE IMMUNOSUPPRESSANTS

Studies in murine models of lupus suggest an important role for the macrolide immunosuppressive agents in the prevention and treatment of nephritis.[245–247] To date, however, clinical information is limited to a small number of trials done with cyclosporine and tacrolimus.

Cyclosporine

Cyclosporine has been widely used to prevent rejection in patients with kidney, liver and heart transplantation. It has also been studied in severe rheumatoid arthritis and less frequently in lupus. It is a cyclic, lypophilic polypeptide produced as a metabolite by the fungus *Beauveria nivea*. The lipid solubility of the drug requires that it be given in a liquid oil base; however, the newer preparation form a microemulsion in aqueous environments. The absorption of the drug from gastrointestinal tract in incomplete and highly variable, although newer microemulsion formula is reported to have better bioavailability and less intrasubject variability. Oral preparations have about 30% bioavailability compared to that of intravenous administration, and peak serum levels are achieved in 3–4 hours. The biliary tract is the major pathway of drug excretion. Neither dialysis nor renal failure appears to significantly alter clearance of the drug. The pharmacokinetic properties vary widely with age of the patient, the presence of concomitant diseases, and the use of many other medications. Serum cyclosporine levels are possible to monitor; however, levels reflect neither the potential for toxicity nor efficacy. The minimal effective daily dose has not been established but is probably less that 5 mg/kg body weight. The two forms of cyclosporine are not bioequivalent, and dose adjustments are required when changing to the microemulsion formulation. A 1:1 dosage is usually started with adjustments made to attain preconversion trough concentrations.

The mechanism of action of cyclosporine has been reported to be the inhibition of T-cell-receptor-induced IL-2 transcription through inhibition of the calcium/calcineurin induced nuclear translocation of NF-ATc.[248] It inhibits the production of Interleukin-2 and multiple other inflammatory cytokines by lymphocytes. Generation of cytotoxic T cells is also suppressed. In animal studies, improvements included survival of skin grafts, reduced swelling of adjuvant arthritis, and modulation of a cell-mediated model of autoimmune uveitis.

Most of the literature on cyclosporine in the treatment of SLE consists of brief reports with small numbers of patients treated in an open-label fashion with short periods of follow-up.[249–253] Although improvements in lupus nephritis have been noted in several series, other studies have failed to document any clinical benefits. Cyclosporine appears to produce remission in adults and children with nephritic syndrome associated with minimal-change disease. It also has been reported to produce remission in children with focal and segmental glomerulosclerois; however, it has been less effective in adults and in individuals resistant to steroids. It appears to reduce proteinuria and to slow progression of renal insufficiency in membranous forms of glomerulonephritis. There have been several reports of the effectiveness of cyclosporine in patients whose disease was either poorly responsive or unresponsive to treatment with steroids, cytotoxic agents, or both.[252,253]

Most improvement in disease activity has been reported with 4–8 weeks of drug initiation, and it has been suggested that prolonged treatment should be aimed more at consolidating clinical results rather than achieving further improvements. Relapses are common after drug discontinuation. Adverse drug reactions encountered included hypertrichosis, paresthesias, gastrointestinal symptoms, tremors, gingival hyperplasia, and a peculiar angioedema with reduction of C1 esterase inhibitor levels. It is the side effects of hypertension[254] and direct nephrotoxicity[255] of cyclosporine that appear to limit the usefulness of the drug in the management of lupus. In particular, studies have shown a clear dose-dependent reduction in renal function that was only partially reversed upon discontinuation of the decrease in dosage of cyclosporine. Risk appears to be increased with initial or maximal doses greater than 5 mg/kg/day; however, even in lower doses nephrotoxicity and hypertension have been reported.[256,257] Toxicity appears to be additive when cyclosporine is used in combination with nonsteroidal anti-inflammatory drugs.[238] Cyclosporine-induced hypertension is generally treated by angiotensin-converting enzyme inhibitors or B-blockers. Metabolism of cyclosporine is reduced with concomitant diltiazem, ketoconazole, rifampin, and phenytoin use. Cyclosprine is rate Category C for use in pregnancy (Table 3)

A review of over two hundred patients (in twelve studies) reported that treatment with doses of 2.5 to 5.0 mg/kd/day were well tolerated.[258] Improvement in the following parameters were reported: proteinuria, cytopenias, complement, double stranded DNA antibody, and general disease activity scores.[258] Adverse events reported were mild nephrotoxicity, hypertension, gingival hyperplasia, and hypertrichosis—most of which improved with discontinuation of the drug. One group has reported efficacy of cyclosporine particularly in patients with membranous nephropathy.[259]

Cyclosporine should be considered in patients with SLE and major organ involvement (especially membranous nephritis) whose disease is either poorly responsive or unresponsive to steroids, cytotoxic drug, or both.

Tacrolimus

Tacrolimus is a potent macrolide immunosupressant, 10–100 times as potent as cyclosporine.[260,261] It is chemically unrelated to cyclosporine, however has a similar mechanism of action in the inhibition of T cell activation.[262] It appears to be efficacious in the treatment of autoimmune, inflammatory, and allergic skin diseases through at least three distinct mechanisms. First, Tacrolimus suppresses the activation of T cells by inhibiting the expression of early T-cell response genes. It binds to a cytosolic FK binding protein, forming a complex which inhibits the ability calcineurin to dephosphorylate a subunit of the transcription factor NF-AT. Thereby preventing the subunit of NF-AT from translocating across the nuclear membrane and associating with its nuclear counterpart, thereby diminishing transcription and expressing of all genes activated by NF-AT (including IL-2) (Figure 1. Second, Tacrolimus possesses strong anti-pruritic activity, and has been shown to inhibit IgE mediated histamine release from mast cells, diminish transcriptional activation of IL-3 & IL-5, and downregulate the expression of leukotrienes.[263] Finally, Tacrolimus has also been demonstrated to inhibit the expression of IgE on Langerhans antigen presenting cells in the epidermis and inhibit their ability to stimulate autologous lymphocytes.[263,264]

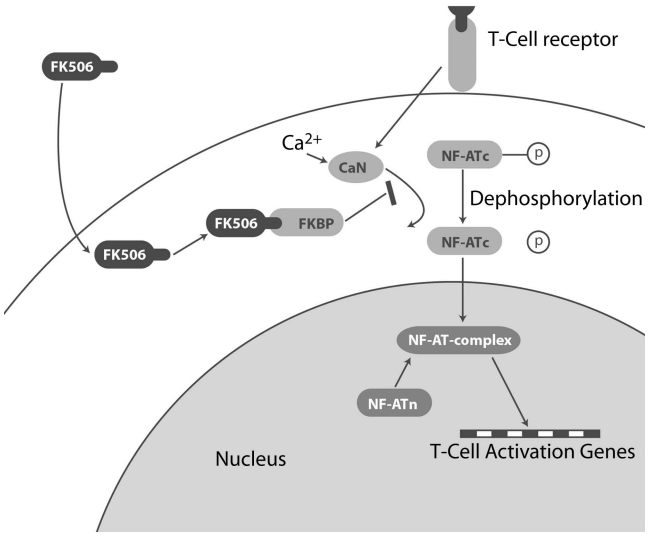


FIGURE 1 Mechanism of action of tacrolimus. Tacrolimus suppresses the immune response by preventing dephosphoralyation of the nuclear transcription factor NF-Atc which is required for nuclear translocation NF-AT. This action then bocks the expression of early T-cell activation genes.

A single oral dose, tacrolimus reaches peak blood levels within 30 to 180 minutes, with a mean bioavailability of 16–22% and half-life of 40 hours.[265] Oral doses of 0.10, 0.15, and 0.20 mg/kg lead to maximum blood levels of 19, 24, and 48 ng/ml respectively in renal transplant recipients.[265] Tacrolimus is strongly bound to plasma proteins and red blood cells. Hepatic metabolism represents the major route of elimination.[265]

As with cyclosporine, the clinical use of systemic tacrolimus has been associated with a dose-dependent and reversible nephrotoxicity as demonstrated in kidney transplant recipients.[260] Following liver transplantation, tacrolimus has also been shown to lead to a lower incidence of hypertension than cyclosporine.[260] Less frequent reported side effects of systemic use of tacrolimus include cardiomyopathy in children, anxiety, seizures, delerium and tremor, diabetes, and hyperlipidemia.[260,261]

Both oral and topical forms of the drug have been studied. To date, with over 5,000 patients studied, the use of topical tacrolimus has not been associated with glucose intolerance, hypertension, nephrotoxicity, or neurotoxicity.[262] In a clinical trial of topical tacrolimus conducted in 180 children 7–16 years of age with atopic dermatitis, no systemic side effects were reported. The most common local adverse events noted were increased pruritis and burning only during the first four days of application.[266] There were no significant changes in laboratory values throughout the 22 days of the study, and no increase in skin infections was observed.[266] These results in pediatric patients were confirmed earlier this year in a long-term safety trial conducted in children ages 2–15 with atopic dermatitis.[267] In the European Tacrolimus Ointment Study Group, 316 adult patients greater than 18 years of age were studied for 6–12 months. The study revealed no significant change in laboratory parameters, with minimal systemic levels of tacrolimus detected (76% with less than 1 ng/ml).[268] Adverse events reported were local irritation, pruritis, erythema, and burning which tended to occur during therapy initiation.[268]

Recently, topical tacrolimus was approved by the FDA for use in atopic dermatitis. In numerous studies, topical tacrolimus has been found to be superior to placebo in improving symptom scores and reducing the skin scores of patients with atopic dermatitis, including several patients with disease refractory to topical cyclosporine and steroids.[260-263,266–268] Doses of 0.03%, 0.1%, and 0.3% were used in one trial for atopic der-

matitis and all doses found to be equally efficacious.[261] Other applications of topical tacrolimus to treat autoimmune skin disease include its successful use for erosive mucosal lichen planus.[269,270] In one series of 6 patients with severe refractory disease, application of topical tacrolimus 0.1% resulted in improvements in all patients, with 3 showing complete resolution of disease.[269] Treatment successes with topical tacrolimus have also been reported for pyoderma gangrenosum and the bald-rat model of alopecia areata.[260,261,271]

Although showing positive results with oral tacrolimus, the treatment of psoriasis has been less impressive than topical preparations. However it was shown to be effective in patients in whom the ointment was applied under occlusion after removal of psoriatic scale. Oral tacrolimus has been found to be effective in several cases series to treat the skin and mucosal manifestations of Behçet's disease.[261,272]

To date, there have been no reports of clinical trials involving the use of oral or topical tacrolimus to treat the manifestations of lupus. In animal models, the intraperitoneal administration of tacrolimus has been found to be effective in reducing the incidence of skin lesions in the MRL/lpr mouse model of SLE.[272] Finally a case series of 3 patients with severe, refractory SLE has been reported showing a benefit in 2/3 patients in controlling manifestations of the disease, including vasculitis. A recent case series reported the efficacy of topical tacrolimus in patients with lupus.[273] Further controlled studies are needed to assess the role of tacrolimus in the treatment of lupus.

Reference

1. Payne, J. F. (1894). A postgraduate lecture on lupus erythematosus. *Clin. J.* **4**, 223.
2. Page, F. (1951). Treatment of lupus erythematosus with mepacrine. *Lancet*. **2**, 755.
3. Fox, R. I. (1993). Mechanisms of action of hydroxychloroquine as an antirheumatic drug. *Semin. Arthritis. Rheum.* **23**(Suppl 1), 82.
4. Doglia, S., Gräslund, A., and Ehrenberg, A. (1986). Specific interactions between quinacrine and self-complementary deoxynucleotides. *Anticancer. Res.* **6**, 1363.
5. Guidos, C., Wong, M., and Lee, K. C. (1984). A comparison of the stimulatory activities of lymphoid dendritic cells and macrophages in T proliferative responses to various antigens. *J. Immunol.* **133**, 1179.
6. Nowell, J., and Quaranta, J. (1985). Chloroquine effects biosynthesis of Ia molecules by inhibiting dissociation of invariant (γ) chains from α-β dimers in B cells. *J. Exp. Med.* **162**, 1371.
7. Papaioannou, M., Fishbein, D. B., Dresen, D. W., *et al*. (1986). Antibody response to preexposure to human diploid-cell rabies vaccine given concurrently with chloroquine. *N. Engl. J. Med.* **314**, 280.
8. Van Der Straeten, C., and Klippel, J. H. (1986). Antimalarials and pneumococcal immunization. *N. Engl. J. Med.* **315**, 712.
9. Ferrante, A., Rowan-Kelly, B., Seow, W. K., and Thong, Y. H. (1986). Depression of human polymorphonuclear leucocyte function by anti-malarial drugs. *Immunology.* **58**, 125.
10. Panus, P. C., and Jones, H. P. (1987). Inhibition of neutrophil response by mepacrine. *Biochem. Pharmacol.* **36**, 1281.
11. Hurst, N. P., French, J. K., Bell, A. L., *et al*. Differential effects of mepacrine, chloroquine and hydroxychloroquine on super-oxide anion generation, phospholipid methylation and arachidonic acid release by human blood monocytes. *Biochem. Pharmacol.* **35**, 3083.
12. Meng, X. W., Feller, J. M., Ziegler, J. B., Pittman, S. M., and Ireland, C. M. (1997). Induction of apoptosis in peripheral blood lymphocytes following treatment in vitro with hydroxychloroquine. *Arthritis. Rheum.* **40**, 927.
13. McCrea, J. M., Robinson, P., and Gerrard, J. M. (1985). Mecaprine (quinacrine) inhibition of thrombin-induced responses can be overcome by lysophosphatidic acid. *Biochem. Biophys. Acta.* **84**, 189.
14. Dubois, E. L. (1954). Quinacrine (Atabrine) in treatment of systemic and discoid lupus erythematosus. *Arch. Intern. Med.* **94**, 131.
15. Christiansen, J. V., and Nielsen, J. P. (1956). Treatment of lupus erythematosus with mepacrine: Results and relapses during a long observation. *Br. J. Dermatol.* **68**, 73.
16. Ziff, M., Esserman, P., and McCune, C. (1958). Observations on the course and treatment of systemic lupus erythematosus. *Arthritis. Rheum.* **1**, 332.
17. Buchanan, R. N., Jr. (1959). Quinacrine in the treatment of discoid lupus erythematosus: A five-year follow-up survey—results and evaluation. *South. Med. J.* **52**, 978.
18. Winkelmann, R. K., Merwin, C. F., and Brunsting, L. A. (1961). Antimalarial therapy of lupus erythematosus. *Ann. Intern. Med.* **55**, 772.
19. Rudnicki, R. D., Gresham, G. E., and Rothfield, N. F. (1975). The efficacy of antimalarials in systemic lupus erythematosus. *J. Rheumatol.* **2**, 323.
20. Callen, J. P. (1982). Chronic cutaneous lupus erythematosus: Clinical, laboratory, therapeutic, and prognostic examination of 62 patients. *Arch. Dermatol.* **118**, 412.
21. Wallace, U. J. (1989). The use of quinacrine (atabrine) in rheumatic diseases: A reexamination. *Semin. Arthritis. Rheum.* **18**, 282.
22. Callen, J. P. (1997). Management of skin disease in lupus. *Bull. Rheum. Dis.* **46**, 4.
23. Callen, J. P. (1997). Management of anti-malarial-refractory cutaneous lupus erythematosus. *Lupus.* **6**, 203.
24. Feldman, R., Salomon, D., and Saurat, J.-H. (1994). The association of the two antimalarials chloroquine and quinacrine for treatment-resistant chronic and subacute cutaneous lupus erythematosus. *Dermatology.* **189**, 425.

25. Williams, H. J., Egger, M. J., Singer, J. Z., *et al*. Comparison of hydroxychloroquine and placebo in the treatment of the arthropathy of mild systemic lupus erythematosus. *J. Rheumatol.* **21**, 1457.
26. Meinao, I. M., Sato, E. I., Andrade, L. E. C., Ferraz, M., and Atra, E. (1996). Controlled trial with chloroquine diphosphate in systemic lupus erythematosus. *Lupus.* **5**, 237.
27. Petri, M., Hochberg, M., Hellman, D., Bell, W., and Goldman, D. (1992). Incidence of and predictors of thrombotic events in SLE. Protective role of hydroxychloroquine (abstract). *Arthritis. Rheum.* **35**, 554.
28. Petri, M., Lakatta, C., Magder, L., and Goldman, D. (1994). Effect of prednisone and hydroxychloroquine on coronary artery disease risk factors in systemic lupus erythematosus: A longitudinal data analysis. *Am. J. Med.* **96**, 254.
29. The Canadian Hydroxychloroquine Study Group: A randomized study of the effect of withdrawing hydroxychloroquine sulfate in systemic lupus erythematosus. *N. Engl. J. Med.* **324**, 150.
30. Johnson, M. W., and Vine, A. K. (1982). Hydroxychloroquine therapy in massive total doses without retinal toxicity. *Am. J. Ophthamol.* **104**, 139.
31. Rynes, R. I. (1983). Ophthalmologic safety of long-term hydroxychloroquine sulfate treatment. *Am. J. Med.* **75**(Suppl 1A), 35.
32. Levy, C. D., Munz, S. J., Paschal, J., *et al*. Incidence of hydroxychloroquine retinopathy in 1,207 patients in a large multicenter outpatient practice. *Arthritis. Rheum.* **40**, 1482.
33. Finbloom, D. S., Silver, K., Newsome, D. A., and Gunkel, R. (1985). Comparison of hydroxychloroquine and chloroquine use and the development of retinal toxicity. *J. Rheumatol.* **12**, 692.
34. Mavrikakis, M., Papazoglou, S., Sfikakis, P. P., Vaiopoulos, G., and Rougas, K. Retinal toxicity in long term hydroxychloroquine treatment. *Ann. Rheum. Dis.* **55**, 187.
35. Easterbrook, M. (1984). The use of Amsler grids in early chloroquine retinopathy. *Ophthalmology.* **91**, 1368.
36. Parke, A. L., and West, B. (1996). Hydroxychloroquine in pregnant patients with systemic lupus erythematosus. *J. Rheumatol.* **23**, 1715.
37. Khamashta, M. A., Buchanan, N. M., and Hughes, G. R. V. (1996). The use of hydroxychloroquine in lupus pregnancy: The British experience. *Lupus.* **5**(Suppl 1), S65.
38. Rynes, R. I. (1997). Antimalarial drugs in the treatment of rheumatological disease. *Br. J. Rheumatol.* **36**, 799.
39. DuBois, E. L. (1954). Nitrogen mustard in treatment of systemic lupus erythematosus. *Arch. Intern. Med*. **93**, 667.
40. Kellum, R. E., and Haserick, J. R. (1963). Mechlorethamine therapy for systemic lupus nephropathy. *Arch. Dermatol*. **87**, 289.
41. Dillard, M. G., Dujovne, I., Pollack, V. E., and Pirani, C. L. (1973). The effect of treatment with prednisolone and nitrogen mustard on the renal lesions and life span of patients with lupus glomerulonephritis. *Nephron*. **10**, 273.
42. Wallace, D. J., and Metzger, A. L. (1995). Successful use of nitrogen mustard for cyclophosphamide resistant diffuse proliferative lupus glomerulonephritis: Report of 2 cases. *J. Rheumatol*. **22**, 801.
43. Doris, A., Vitali, C., Tincani, A., *et al*. International survey on the management of patients with SLE. III. The results of a questionnaire regarding renal involvement. *Clin. Exp. Rheumatol.* **14**(Suppl 16), S31.
44. Martinelli, R., Pereira, L. J. C., Santos, E. S. C., and Rocha, H. (1996). Clinical effects of intermittent, intravenous cyclophosphamide in severe systemic lupus erythematosus. *Nephron.* **74**, 313.
45. Martin-Suarez, I., D'Cruz, D., Mansoor, M., *et al*. (1997). Immunosuppressive treatment in severe connective tissue diseases: Effects of low dose intravenous cyclophosphamide. *Ann. Rheum. Dis*. **56**, 481.
46. D'Cruz, D., Cuadrado, M. J., Mujic, F., *et al*. (1997). Immunosuppressive therapy in lupus nephritis. *Clin. Exp. Rheumatol*. **15**, 275.
47. Ramos, P. C., Mendez, M. J., Ames, P. R., *et al*. (1996). Pulse cyclophosphamide in the treatment of neuropsychiatric systemic lupus erythematosus. *Clin. Exp. Rheumatol*. **14**, 295.
48. Houssiau, F. A., Vasconcelos, C., D'Cruz, D., *et al*. (2002). Immunosuppressive therapy in lupus nephritis: the Euro-Lupus Nephritis Trial, a randomized trial of low-doe versus high-dose intravenous cyclophosphamide. *Arthritis. Rheum*. **46**, 2121.
49. Dawisha, S. M., Yarboro, C. H., Vaughan, E. M., *et al*. (1996). Outpatient monthly oral bolus cyclophosphamide therapy in systemic lupus erythematosus. *J. Rheumatol*. **23**, 212.
50. Sigal, L. H. (1989). Chronic inflammatory polyneuropathy complicating SLE: Successful treatment with monthly oral pulse cyclophosphamide. *J. Rheumatol*. **16**, 1518.
51. Balow, J. E., Austin, H. A., Muenz, L. R., *et al*. (1984). Effect of treatment on the evolution of renal abnormalities in lupus nephritis. *N. Engl. J. Med*. **311**, 491.
52. Carette, S., Klippel, J. H., Decker, J. L., *et al*. (1983). Controlled studies of oral immunosuppressive drugs in lupus nephritis: A long-term follow-up. *Ann. Intern. Med*. **99**, 1.
53. Boumpas. D. T., Austin, H. A., Vaughan, E. M., *et al*. (1992). Severe lupus nephritis: Controlled trial of pulse methylprednisolone versus two different regimens of pulse cyclophosphamide. *Lancet*. **340**, 741.
54. Gourley, M. F., Austin, H. A., Scott, D., *et al*. (1996). Methylprednisolone and cyclophosphamide, alone or in combination, in patients with lupus nephritis. A randomized, controlled trial. *Ann. Intern. Med.* **125**, 549.
55. Austin, H. A., Klippel, J. H., Balow, J. E., *et al*. (1986). Therapy of lupus nephritis: Controlled trial of prednisone and cytotoxic drugs. *N. Engl. J. Med*. **314**, 614.
56. Faedda, R., Palomba, D., Satta, A., *et al*. (1995). Immunosuppressive treatment of the glomerulonephritis of systemic lupus. *Clin. Nephrol*. **44**, 3678.
57. Valeri, A., Radhakrishnan, J., Estes, D., *et al*. (1994). Intravenous pulse Cyclophosphamide treatment of severe

lupus nephritis: A prospective five-year study. *Clin. Nephrol*. **42**, 71.
58. Sesso, R., Monteiro. M., Sato, E., *et al*. (1994). A controlled trial of pulse cyclophosphamide versus pulse methylprednisolone in severe lupus nephritis. *Lupus*. **3**, 107.
59. Illei, G. G., Austin, H. A., Crane, M., *et al*. (2001). Combination therapy with pulse cyclophosphamide plus pulse methylprednisolone improves long-term renal outcome without adding toxicity in patients with lupus nephritis. *Ann. Intern. Med*. **135**, 248.
60. Baqi, N., Moazami, S., Singh, A., *et al*. (1996). Lupus nephritis in children: A longitudinal study of prognostic factors and therapy. *J. Am. Soc. Nephrol*. **7**, 924.
61. Silverman, E. (1996). What's new in the treatment of pediatric SLE. *J. Rheumatol*. **23**, 1657.
62. Lehman, T. J. (1995). A practical guide to systemic lupus erythematosus. Pediatr. *Clin. North. Am*. **42**, 1223.
63. Yan, D. C., Chou, C. C., Tsai, M. J., *et al*. (1995). Intravenous cyclophosphamide pulse therapy on children with severe active lupus nephritis. *Acts. Paediatr. Sin*. **36**, 203.
64. Barhano, G., Gusmanu, R., Damasio, B., *et al*. (2002). Childhood-onset lupus nephritis: a single-center experience of pulse intravenous cyclophosphamide therapy. *J. Nephrol*. **15**, 123.
65. Ciruelo, E., de la Cruz, J., Lopez, I., and Gomez-Reino, J. J. (1996). Cumulative rate of relapse of lupus nephritis after successful treatment with cyclophosphamide. *Arthritis. Rheum*. **39**, 2028.
66. Pablos, J. L., Gutierrez-Millet, V., and Gomez-Reino, J. J. (1994). Remission of lupus nephritis with cyclophosphamide and late relapses following therapy withdrawal. *Scan. Rheumatol*. **23**, 142.
67. Illei, G. G., Takada, K., Parkin, D., *et al*. (2002). Renal flares are common in patients with severe proliferative lupus nephritis treated with pulse immunosuppressive therapy: long-term follow-up of a cohort of 145 patients participating in randomized controlled studies. *Arthritis. Rheum*. **46**, 995.
68. Ioannidis, J. P., Boki, K. A., Katsorida, M. E., *et al*. (2000). Remission, relapse, and re-remission of proliferative lupus nephritis treated with cyclophosphamide. *Kidney. Int*. **57**, 258.
69. Belmont, H. M., Storch, M., Buyon, J., and Abramson, S. (1995). Intravenous cyclophosphamide treatment: Efficacy in steroid unresponsive lupus nephritis. *Lupus*. **4**, 104.
70. Dooley, M. A., Hogan, S., Jennette, C., and Falk, R. (1997). Cyclophosphamide therapy for lupus nephritis: Poor renal survival in black Americans. *Kidney. Int*. **51**, 1188.
71. Conlon, P. J., Fischer, C. A., Levesque, M. C., *et al*. (1996). Clinical, biochemical and pathological predictors of poor response to intravenous cyclophosphamide in patients with proliferative lupus nephritis. *Clin. Nephrol*. **46**, 170.
72. Propper, D. J., and Bucknall, R. C. (1989). Acute transverse myelopathy complicating systemic lupus erythematosus. *Ann. Rheum. Dis*. **48**, 512.
73. Boumpas, D. T., Patronas, N., Dalakas, M. C., *et al*. (1989). Acute transverse myelitis in systemic lupus erythematosus: Magnetic resonance imaging and review of the literature. *J. Rheumatol*. **17**, 89.
74. Chan, K. F., and Boey, M. L. (1996). Transverse myelopathy in SLE: Clinical features and functional outcomes. *Lupus*. **5**, 294.
75. Stahl, H. D., Ettlin, T. H., Plohmann, A., *et al*. (1994). Central Nervous system lupus: Concomitant occurrence of myelopathy and cognitive dysfunction. *Clin. Rheumatol*. **13**, 273.
76. Martinez-Taboada, V. M., Alonso, R. B., Armona, J., *et al*. (1996). Mononeuritis multiplex in systemic lupus erythematosus: Response to pulse intravenous cyclophosphamide. *Lupus*. **5**, 74.
77. Neuwelt, C. M., Lacks, S., Kaye, B. R., Ellman, J. B., and Borenstein, D. G. (1995). Role of intravenous cyclophosphamide in the treatment of severe neuropsychiatric systemic lupus erythematosus. *Am. J. Med*. **98**, 32.
78. Rosenbaum, J. T., Simpson, J., and Neuwelt, C. M. (1997). Successful treatment of optic neuropathy in association with systemic lupus erythematosus using intravenous cyclophosphamide. *Br. J. Ophthalmol*. **81**, 130.
79. Walport, M. J., Hubbard, W. N., and Hughes, G. R. V. (1982). Reversal of aplastic anemia secondary to systemic lupus erythematosus by high-dose cyclophosphamide. *BMJ* **285**, 769.
80. Winkler, A., Jackson, R. W., Kay, D. S., *et al*. (1998). High-dose intravenous cyclophosphamide treatment of systemic lupus erythematosus-associated aplastic anemia. *Arthritis. Rheum*. **31**, 693.
81. Boumpas, D. T., Barez, S., Klippel, J. H., and Balow, J. E. (1990). Intermittent cyclophosphamide for the treatment of autoimmune thrombocytopenia in systemic lupus erythematosus. *Ann. Intern. Med*. **112**, 674.
82. McCune, W. J., Golbus, J., Zeldes, W., *et al*. (1988). Clinical and immunologic effects of monthly administration of intravenous cyclophosphamide in severe systemic lupus erythematosus. *N. Engl. J. Med*. **318**, 1423.
83. Liang, T. J. (1988). Gastrointestinal vasculitis and pneumatosis intestinalis due to systemic lupus erythematosus: Successful treatment with pulse intravenous cyclophosphamide. *Am. J. Med*. **85**, 555.
84. Perez, M.C., Wilson, W. A., and Scopelitis, E. (1989). Cyclophosphamide use in a young woman with antiphospholipid antibodies and recurrent cerebrovascular accident. *South. Med. J*. **82**, 1421.
85. Jara, L. J., Capin, N. R., and Lavalle, C. (1989). Hyperviscosity syndrome as the initial manifestation of systemic lupus erythematosus. *J. Rheumatol*. **16**, 225.
86. Kono, D. H., Klashman, D. J., and Gilbert, R. C. (1990). Successful IV pulse cyclophosphamide in refractory PM in 3 patients with SLE. *J. Rheumatol*. **17**, 982.
87. Eiser, A. R., and Shanies, H. M. (1994). Treatment of lupus interstitial lung disease with intravenous cyclophosphamide. *Arthritis. Rheum*. 37, 428.
88. Fukuda, M., Kamiyama, Y., Kawahara, K., *et al*. (1994). The favourable effect of cyclophosphamide pulse therapy in the treatment of massive pulmonary haemor-

rhage in systemic lupus erythematosus. *Eur. J. Pediatr*. **153**, 167.
89. Tanska, E., Harigai, M., Tanaka, M., *et al*. (2002). Pulmonary hypertension and systemic lupus erythematosus: evaluation of clinical characteristics and response to treatment. *J. Rheumatol*. **29**, 282.
90. Naarendorp, M., Kerr, A. S., *et al*. (1999). Dramatic improvement of left ventricular function after cytotoxic therapy in lupus patients with acute cardiomyopathy: report of 6 cases. *J. Rheumatol*. **26**, 2257.
91. Ginzler, E., Diamond, H., Guttadauria, M., and Kaplan, D. (1976). Prednisone and azathioprine compared to prednisone plus low-dose azathioprine and cyclophosphamide in the treatment of diffuse lupus nephritis. *Arthritis. Rheum*. **19**, 693.
92. Bansal, V. K., and Beto, J. A. (1997). Treatment of lupus nephritis: A metaanalysis of clinical trials. *Am. J. Kidney. Dis*. **29**, 193.
93. Mokm, C. C., Ho, C. T., Siu, Y. P., *et al*. (2001). Treatement of diffuse proliferative lupus glomerulonephritis: a comparison of two cyclophosphamide-containing regimens. *Am. J. Kid. Dis*. **38**, 256.
94. Mok, C. C., Ho, C. T., Chan, K. W., *et al*. (2002). Outcome and prognostic indicators of diffuse proliferative lupus glomerulonephritis treated with sequential oral cyclophosphamide and azathioprine. *Arthritis. Rheum*. **46**, 1003.
95. Abud-Mendoza, C., Sturbaum, A. K., Vazquez-Compean, R., Gonzalez-Amaro, R. (1993). Methotrexate therapy in childhood systemic lupus erythematosus. *J. Rheumatol*. **20**, 731.
96. Wallace, D., Goldfinger, D., Pepkowitz, S. (1997). A prospective, controlled trial of pulse synchronization cyclophosphamide (CTX)/apheresis for proliferative lupus nephritis. *Arthritis. Rheum*. **40**, S58.
97. Schroeder, J. O., Schwab, U., Zeuner, R., Fastenrath, S., Euler, H. H. (1997). Plasmapheresis and subsequent pulse cyclophosphamide in severe systemic lupus erythematosus. Preliminary results of the LPSG-Trial. *Arthritis. Rheum*. **40**, S324.
98. Marmont, A. M., van Lint, M. T., Gualandi, F., Bacigalupo, A. (1997). Autologous marrow stem cell transplantation for severe systemic lupus erythematosus of long duration. *Lupus*. **6**, 545.
99. Traynor, A. E., Schroeder, J., Rosa, R. M., *et al*. (2000). Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: a phase I study. *Lancet*. **356**, 701.
100. Trysberg, E., Lindgren, I., Tarkowshi, A. (2000). Autologous stem cell transplantation in a case of treatment resistant central nervous system lupus. *Ann. Rheum. Dis*. **59**, 36.
101. Brunner, M., Greinix, H. T., Redlich, K., *et al*. (2002). Autologous blood stem cell transplantation in refractory systemic lupus erythematosus with severe pulmonary impairment: a case report. *Arthritis. Rheum*. **46**, 1580.
102. Tyndall, A. (2001). Immunoablation and haemopoietic stem cell transplantation for severe autoimmune disease with special reference to systemic lupus erythematosus. *Lupus*. **10**, 214.
103. Brodsky, R. A., Petri, M., Smith, B. D., *et al*. (1998). Immunoablative high-dose cyclophosphamide without stem-cell rescue for refractory, severe autoimmune disease. *Ann. Intern. Med*. **129**, 1031.
104. Brodsky, R. A. (2002). High-dose cyclophosphamide for aplastic anemia and autoimmunity. *Curr. Opin. Oncol*. **14**, 143.
105. Fraenkel, L., Bogardus, S., Conato, J. (2002). Patient preferences for treatment of lupus nephritis. *Arthritis. Rheum*. **47**, 421.
106. Yarboro, C. H., Wesley, R., Amantea, M. A., Klippel, J. H., and Pucino, F. (1996). Modified oral ondansetron regimen for cyclophosphamide-induced emesis in lupus nephritis. *Ann. Pharmacother*. **30**, 752.
107. Kirshon, B., Wasserstrum, N., Willis, R., Herman, G. E., and McCabe, E. R. (1988). Teratogenic effects of first-trimester cyclophosphamide therapy. *Ostet. Gynecol*. **72**, 462.
108. Ramsey-Goldman, R., Achilling, E. (1997). Immunosuppressive drug use during pregnancy. *Rheum. Dis. Clin. North. Am*. **23**, 149.
109. Katsifis, G. E., Tzioufas, A. G., Vlachoyiannopoulos, P. G., *et al*. (2002). Risk of myelotoxicity with intravenous cyclophosphamide with systemic lupus erythematosus. *Rheumatology* **41**, 780.
110. Bradley, J. D., Brandt, K. D., Katz, B. P. (1989). Infectious complications of cyclophosphamide treatment for vasculitis. *Arthritis. Rheum*. **32**, 45.
111. Kattwinkel, N., Cook, L., Agnello, V. (1991). Overwhelming fatal infection in a young woman after intravenous cyclophosphamide therapy for lupus nephritis. *J. Rheumatol*. **18**, 79.
112. Segal, B. H., Sneller, M. C. (1997). Infectious complications of immunosuppressive therapy in patients with rheumatic diseases. *Rheum. Dis. Clin. North. Am*. **23**, 219.
113. Pryor, B. D., Bologna, S. G., Kahl, L. E. (1996). Risk factors for serious infection during treatment with cyclophosphamide and high-dose corticosteroids for systemic lupus erythematosus. *Arthritis. Rheum*. **39**, 1475.
114. Ognibene, F. P., Shelhammer, J. H., Hoffman, G. S., *et al*. (1995). Pneumocystis carinii pneumonia: A major complication of immunosuppressive therapy in patients with Wegener's granulomatosis. *Am. J. Respir. Crit. Care. Med*. **151**, 795.
115. Noel, V., Lortholary, O., Casassus, P., *et al*. (2001). Risk factors and prognostic influence of infection in a single cohort of 87 adults with systemic lupus erythematosus. *Ann. Rheum. Dis*. **60**, 1141.
116. Qureshi, M. S. A., Pennington, J. H., Goldsmith, H. J., Cox, P. E. (1972). Cyclophosphamide therapy and sterility. *Lancet*. **2**, 1290.
117. Warne, G. L., Fairley, K. F., Hobbs, J. B., Martin, R. I. (1973). Cyclophosphamide-induced ovarian failure. *N. Engl. J. Med*. **289**, 1159.
118. Sherins, R. J., DeVita, V. T. (1973). Effect of drug treatment for lymphoma on make reproductive capacity.

Studies of men in remission after therapy. *Ann. Intern. Med*. **79**, 216.
119. Roeser, H. P., Stocks, A. E., Smith, A. J. (1978). Testicular damage due to cytotoxic drugs and recovery after cessation of therapy. *Aust. N. Z. J. Med*. **8**, 250.
120. Scholsky, R. L., Lewis, B. J., Sherins, R. J., Young, R. C. (1980). Gonadal dysfunction in patients receiving chemotherapy for cancer. *Ann. Intern. Med*. **93**, 109.
121. Boumpas, D. T., Austin, H. A., Vaughan, E. M., *et al*. (1993). Risk for sustained amenorrhea in patients with systemic lupus erythematosus receiving intermittent pulse cyclophosphamide. *Ann. Intern. Med*. **119**, 366.
122. McDermott, E. M., Powell, R. J. (1996). Incidence of ovarian failure in systemic lupus erythematosus after treatment with pulse cyclophosphamide. *Ann. Rheum. Dis*. **55**, 224, 29.
123. Gonzalez-Crespo, M. R., Gomez-Reino, J. J., Merino, R., *et al*. (1995). Menstrual disorders in girls with systemic lupus erythematosus. *Br. J. Rheumatol*. **34**, 737.
124. Wang, C. L., Wang, F., Bosco, J. J. (1995). Ovarian failure in oral cyclophosphamide treatment for systemic lupus erythematosus. *Lupus*. **4**, 11.
125. Mok, C. C., Lau, C. S., Wong, R. W. (1998). Risk factors for ovarian failure in patients with systemic lupus erythematosus receiving cyclophosphamide therapy. *Arthritis. Rheum*. **41**, 831.
126. Masala, A., Faedda, R., Alagna, S., *et al*. (1997). Use of testosterone to prevent cyclophosphamide-induced azoospermia. *Ann. Intern. Med*. **126**, 292.
127. Blumenfeld, Z., Shapiro, D., Shteinberg, M., *et al*. (2000). Preservation of fertility and ovarian function and minimizing gonadotoxicity in young women with systemic lupus erythematosus treated with chemotherapy. *Lupus*. **9**, 401.
128. Talar-Williams, C., Hijazi, Y. M., Walther, M. M., *et al*. (1996). Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegner granulomatosis. *Ann. Intern. Med*. **124**, 477.
129. Johnson, W. W., Meadows, D. C. (1971). Urinary bladder fibrosis and telangiectasia associated with long-term cyclophosphamide therapy. *N. Engl. J. Med*. **284**, 290.
130. Elliott, R. W., Essenhigh, D. M., Morley, A. R. (1982). Cyclophosphamide treatment of systemic lupus erythematosus: Risk of bladder cancer exceeds benefit. *BMJ* **284**, 1160.
131. Pedersen-Bjergaard, J., Ersbll, J., Hansen, V. L., *et al*. (1988). Carcinoma of the urinary bladder after treatment with cyclophosphamide for non-Hodgkin's lymphoma. *N. Engl. J. Med*. **318**, 1028.
132. Thrasher, J. B., Miller, G. J., Wettlaufer, J. N. (1990). Bladder leiomyosarcoma following cyclophosphamide therapy for lupus nephritis. *J. Urol*. **143**, 119.
133. Radis, C. D., Kahl, L. E., Baker, G. L., *et al*. (1996). Effects of cyclophosphamide on the development of malignancy and on long-term survival of patients with rheumatoid arthritis. *Arthritis. Rheum*. **38**, 1120.
134. Cox, P. J. (1979). Cyclophosphamide cystitis: Identification of acrolein as causative agent. *Biochem. Pharmacol*. **28**, 2045.
135. Hows, J. M., Mehta, A., Ward, L., *et al*. (1984). Comparison of mesna with forced diuresis to prevent cyclophosphamide induced haemorrhagic cystitis in marrow transplantation: A prospective randomised study. *Br. J. Cancer*. **50**, 753.
136. Fraiser, L. H., Kanekal, S., Kehrer, J. P. (1991). Cyclophosphamide toxicity. Characterising and avoiding the problem. *Drugs*. **42**, 781.
137. Kinlen, L. J., Sheil, A. G. R., Peto, J., Doll, R. (1979). Collaborative United Kingdom-Australasian study of cancer in patients treated with immunosuppressive drugs. *BMJ* **282**, 474.
138. Kinlen, L. J., Peto, J., Doll, R., Sheil, A. G. (1981). Cancer in patients treated with immunosuppressive drugs. *BMJ*, **282**, 474.
139. Tannenbaum, H., Schur, P. H. (1974). Development of reticulum cell sarcoma during cyclophosphamide therapy. *Arthritis. Rheum*. **17**, 15.
140. Cras, P., Franckx, C., Martin, J. J. (1989). Primary lymphoma in systemic lupus erythematosus treated with immunosuppressives. *Clin. Neuropathol*. **8**, 200.
141. Gibbons, R. B., Westerman, E. (1988). Acute nonlymphocytic leukemia following short-term, intermittent, intravenous cyclophosphamide treatment of lupus nephritis. *Arthritis. Rheum*. **31**, 1552.
142. Paolozzi, F. P., Goldberg, J. (1985). Acute granulocytic leukemia following systemic lupus erythematosus. *Am. J. Med. Sci*. **290**, 32.
143. Lishner, M., Hawker, G., Amato, D. (1990). Chronic lymphocytic leukemia in a patient with systemic lupus erythematosus. *Acta. Hematol*. **84**, 38.
144. Vasquez, S., Kavanaugh, A. F., Scheider, N. R., Wacholtz, M. C., Lipsky, P. E. (1992). Acute nonlymphocytic leukemia after treatment of systemic lupus erythematosus with immunosuppressive agents. *JH Rheumatol*. **19**, 1625.
145. Lugassy, G., Lishner, M., Polliack, A. (1992). Systemic lupus erythematosus and chronic lymphocytic leukemia: Rare coexistence in three patients, with comments on pathogenesis. *Leukemia. Lymphoma*. **8**, 243.
146. Pryor, B., Bologna, S., Ernst, C., *et al*. (1993). Risk of malignancy in cyclophosphamide-treated patients. *Arthritis. Rheum*. **36**, S91.
147. Pando, J. A., Gourley, M. F., Boumpas, D. T., *et al*. (1994). Risks of malignancy in patients with lupus nephritis treated with bolus cyclophosphamide. *Arthritis. Rheum*. **37**, S179.
148. Spector, J. I., Zimbler, H., Ross, J. S. (1979). Early-onset cyclophosphamide-induced interstitial pneumonitis. *JAMA* **242**, 2852.
149. Mills, B. A., Roberts, R. W. (1979). Cyclophosphamide-induced cardiomyopathy. *Cancer*. **43**, 2223.
150. Goldberg, J. W., Lidsky, M. D. (1985). Cyclophosphamide-associated hepatotoxicity. *South. Med. J*. **78**, 222.
151. Bressler, R. B., Huston, D. P. (1983). Water intoxication following moderate-dose intravenous cyclophosphamide. *Arch. Intern. Med*. **145**, 548.
152. Martin Santos, J. M., Terroba Larumbe, M. C., Dib, B., Armentia Medina, A. (1996). Systemic lupus erythe-

matosus and the syndrome of inappropriate secretion of antidiuretic hormone. *Clin. Exp. Rheumatol*. **14**, 578.
153. Lakin, J. D, and Cahill, R. A. (1976). Generalized urticaria to cyclophosphamide: Type 1 hypersensitivity to an immunosuppressive agent. *J. Allergy. Clin. Immunol.* **58**, 160.
154. Karchmer, R. K., Hansen, V. L. (1977). Possible anaphylactic reaction to intravenous cyclophosphamide: Report of a case. *JAMA* **237**, 475.
155. Smith, M. I., Holt, J. M., Oliver, D. O., Dunnill, M. S. (1973). Treatment of patients with systemic lupus erythematosus including nephritis with chlorambucil. *BMJ* **2**, 197.
156. Sabbour, M. S., Osman, L. M. (1979). Comparison of chlorambucil, azathioprine or cyclophosphamide combined with corticosteroids in the treatment of lupus nephritis. *Br. J. Dermatol*. **100**, 113.
157. Ellman, J. B., Whiting-O'Keefe, Q. E., Epstein, W. V. (1980). Chlorambucil (CAB) treatment in systemic lupus erythematosus (SLE). *Arthritis. Rheum*. **23**, S70.
158. Ivanova, M. M., Nassonova, V. A., Solovev, S. K., Akhnazarova, V.D., Speranskii, A. I. (1981). Controlled trial of cyclophosphamide, azathioprine, and chlorambucil in lupus nephritis (a double-blind trial) (In Russian). *Vopr. Revum. NS* **2**, 11.
159. Ponticelli, C., Zuccgelli, P., Passerini, P., *et al*. (1995). A 10-year follow-up of randomized study with methylprednisolone and chlorambucil in membranous Nephropathy. *Kidney. Int*. **48**, 1600.
160. Piccoli, A., Pillon, L., Passerini, P., Ponticelli, C. (1994). Therapy for idiopathic membranous nephropathy: Tailoring the choice be decision analysis. *Kidney. Int*. **45**, 1193.
161. Patapanian, H., Graham, S., Sambrook, P. N., *et al*. (1988). The oncogenicity of chlorambucil in rheumatoid arthritis. *Br. J. Rheumatol*. **27**, 44.
162. Eisen, B., Demis, D. J., and Crosby, W. H. (1962). Thioguanine therapy. Systemic lupus erythematosus, atopic dermatitis, and other non-malignant diseases. *JAMA* **179**, 789.
163. Carpenter, R. R. (1963). Preliminary experience with administration of 6-thioguanine in lupus erythematosus. In Goodman, H. C. (Moderator): Current studies on the effect of antimetabolites in nephrosis, other non-neoplastic diseases, and experimental animals. *Ann. Intern. Med.* **59**, 388.
164. Demis, D. J., Brown, C. S., and Crosby, W. H. (1964). Thioguanine in the treatment of certain autoimmune, immunologic and related diseases. *Am. J. Med.* **37**, 195.
165. Shearn, M. A. (1965). Mercaptopurine in the treatment of steroid-resistant nephrotic syndrome. *N. Engl. J. Med.* **273**, 943.
166. Rundles, R. E. (1966). Effects of allopurinol on 6-mercaptopurine therapy in neoplastic diseases. *Ann. Rheum. Dis.* **25**, 655.
167. American College of Rheumatology Ad Hoc Committee on Clinical Guidelines. (1996). Guidelines for monitoring drug therapy in rheumatoid arthritis. *Arthritis. Rheum.* **39**, 723.
168. Corley, C. C., Lessner, H. E., Larsen, H. E., and Larsen, W. E. (1966). Azathioprine therapy of "autoimmune" diseases. *JAMA* **41**, 404.
169. Adams, D. A., Gordon, A., and Mazwell, M. H. (1967). Azathioprine treatment of immunological renal disease. *JAMA* **199**, 459.
170. Maher, J. F., and Schreiner, G. E. (1973). Treatment of lupus nephritis with azathioprine. *Arch. Intern. Med.* **125**, 293.
171. Drinkard, J. P., Stanley, T. M., Dornfield, L., *et al*. (1970). Azathioprine and prednisone in the treatment of adults with lupus nephritis. *Medicine.* **49**, 411.
172. Shelp, W. D., Bloodworth, J. M. B., and Rieselbach, R. E. (1971). Effects of azathioprine on renal histology and function in lupus nephritis. *Arch. Intern. Med.* **128**, 566.
173. Hayslett, J. P., Kashgarian, M., Cook, C. D., and Sparga, B. H. (1972). The effect of azathioprine on lupus glomerulonephritis. *Medicine.* **51**, 393.
174. Sharon, E., Kaplan, D., and Diamond, H. S. Exacerbation of systemic lupus erythematosus after withdrawal of azathioprine therapy. *N. Engl. J. Med.* **288**, 122.
175. Ginzler, E., Sharon, E., Diamond, H., and Kaplan, D. (1975). Long-term maintenance therapy with azathioprine in systemic lupus erythematosus. *Arthritis. Rheum.* 18, 27.
176. Lindeman, R. D., Pederson, J. A., Matter, B. J., Laughlin, L. O., and Mandal, A. K. (1976). Long-term azathioprine-corticosteroid therapy in lupus nephritis and idiopathic nephrotic syndrome. *J. Chron. Dis.* **29**, 189.
177. Barnett, E. V., Dornfield, L., Lee, D. B. N., and Liebling, M. R. (1978). Long-term survival of lupus nephritis patients treated with azathioprine and prednisone. *J. Rheumatol.* **5**, 257.
178. Laitman, R. S., Glicklich, D., Sablay, L. B., *et al*. (1989). Effect of long-term normalization of serum complement levels on the course of lupus nephritis. *Am. J. Med.* **87**, 132.
179. Hollingsworth, P., DeVere Tyndall, A., Ansell, B. M., *et al*. (1982). Intensive immunosuppression versus prednisolone in the treatment of connective tissue diseases. *Ann. Rheum. Dis.* **41**, 557.
180. Balow, J. E., Austin, H. A., Muenz, L. R., *et al*. (1984). Effect of treatment on the evolution of renal abnormalities in lupus nephritis. *N. Engl. J. Med.* **311**, 491.
181. Carette, S., Klippel, J. H., Decker, J. L., *et al*. (1983). Controlled studies of oral immunosuppressive drugs in lupus nephritis: A long-term follow-up. *Ann. Intern. Med.* **99**, 1.
182. Austin, H. A., Klippel, J. H., Balow, J. E., *et al*. Therapy of lupus nephritis: Controlled trial of prednisone and cytotoxic drugs. *N. Engl. J. Med.* **314**, 614.
183. Sztejnbok, M., Stewart, A., Diamond, H., and Kaplan, D. (1971). Azathioprine in the treatment of systemic lupus erythematosus. A controlled study. *Arthritis. Rheum.* **14**, 639.
184. Cade, R., Spooner, G., Schlein, E., *et al*. (1973). Comparison of azathioprine, prednisone, and heparin alone or combined in treating lupus nephritis. *Nephron.* **10**, 37.
185. Donadio, J. V., Holley, K. E., Wagoner, R. D., Ferguson, R. H., and Mc Duffie, F. C. (1972). Treatment of lupus

nephritis with prednisone and combined prednisone and azathioprine. *Ann. Intern. Med.* **77**, 829.
186. Donadio, J. V., Holley, K. E., Wagoner, R. D., Ferguson, R. H., and Mc Duffie, F. C. (1974). Further observations on the treatment of lupus nephritis with prednisone and combined prednisone and azathioprine. *Arthritis. Rheum.* **17**, 573.
187. Hahn, B. H., Kantor, O. S., and Osterland, C. K. (1975). Azathioprine plus prednisone compared with prednisone alone in the treatment of systemic lupus erythematosus. Report of a prospective trial in 24 patients. *Ann. Intern. Med.* **83**, 597.
188. Ginzler, E., Diamond, H., Guttadauria, M., and Kaplan, D. (1976). Prednisone and azathioprine compared to prednisone plus low-dose azathioprine and cyclophosphamide in the treatment of diffuse lupus nephritis. *Arthritis. Rheum.* **19**, 693.
189. de Glas-Vos, J. W., Krediet, R. T., Weening, J. J., and Arisz, L. (1995). Treatment of proliferative lupus nephritis with methylprednisolone pulse therapy and oral azathioprine. *Neth. J. Med.* **46**, 4.
190. Singh, G., Fries, J. F., Spitz, P., and Williams, C. A. (1989). Toxic effects of azathioprine in rheumatoid arthritis. A national post-marketing perspective. *Arthritis. Rheum.* **32**, 837.
191. Nossent, J. C., and Swaak, A. J. (1990). Pancytopenia in systemic lupus erythematosus related to azathioprine. *J. Intern. Med.* **227**, 69.
192. Jeurissen, M. E. C., Boerbooms, A. M. Th., van de Putte, L. B. A., and Kruijsen, M. W. M. (1990). Azathioprine-induced fever, chills, rash, and hepatotoxicity in rheumatoid arthritis. *Ann. Rheum. Dis.* **49**, 25.
193. Aissaoui, M., Mounedji, N., Mathelier-Fusade, P., and Leynadier, F. (1996). Pancreatitis caused by azathioprine: Immuno-allergy? (letter). *Presse. Med.* **25**, 1650.
194. Wilmink, T., and Frick, T. W. (1996). Drug-induced pancreatitis. *Drug. Saf.* **14**, 406.
195. Tragnone, A., Bazzocchi, G., Aversa, G., *et al*. (1996). Acute pancreatitis after azathioprine treatment for ulcerative colitis. *Ital. J. Gastroenterol.* **28**, 102.
196. Lankisch, P. G., Droge, M., and Gottesleben, F. (1995). Drug-induced acute pancreatitis: Incidence and severity. *Gut.* **37**, 565.
197. Kolk, A., Horneff, G., Wilgenbus, K. K., Wahn, V., and Gerhar, C. D. (1995). Acute lethal necrotising pancreatitis in childhood systemic lupus erthematosus—possible toxicity of immunosuppressive therapy. *Clin. Exp. Rheumatol.* **13**, 399.
198. Knowles, S. R., Gupta, A. K., Shear, N. H., and Sauder, D. (1995). Azathioprine hypersensitivity-like reactions—a case report and a review of the literature. *Clin. Exp. Dermatol.* **20**, 353.
199. Caramaschi, P., Biasi, D., Carletto, A., and Bambara, L. M. (1997). Azathioprine hypersensitivity in a patient affected by systemic lupus erythematosus. *Lupus.* **6**, 616.
200. Key, T. C., Resnik, R., Dittrich, H. C., and Reisner, L. S. (1989). Successful pregnancy after cardiac transplantation. *Am. J. Obstet. Gynecol.* **160**, 367.
201. Alstead, E. M., Ritchie, J. K., Lennard-Jones, J. E., Furthing, M. J., and Clark, M. L. (1990). Safety of azathioprine in pregnancy in inflammatory bowel disease. *Gastroenterology.* **99**, 443.
202. Khamashta, M. A., Ruiz-Irastorza, G., and Hughes, G. R. (1997). Systemic lupus erythematosus flares during pregnancy. *Rheum. Dis. Clin. North. Am.* **23**, 15.
203. Cote, C. J., Meuwissen, H. J., and Pickering, R. J. (1974). Effects on the neonate of prednisone and azathioprine administered to the mother during pregnancy. *J. Pediatr.* **85**, 324.
204. Mascola, M. A., and Repke, J. T. (1997). Obstetric management of the high-risk lupus pregnancy. *Rheum. Dis. Clin. North. Am.* **23**, 119.
205. Price, H. V., Salaman, J. R., Laurence, K. M., and Langmaid, H. (1976). Immunosuppressive drugs and the foetus. *Transplantation.* **21**, 294.
206. Ramsey-Goldman, R., and Achilling, E. (1997). Immunosuppressive drug use during pregnancy. *Rheum. Dis. Clin. North. Am.* **23**, 149.
207. Williamson, R. A., and Karp, L. E. (1981). Azathioprine teratogenicity: Review of the literature and case report. *Obstet. Gynecol.* **58**, 247.
208. De Witte, D. B., Buik, M. K., Cyran, S. E., and Maisels, M. J. (1984). Neonatal pancytopenia and severe combined immunodeficiency associated with antenatal administration of azathioprine and prednisone. *J. Pediatr.* **105**, 625.
209. Krogh Jensen, M. (1970). Effect of azathioprine on the chromosome complement of human bone marrow cells. *Int. J. Cancer.* **5**, 147.
210. McAdam, L., Paulus, H. E., and Peter, J. B. (1974). Adenocarcinoma of the lung during azathioprine therapy. *Arthritis. Rheum.* **17**, 92.
211. Canoso, J. J., and Cohen, A. S. (1974). Malignancy in a series of 70 patients with systemic lupus erythematosus. *Arthritis. Rheum.* **17**, 383.
212. Walden, P. A., Philalithis, P. E., Joekes, A. M., and Bagshawe, K. D. (1977). Development of a lymphocytic lymphoma during immunosuppressive therapy with azathioprine for systemic lupus erythematosus with renal involvement induced by phenylbutazone. *Clin. Nephrol.* **8**, 317.
213. Grunwald, H. W., and Rosner, F. (1979). Acute leukemia and immunosuppressive drug use. A review of patients undergoing immunosuppressive therapy for non-neoplastic diseases. *Arch. Intern. Med.* **139**, 461.
214. Vismans, J. J., Briet, E., Meijer, K., and den Ottolander, G. J. (1980). Azathioprine and subacute myelomonocytic leukemia. *Acta. Med. Scand.* **207**, 315.
215. Woolf, A. S., and Conway, G. (1987). Systemic lupus erythematosus and primary cerebral lymphoma. *Postgrad. Med. J.* **63**, 569.
216. Nyberg, G., Eriksson, O., and Westberg, N. G. (1981). Increased incidence of cervical atypia in women with systemic lupus erythematosus treated with chemotherapy. *Arthritis. Rheum.* **24**, 648.
217. Kinlen, L. J., Sheil, A. G. R., Peto, J., and Doll, R. (1979). Collaborative United Kingdom–Australasian study of

cancer in patients treated with immunosuppressive drugs. *BMJ* **2**, 1461.
218. Kinlen, L. J., Peto, J., Doll, R., and Sheil, A. G. (1981). Cancer in patients treated with immunosuppressive drugs. *BMJ* **282**, 474.
219. Sollinger, H. W. (1995). Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. U. S. Renal Transplant Mycophenolate Mofetil Study Groupp. *Transplantation*. **60**(3): 225–232.
220. Van Bruggen, M. C., *et al*. (1998). Attenuation of murine lupus nephritis by mycophenolate mofetil. *J. Am. Soc. Nephrol*. **9**(8): 1407–1415.
221. Jonsson, C. A., Svensson, L., and Carlsten, H. (1999). Beneficial effect of the inosine monophosphate dehydrogenase inhibitor mycophenolate mofetil on survival and severity of glomerulonephritis in systemic lupus erythematosus (SLE)-prone MRLlpr/lpr mice. *Clin. Expp. Immunol*. **116**(3): 534–541.
222. Glicklich, D., and Acharya, A. (1998). Mycophenolate mofetil therapy for lupus nephritis refractory to intravenous cyclophosphamide. *Am. J. Kidney. Dis.* **32**(2): 318–322.
223. Dooley, M. A., *et al*. (1999). Mycophenolate mofetil therapy in lupus nephritis: clinical observations. *J. Am. Soc. Nephrol*. **10**(4): 833–839.
224. Chan, T. M., *et al*. (2000). Efficacy of mycophenolate mofetil in patients with diffuse proliferative lupus nephritis. Hong Kong-Guangzhou Nephrology Study Groupp. *N. Engl. J. Med*. **343**(16): 1156–1162.
225. Abud-Mendoza, C., Sturbaum, A. K., Vazquez-Compean, R., and Gonzalez-Amaro, R. (1993). Methotrexate therapy in childhood systemic lupus erythematosus. *J. Rheumatol.* **20**, 731.
226. Miescher, P. A., and Riethmuller, D. (1965). Diagnosis and treatment of systemic lupus erythematosus. *Semin. Hematol.* **2**, 1.
227. Swanson, M. A., and Schwartz, R. S. (1967). Immunosuppressive therapy. The relationship between clinical response and immunologic competence. *N. Engl. J. Med.* **277**, 163.
228. Kersley, G. D. (1968). Amethopterin (methotrexate) in connective tissue disease: Psoriasis and polyarthritis. *Ann. Rheum. Dis.* **27**, 64.
229. Rothenberg, R. J., Graziano, F. M., Grandone, J. T., *et al*. (1988). The use of methotrexate in steroid-resistant systemic lupus erythematosus. *Arthritis. Rheum.* **31**, 612.
230. Wilke, W. S., Krall, P. L., Scheetz, R. J., *et al*. (1991). Methotrexate for systemic lupus erythematosus: A retrospective analysis of 17 unselected cases. *Clin. Exp. Rheumatol.* **9**, 581.
231. Galarza, D., Esquivel, J., Villareal, M., de la Garza, N., and Garza, M. (1992). Methotrexate in lupus nephritis: An uncontrolled study, preliminary results. *Arthritis. Rheum.* **34**, 5187.
232. Wilson, K., and Abeles, M. (1994). A 2-year, open-ended trial of methotrexate in systemic lupus erythematosus. *J. Rheumatol.* **21**, 1674.
233. Walz LeBlanc, B. A., Dagenais, P., Urowitz, M. B., and Gladman, D. D. (1994). Methotrexate in systemic lupus erythematosus. *J. Rheumatol.* **21**, 836.
234. Wise, C. M., Vuyyuru, S., and Roberts, W. N. (1996). Methotrexate in nonrenal lupus and undifferentiated connective tissue disease—a review of 36 patients. *J. Rheumatol.* **23**, 1005.
235. Gansauge, S., Breitbart, A., Rinaldi, N., and Schwarz-Eywill, M. (1997). Methotrexate in patients with moderate systemic lupus erythematosus (exclusion of renal and central nervous system disease). *Ann. Rheum. Dis.* **56**, 382.
236. Cronstein, B. N. (1996). Molecular therapeutics. Methotrexate and its mechanism of action. *Arthritis. Rheum.* **39**, 1951.
237. Segal, R., Dayan, M. I., Zinger, H., and Mozes, E. (1995). Methotrexate treatment in murine experimental systemic lupus erythematosus (SLE); clinical benefits associated with cytokine manipulation. *Clin. Exp. Immunol.* **101**, 66.
238. Carneiro, J. R., and Sato, E. I. (1999). Double blind, randomized, placebo controlled clinical trial of methotrexate in systemic lupus erythematosus. *J. Rheumatol*. **26**(6): 1275–1279.
239. Sharp, J. T., *et al*. (2000). Treatment with leflunomide slows radiographic progression of rheumatoid arthritis: results from three randomized controlled trials of leflunomide in patients with active rheumatoid arthritis. Leflunomide Rheumatoid Arthritis Investigators Groupp. *Arthritis. Rheum*. **43**(3): 495–505.
240. Strand, V., *et al*. (1999). Treatment of active rheumatoid arthritis with leflunomide compared with placebo and methotrexate. Leflunomide Rheumatoid Arthritis Investigators Groupp. *Arch. Intern. Med*. **159**(21): 2542–2550.
241. Cohen, S., *et al*. (2001). Two-year, blinded, randomized, controlled trial of treatment of active rheumatoid arthritis with leflunomide compared with methotrexate. Utilization of Leflunomide in the Treatment of Rheumatoid Arthritis Trial Investigator Groupp. *Arthritis. Rheum*. **44**(9): 1984–1992.
242. Thoenes, G. H., *et al*. (1989). Leflunomide (HWA 486) inhibits experimental autoimmune tubulointerstitial nephritis in rats. *Int. J. Immunopharmacol*. **11**(8): 921–929.
243. Bartlett, R. R., Popovic, S., and Raiss, R. X. (1988). Development of autoimmunity in MRL/lpr mice and the effects of drugs on this murine disease. *Scand. J. Rheumatol. Suppl*. **75**: 290–299.
244. Remer, C. F., Weisman, M. H., and Wallace, D. J. (2001). Benefits of leflunomide in systemic lupus erythematosus: a pilot observational study. *Lupus*. **10**(7): 480–483.
245. Bergijk, E. C., Baelde, H. J., de Heer, E., *et al*. (1994). Prevention of glomerulosclerosis by early cyclosporine treatment of experimental lupus nephritis. *Kidney. Int.* **46**, 1663.
246. Warner, L. M., Adams, L. M., and Sehgal, S. N. (1994). Rapamycin prolongs survival and arrests pathophysio-

logic changes in murine systemic lupus erythematosus. *Arthritis. Rheum.* **37**, 289.
247. Woo, J., Wright, T. M., Lemster, B., *et al*. (1995). Combined effects of FK506 (tacrolimus) and cyclophosphamide on atypical B220+ T cells, cytokine gene expression and disease activity in MRL/MpJ-lpr/lpr mice. *Clin. Exp. Immunol.* **100**, 118.
248. Ho, S., Clipstone, N., Timmermann, L., *et al*. (1996). The mechanism of action of cyclosporin A and FK506. *Clin. Immunol. Immunopathol.* **80**, 540.
249. Isenberg, D. A., Snaith, M. L., Morrow, W. J. W., *et al*. (1981). Cyclosporin A for the treatment of systemic lupus erythematosus. *Int. J. Immunopharmacol.* **3**, 163.
250. Favre, H., Miescher, P. A., Huang, Y. P., Chatelanat, F., and Mihatsch, M. J. (1989). Ciclosporin in the treatment of lupus nephritis. *Am. J. Nephrol.* **9**(Suppl 1), 57.
251. Hammond, J. M., and Bateman, E. D. (1990). Successful treatment of life-threatening steroid-resistant pulmonary sarcoidosis with cyclosporin in a patient with systemic lupus erythematosus. *Respir. Med.* **84**, 77.
252. Caccavo, D., Lagana, B., Mitterhofer, A. P., *et al*. (1997). Long-term treatment of systemic lupus erythematosus with cyclosporin A. *Arthritis. Rheum.* **40**, 27.
253. Manger, K., Kalden, J. R., and Manger, B. (1996). Cyclosporin A in the treatment of systemic lupus erythematosus: Results of an open clinical study. *Br. J. Rheumatol.* **35**, 669.
254. Scherrer, U., Vissing, S. F., Morgan, B. J., and Rollins, J. A. (1990). Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. *N. Engl. J. Med.* **323**, 693.
255. Palestine, A. G., Austin, H. A., III, Balow, J. E., and Antonovych, T. T. (1986). Renal histopathologic alterations in patients treated with cyclosporin for uveitis. *N. Engl. J. Med.* **314**, 1293.
256. Altman, R. D., Perez, G. O., and Sfakianakis, G. N. (1992). Interaction of cyclosporine A and nonsteroidal anti-inflammatory drugs on renal function in patients with rheumatoid arthritis. *Am. J. Med.* **93**, 396.
257. Deray, G., Benhmida, M., Le Hoang, P., *et al*. (1992). Renal function and blood pressure in patients receiving long-term low-dose cyclosporine therapy for idiopathic autoimmune uveitis. *Ann. Intern. Med.* **117**, 578.
258. Hallegua, D., *et al*. (2000). Cyclosporine for lupus membranous nephritis: experience with ten patients and review of the literature. *Lupus*. **9**(4): 241–251.
259. Balow, J. E., and Austin, H. A. 3rd, (2000). Progress in the treatment of proliferative lupus nephritis. *Curr. Opin. Nephrol. Hypertens*. **9**(2): 107–115.
260. Assmann, T., Homey, B., and Ruzicka, T. (2000). Applications of tacrolimus for the treatment of skin disorders. *Immunopharmacology*. **47**(2–3): 203–213.
261. Nasr, I. S. (2000). Topical tacrolimus in dermatology. *Clin. Exp. Dermatol*. **25**(3): 250–254.
262. Cather, J. C., Abramovits, W., and Menter, A. (2001). Cyclosporine and tacrolimus in dermatology. *Dermatol. Clin*. **19**(1): 119–137, ix.
263. Smith, C. H. (2000). New approaches to topical therapy. *Clin. Exp. Dermatol*. **25**(7): 567–574.
264. Wollenberg, A., *et al*. (2001). Topical tacrolimus (FK506) leads to profound phenotypic and functional alterations of epidermal antigen-presenting dendritic cells in atopic dermatitis. *J. Allergy. Clin. Immunol*. **107**(3): 519–525.
265. Assmann, T., Homey, B., and Ruzicka, T. (2001). Topical tacrolimus for the treatment of inflammatory skin diseases. *Expert. Opin. Pharmacother*. **2**(7): 1167–1175.
266. Boguniewicz, M., *et al*. (1998). A randomized, vehicle-controlled trial of tacrolimus ointment for treatment of atopic dermatitis in children. Pediatric Tacrolimus Study Groupp. *J. Allergy. Clin. Immunol*. **102**(4 Pt 1): 637–644.
267. Kang, S., *et al*. (2001). Long-term safety and efficacy of tacrolimus ointment for the treatment of atopic dermatitis in children. *J. Am. Acad. Dermatol*. **44**(1 Suppl): S58–S64.
268. Reitamo, S., *et al*. (2000). Safety and efficacy of 1 year of tacrolimus ointment monotherapy in adults with atopic dermatitis. The European Tacrolimus Ointment Study Groupp. *Arch. Dermatol*. **136**(8): 999–1006.
269. Vente, C., *et al*. (1999). Erosive mucosal lichen planus: response to topical treatment with tacrolimus. *Br. J. Dermatol*. **140**(2): 338–342.
270. Lener, E. V., *et al*. (2001). Successful treatment of erosive lichen planus with topical tacrolimus. *Arch. Dermatol*. **137**(4): 419–422.
271. Schuppe, H. C., *et al*. (1998). Topical tacrolimus for pyoderma gangrenosum. *Lancet*. **351**(9105): 832.
272. Furukawa, F., Imamura, S., and Takigawa, M. (1995). FK506: therapeutic effects on lupus dermatoses in autoimmune-prone MRL/Mp-lpr/lpr mice. *Arch. Dermatol. Res*. **287**(6): 558–563.
273. Yoshimasu, T., *et al*. (2002). Topical FK506 (tacrolimus) therapy for facial erythematous lesions of cutaneous lupus erythematosus and dermatomyositis. *Eur. J. Dermatol*. **12**(1): 50–52.

48

UNPROVEN AND EXPERIMENTAL THERAPIES

Ronald F. van Vollenhoven

INTRODUCTION

While established therapies are successful in many patients with systemic lupus erythematosus (SLE), there is still a great need for more effective and safer treatments, particularly with respect to the long-term outcomes and quality of life. New data continue to emerge regarding unproven but widely employed therapies, such as intravenous gammaglobulin and plasmapheresis, while several immunosuppressive and immunomodulatory agents originally developed for use in other diseases are being evaluated for their potential in SLE, in particular, the agent mycophenolate mofetil that in one study showed promising results in the treatment of lupus nephritis. Appropriately, a large number of entirely new therapies for SLE are currently being tested in animal models and in human clinical trials. These include the hormonal agent dehydroepiandrosterone (DHEA) which has gone through extensive clinical trials with positive results. A number of biological agents, such as monoclonal antibodies targeting B lymphocytes (rituximab), various "second signal" inhibitors (including CTLA4-Ig), and anti-IL10 have also been tested in humans, with varying results. The B-cell toleragen LJP394 is being evaluated for its ability to prevent nephritis flares. In addition, a range of "alternative" treatments including herbs and nutritional supplements are available to the general public. Thus, while the proper evaluation of new treatments for SLE remains a formidable challenge, the prospects are excellent for more successful treatments in the not too distant future.

> All who partake of this remedy recover in a short time except those whom it does not help, who all die. Therefore, it is obvious that it fails only in incurable cases.
>
> [Galen, second century A.D.]

OVERVIEW

Evaluating New Therapies for SLE

Numerous potential new treatments for SLE have been proposed in both the scientific and the lay literature over the past decades. A survey of these treatments might easily leave one bewildered and wondering where they are now, given the time that has elapsed. While evaluation of new treatments for any disease engenders certain difficulties and pitfalls, it is particularly challenging in the case of SLE, because of several characteristic features of this disease.

1. SLE is a chronic disease, and treatments must be evaluated based on their ability to bring about a meaningful change in the long-term rather than the short-term outcome.
2. SLE is characterized by morbidity as well as mortality. In most patients the main focus is on improving quality rather than length of life; thus, longer-term outcomes and quality-of-life issues play a major role.
3. SLE is highly heterogeneous. In view of this, some clinical trials single out one specific organ manifestation of SLE, most often nephritis, to investigate the efficacy of an intervention. However, it is not necessarily true that a treatment for one such organ manifestation is also

effective for other symptoms or for the disease as a whole. In order to deal with this problem, a number of indices for *global* disease activity have been developed [1, 2], that are now being used in therapeutic trials.

4. SLE has a variable and unpredictable course. Any clinician can attest to the fact that some patients with very severe disease have made remarkable recoveries, while others have succumbed to what appeared to be manageable disease severity. In view of this, case reports and small series of 'successful' interventions need to be regarded with caution, and controlled studies of sufficient size are critically important.

Challenges in SLE Therapy

The currently available therapies for SLE fall considerably short of achieving the desired objectives in many patients. Some patients still die of lupus, and organ damage accrues over time in many patients. Moreover, therapy for SLE often does not achieve control of persistent "smoldering" disease activity, and the patients who attain complete remissions of disease activity, even when treated aggressively, may well be in the minority. Clinical lupus practice is frequently dominated by patients who, despite adequate treatment for the most serious, organ-threatening manifestations of SLE, remain symptomatic, often with "minor" or "systemic" manifestations of the disease. While lupus therapeutics has been dominated for many years by the emphasis on preventing death, renal failure, and other end-organ damage, a number of more recent studies have deliberately focused on agents and treatments that may improve the less severe but more common symptoms of SLE, such as UV-A1 light, bromocriptine, and DHEA.

Unproven and Experimental Therapies: An Overview

Unproven and experimental therapies for SLE are found in various stages of development (Table 1). Many new biological and pharmacological therapies have been studied in mouse models of SLE only, while some have been used in small numbers of patients with SLE; and a few such novel therapies are currently in controlled clinical trials. Some "new" treatments, such as the immunosuppressive mycophenolate mofetil or the hormonal agent bromocriptine, are established in the treatment of other diseases, and are being tested in clinical trials involving patients with lupus. These types of treatment are, therefore, available to physicians for use in individual patients (although insurance coverage may not be extended for their use). Finally, most "alternative" treatments, such as nutritional interventions, herbs, and acupuncture, are generally available to the patient without having received any kind of formal regulatory approval.

NONIMMUNOSUPPRESSIVE IMMUNOMODULATORY TREATMENTS

Intravenous Immunoglobulin

Intravenous infusion of pooled human donor-derived immunoglobulin (IVIG) has become a well-established treatment for several human diseases with immunological mechanisms, but remains controversial in the therapy of SLE [3]. The most successful roles for IVIG are in the management of idiopathic thrombocytopenic purpura (ITP) where rapid and dramatic, albeit transient, improvements of platelet counts are almost invariably seen [4]; and in Kawasaki's disease, where significantly improved long-term outcomes are observed following such therapy [5]. In the wake of these successes, IVIG has been used in various autoimmune diseases, but with few controlled data so far.

In SLE, the use of IVIG in patients that manifest lupus-associated thrombocytopenia is generally accepted. The mechanism of this therapy is believed to be blockage of Fc receptors in the reticuloendothelial system, thereby preventing the phagocytosis of antibody-coated platelets [6]. Whether IVIG is useful for the treatment of SLE other than in this specific setting remains controversial. In 1984, Gaedicke *et al*. [7] reported on 2 patients with juvenile SLE, 1 of whom showed impressive improvement of multiple SLE manifestations following IVIG administration. Francioni *et al*. [8] reported on 12 patients with moderately severe lupus who were treated with monthly IVIG infusions for up to 2 years; 11 of their patients had progressively improving disease courses during the trial. Several case reports suggested remarkable benefits in patients with CNS-lupus [9–12]. Other reports have stressed benefits for lupus nephritis [8, 13]. While most reports in the literature describe patients with severe disease who have failed conventional therapy, Schroeder *et al*. [14] reported on 12 patients with mild to moderate disease, who were treated with two series of IVIG infusions (approximately 2 g/kg × 2 total) in lieu of standard therapy; 9 of the 12 patients improved, and 3 had sustained responses for 5–12 months. Successful treatment with IVIG has also been reported for lupus-related myelofibrosis [15], lupus-related acquired factor VIII inhibitor [16], lupus-related cardiomyopathy [17], and lupus-related polyradiculoneuropathy [18]; but treatment failures, which are far less likely to be reported in

TABLE 1 Unproven and Experimental Therapies: An Overview

Studied in mouse models	Studied in patients with SLE; uncontrolled data	Studied in controlled clinical trial(s)	Available to treating physician	Available to public
	IVIG	IVIG	IVIG	
LJP394	LJP394	LJP394		
	Plasmapheresis	Plasmapheresis[a]	Plasmapheresis	
	Selective immunoadsorption		Selective immunoadsorption[b]	
Total lymphoid irradiation	Total lymphoid irradiation		Total lymphoid irradiation[b]	
	High-dose cyclophosphamide and myeloablative therapy with autologous stem-cell transplantation		High-dose cyclophosphamide and myeloablative therapy with autologous stem-cell transplantation[b]	
DHEA (dehydroepiandrosterone)	DHEA	DHEA	DHEA[c]	DHEA[d]
	Danazol	Danazol	Danazol	
	(Nor)testosterone		(Nor)testosterone[e]	
	Leuprolide	Leuprolide	Leuprolide	
	Bromocriptine	Bromocriptine	Bromocriptine	
Tamoxifen	Tamoxifen[f]		Tamoxifen	
Anti-T cell mAb	Anti-CD4, anti-CD5-ricin			
Anti-B cell mAb	Rituximab (anti-CD20)		Rituximab (Rituxin, Mabthera)	
Anti-IL6 receptor				
Anti-IL10	Anti-IL-10			
Anti-interferon α				
	Thymosin			
CTLA4-Ig				
Anti-CD154 (CD40 ligand)	Anti-CD154[g]			
Anti-IgD				
Anti-idiotype				
Anti-Fas, apoptosis inhibitor				
Anti-complement C5				
Antisense oligonucleotides				
Mycophenolate mofetil	Mycophenolate mofetil	Mycophenolate mofetil	Mycophenolate mofetil	
Tacrolimus (FK506)	Tacrolimus		Tacrolimus	
Frentizole	Frentizole[h]			
Mizoribine	Mizoribine			
Rapamycin				
	Thalidomide	Thalidomide[i]	Thalidomide	
	Zileuton	Zileuton	Zileuton	
	UVA light therapy	UVA light therapy	UVA light therapy[b]	
Chinese herbs	Chinese herbs			Chinese herbs
Ganoderma tsugae				
Tripterygium wilfordii Hook f				
Caloric restriction; other dietary manipulation	Fish oil[f]			Fish oil, other dietary products
Flaxseed	Flaxseed	Flaxseed[f]		Flaxseed
Antioxidants: *N*-acetylcysteine, pycnogenol, lobenzarit	Lobenzarit	Pycnogenol		Various antioxidants

[a] Controlled trial(s) with *negative* results.
[b] Available only in specialized centers.
[c] FDA is reviewing new drug application for DHEA (Prasterone, Aslera). Physicians can prescribe DHEA to be compounded by specialized pharmacies.
[d] Non-prescription products labeled as containing DHEA have been found to contain inconsistent and variable amounts of DHEA and may contain other hormones.
[e] Possibly some benefits in male patients with testosterone but worsening disease with nortestosterone; doubtful if any efficacy in females.
[f] *Negative* study.
[g] One small negative study, other study terminated due to serious side effects.
[h] development halted due to severe, life-threatening side effects.
[i] effective in *cutaneous* lupus.

the literature, were also noted [19, 20]. No controlled trials have been performed of IVIG in SLE. Because IVIG is not immunosuppressive, its use could be considered in patients with concurrent infection, particularly with encapsulated organisms where IVIG may have additional therapeutic benefit [21]. IVIG appears to be safe during pregnancy, and its use in this setting has engendered significant interest, particularly when SLE was accompanied by the anti-phospholipid antibody syndrome [22].

Various therapeutic mechanisms of IVIG in SLE have been proposed (reviewed in Ref. 23), including down-regulation of autoantibody production, dissociation and/or increased clearance of immune complexes; RES blockade; and blockade of autoantibodies by anti-idiotypic antibodies in the IVIG [24]. However, most of these possibilities remain to be formally tested.

IVIG is perceived as safe, but some toxicities have been noted. Mild to moderate infusion reactions are fairly common. Leukopenia and other hematological side effects occur in 2–3% of patients. Acute renal failure is an uncommon but potentially serious side effect [25]. A 6-year-old patient with SLE developed severe vasculitis following IVIG therapy [26]. The most notable side effect of IVIG is "pain in the pocket book": the retail cost of a single treatment course of IVIG (2 g/kg) ranges from $2000 to $3500, and the additional expenses involved in the administration of an intravenous drug, such as nursing time and equipment use, add substantially to this figure. One would hope that the considerable financial gains posted by the manufacturers of commercially available IVIG will to some extent be used to support well-designed clinical trials of IVIG in appropriate clinical settings.

Thus, IVIG remains an as yet unproven but interesting therapeutic option in SLE. In clinical practice, one might consider IVIG particularly in those situations where the patient is critically ill despite appropriate corticosteroid and immunosuppressive therapy; in cases of severe CNS disease; in patients with concomitant SLE and infection, particularly with encapsulated organisms; and in pregnant women with severe SLE.

LJP394: DNA-Toleragen

Anti-(double-stranded) DNA antibodies are unique among the autoantibodies associated with human autoimmune diseases in that they are virtually pathognomonic of SLE, are seen mostly in patients with nephritis, and in that their levels may mirror or even predict SLE disease activity [27]. Moreover, anti-DNA may play a pathogenetic role in lupus nephritis through formation of immune complexes with circulating native DNA, through binding of DNA within the glomeruli, or through cross-reactivity with glomerular structures. Thus, the objective of lowering anti-DNA antibody levels in patients with SLE is scientifically appealing. The most straightforward approach to this problem, removal of anti-DNA antibodies from the circulation by plasmapheresis, has met with limited success (discussed elsewhere in this chapter). An approach that would selectively down-regulate the anti-DNA producing B cells is therefore a logical goal.

Experimentally, B-cell tolerance can often be achieved through the administration of antigens intravenously [28–30]. One of the mechanisms for such tolerance is the binding of soluble antigen by the B cell in the absence of a costimulatory signal provided by T cells, resulting in B-cell anergy [31]. The molecule LJP394 was engineered for this purpose. LJP394 is a triethylene glycol-platform with four 20-mer oligonucleotides, consisting of alternating deoxycytidine and deoxyadenosine nucleotides, covalently attached. This molecule was shown to have high affinity for anti-DNA antibodies and to decrease anti-oligonucleotide antibody formation in oligonucleotide immunized mice [32]. Moreover, LJP394 reduced anti-DNA antibody formation in the BXSB mouse model of SLE, and decreased glomerulonephritis, nephrosis, and death in these mice [33]. In human SLE patients treated with LJP394 50-mg IV once weekly for 16 weeks, anti-DNA titers were decreased by more than 50%, and levels of anti-DNA remained suppressed during an additional 2-month follow-up period without further treatment [34]. In a similar but larger dose-finding study the reduction in anti-DNA was around 40% at the highest dosage level [35]. In a subsequent multicenter randomized placebo-controlled clinical trial the ability of weekly intravenous injections with LJP394 to *prevent* flares of lupus nephritis was evaluated. While the results of this trial have not yet been published, the main findings have been made public and indicate that a meaningful reduction of nephritis flares was seen in those patients who had anti-DNA antibodies with high binding affinity for LJP394. A subsequent phase III clinical trial to confirm this possibility is currently underway. If successful, LJP394 may become the first in a new class of drugs, designed to reduce the burden of pathogenic autoantibodies and thereby prevent disease relapses. This principle could then be applied to other diseases as well.

It is as yet unclear if LJP394 would exert its effects by binding circulating anti-DNA, facilitating clearance of these antibodies, or by actually down-regulating autoreactive anti-DNA producing B lymphocytes (as originally intended). While the former mechanism might be equally effective, only the latter possibility would suggest that a major treatment benefit could be

maintained after therapy is discontinued. And even if anergy is in fact induced, it is unknown how long such anergy might persist following cessation of the treatment.

NONPHARMACOLOGICAL IMMUNOSUPPRESSIVE APPROACHES

Plasmapheresis

Conventional Plasmapheresis

Plasmapheresis or plasma exchange for the treatment of SLE appears to have gone through several cycles of enthusiasm and disappointment. The assumption that removal of harmful autoantibodies would be beneficial to patients with lupus could not be more straightforward, and uncontrolled observations in the 1970s suggested that plasmapheresis was indeed an important therapeutic tool for the management of this disease.[1] However, several controlled studies failed to show benefit for plasmapheresis. A 2-year randomized trial in 18 patients showed similar outcomes with or without plasma exchange [36]. In a pivotal clinical trial of this treatment, the Lupus Nephritis Collaborative Study Group randomized 86 patients with severe lupus nephritis to receive either corticosteroids plus cyclophosphamide, or corticosteroids plus cyclophosphamide plus plasmapheresis three times a week for the first 4 weeks [37]. After a mean follow-up of 136 weeks, there were no statistically significant differences between the two groups in terms of mortality, progression to renal failure, or improvements in creatinine clearance and proteinuria.

Plasmapheresis can engender a rapid "rebound," an increase in antibody levels that even exceed the previous ones, due to expansion of autoreactive B cells after withdrawal of the negative feedback exerted by the antibodies in the circulation. Therefore, plasmapheresis is almost exclusively used in settings where strong immunosuppressive therapy can be given as well (most often cyclophosphamide). Some studies even suggested that it might be beneficial to "synchronize" plasma exchange with cytotoxic therapy. However, in an ambitiously designed, multicentered, randomized clinical trial by the Lupus Plasmapheresis Study Group (LPSG), patients with severe SLE (including a majority of patients with nephritis, but not excluding other lupus manifestations) were randomized to regimes of monthly infusions with cyclophosphamide vs synchronized plasmapheresis followed by cyclophosphamide, for 12 months. Contrary to expectations, the results of the trial showed that the treatment with synchronized plasmapheresis plus cytotoxic therapy was *not* superior to cytotoxic therapy alone with respect to the primary outcome of interest, improvement in systemic lupus activity measure (SLAM; Ref. 1) score, or to organ-specific outcomes (reported in abstract form, Ref. 38. An analysis of the subset of patients with nephritis was also published with similar results [39]. From a scientific point of view, it would be important to determine if the synchronized treatment did, in fact, decrease the numbers or activity of autoantibody producing B cells, in order to determine if the negative results of this trial were due to an incorrect premise (i.e., anti-DNA producing B cells are not critical for the outcome of SLE), or due to an inadequate treatment (i.e., inability to down-regulate such cells with this type of therapy).

Thus, plasmapheresis given in a variety of manners does not appear to improve the outcome of lupus overall or of severe lupus nephritis. However, there may still be a role for plasmapheresis in the management of certain patients with SLE, especially since it has become increasingly recognized that some patients with SLE exhibit features of thrombotic microangiopathy [40, 41], such as thrombotic thrombocytopenic purpura (TTP), or hemolytic–uremic syndrome (HUS).

Because of the established role of plasmapheresis in the treatment of these disorders, it would seem reasonable at this time to include plasmapheresis in the therapeutic regimen for severely ill patients with SLE who have evidence for TTP or HUS [42]. Furthermore, patients with SLE who exhibit concomitant conditions amenable to treatment with plasmapheresis, such as cryoglobulinemia, or the catastrophic anti-phospholipid syndrome [43] are also reasonable candidates for such therapy.

Plasmapheresis is not without potential risks. Side effects during the procedure itself include hemodynamic complications, electrolyte disturbances, access-related complications such as bleeding and infections, and others. A small retrospective comparison of lupus patients treated with or without plasmapheresis unexpectedly suggested a significantly higher mortality due to infectious causes for patients given plasmapheresis [44].

Selective Immunoadsorption

A variant of plasmapheresis is immunoadsorption, whereby specific subsets of antibodies can be extracted from the patients' blood. Various methodologies

[1]So much so that, when the Lupus Nephritis Collaborative Study Group proposed doing a controlled trial of plasmapheresis, some prospective participants argued that it was unethical to withhold this treatment from a group of patients for the purposes of this study.

employing membranes and columns have been used over the years [45–52] and the fact that they accomplished the desired reduction in specific antibody titers has been demonstrated. For example, immunoadsorption using a dextran sulfate column effectively removed anti-DNA antibodies from the circulation of patients with SLE [46]. Only individual cases and small series of patients with SLE have been reported [47–50]. Immunoadsorption with a protein A column was effective in the treatment of a patient with SLE-related thrombocytopenia, presumably by binding the antiplatelet antibodies [51]. Successful treatment in a patient with SLE and transverse myelitis was also reported [52].

The complement component C1q has a central role in immune complex mediated inflammation. In a pilot study, treatment with a C1q immunoadsorption column gave excellent responses of long duration in three of eight treated patients [53]. Similarly, rapid improvement of cutaneous lupus was seen in one patient with the same treatment [54].

Total Lymphoid Irradiation (TLI)

X-ray irradiation of the lymphoid tissues results in dose-related immunosuppression [55], and suppresses lupus-like disease in NZB/NZW mice [56, 57]. Total lymphoid irradiation (TLI) in humans resulted in long-lasting immune suppression, reflected by decreased mitogen and antigen responses, and reductions in peripheral blood T-cell counts, particularly $CD4^+$ T cells [58]. TLI with 2000 rads administered to all the lymphoid tissues above and below the diaphragm over a period of around 6 weeks, and with mantel-field shielding of the bone marrow and lungs analogous to the treatment of Hodgkin's disease, has been used in small numbers of patients with SLE at Stanford University over the past 20 years. In the initial description of 15 patients, favorable 1–2 year results were reported [59, 60]: most patients attained significant improvement of renal disease, and many were able to discontinue corticosteroid therapy. However, 2 patients with SLE treated at another institution did not do well [61]. It was also noted that, in patients with rheumatoid arthritis who were treated with TLI, encouraging 2- to 5-year results were followed by 10-year outcomes that were significantly less favorable than with cytotoxic therapy [62]. Long-term follow-up of the Stanford TLI cohort was more recently published [63]. Out of a total of 21 patients treated with TLI for lupus nephritis, 6 died after an average of 6 years. Nine patients (43%) survived >10 years without progressing to renal failure. Of these, 4 patients did not require additional cytotoxic therapy. Inasmuch as these patients were characterized by high severity and, in most cases, failure to achieve disease control with conventional agents, these results may be viewed as favorable. Infections seen among the 21 patients treated with TLI included herpes zoster in 5, and fungal infections in 2. Four malignancies were seen (two lymphoma, one thyroid cancer, one cervical cancer), all of which were successfully treated.

In summary, TLI has been used to date primarily in patients with severe and/or refractory lupus with nephritis. In this group of patients, overall results and toxicities were similar to those seen with aggressive immunosuppressive drug therapy (e.g., with cyclophosphamide). It is unclear at present how TLI would compare if used instead of, rather than in those patients who have failed, cytotoxic therapy. Long-term benefits in a small number of patients who had failed standard cytotoxic regimens suggest that TLI may have a role in the management of patients with refractory lupus nephritis.

HIGH-DOSE AND MYELOABLATIVE CHEMOTHERAPY

It is a rather striking fact that, whereas the chemotherapy agents cyclophosphamide and azathioprine have become mainstays of lupus therapeutics, their usage has been as single agents (along with corticosteroids) and at dosage levels much below those used in oncology.[2] However, there has been a surge of interest in various forms of high-intensity chemotherapy for SLE.

Higher-Than-Conventional Dosages of Cyclophosphamide

A treatment protocol for severe SLE was developed in Kiel, Germany, consisting of plasma exchange, IVIG, and cyclophosphamide at 1200–2100 mg/m^2 given over 3 days, followed by oral cyclophosphamide for 6 months. The initial results with this protocol showed a remarkable percentage of patients with durable treatment-free remissions [64]: of 14 patients treated with this regimen, 8 were in remission without treatment for 4–8 years. A later update on the use of this protocol in a number of centers [65] shows that overall results were somewhat less impressive: while 16 of 52 patients attained treatment-free remissions, toxicities were severe, including a >10% mortality, frequent major infections, hemorrhagic cystitis, severe thrombocytopenia, and major hemorrhage.

[2]The historical reason for this may be the success of low-dose daily cyclophosphamide in the treatment of Wegener's granulomatosis.

Petri and colleagues [66] treated 14 patients with severe and refractory SLE (9 with nephritis) using cyclophosphamide 50 mg/kg daily for 4 days followed by G-CSF. Global disease indices improved significantly. Five of the 14 patients were considered complete responders and 7 partial responders. After up to 4 years of follow-up, some patients had durable responses but others had relapsed. No deaths and no premature ovarian failure were seen. Inasmuch as these results were obtained in patients previously treated with conventional immunosuppressive regimens with inadequate responses, the results are impressive. However, the lack of response in some, and the occurrence of relapses in others, underscores the need for better ways of identifying those patients most likely to benefit from aggressive therapeutic strategies. A trial randomly assigning patients with severe lupus to conventional cytotoxic therapy vs high-intensity chemotherapy, is currently underway.

Bone-Marrow and Stem-Cell Transplantation

The use of allogeneic bone-marrow transplantation for a range of malignancies has been associated with a few scattered reports of remissions in patients who also suffered from autoimmune diseases, for example, rheumatoid arthritis (RA) [67]. However, the 20–30% mortality associated with allogeneic transplantation precludes it from being considered as a reasonable therapeutic option in SLE. In contrast, autologous stem-cell transplantation (ASCT), having a much lower mortality, is under intense investigation for the treatment of autoimmune diseases [68–70]. The rationale for ASCT is based on two separate premises: First, it can be postulated that diseases such as SLE might respond better to very high-dose, myeloablative chemotherapy, which must then be followed by "rescue" ASCT. This premise would follow logically on the demonstration that better results can be obtained in SLE with higher-intensity chemotherapy. Second, it can be argued that ASCT reestablishes the immune system, replacing the malfunctioning lymphocytes with a freshman class of soon-to-be-educated B and T cells. Whether either of these two rationales is correct remains to be seen. In one reported series, five patients with autoimmune diseases, one of whom had SLE, were given ASCT with unmanipulated grafts [71]. In all five patients the serological and clinical evidence for autoimmune disease persisted or recurred after the transplant. The lack of success in these patients was interpreted as underscoring the need for "purging" of the graft from mature lymphocytes prior to reintroduction, but it is also conceivable that more intensive treatment of the host in order to eradicate autoreactive lymphocytes may be necessary to accomplish better results. Several reports of ASCT with purged grafts for SLE were published, including in childhood SLE [72, 73]. A larger series was reported by Traynor *et al*. [74]. Of nine patients with SLE selected for treatment with ASCT, two patients underwent stem-cell mobilization but could not be transplanted due to infections, and both died. Of the remaining seven, after a follow-up of 12–40 months, all were reported to be free of signs of active lupus and to require no other immunosuppressive therapy than small dosages of prednisone.

The overall experience worldwide with ASCT for SLE was reviewed by Tyndall [75]. Thirty-two patients with SLE have now been treated with ASCT, mostly receiving myeloablation with cyclophosphamide plus anti-thymocyte globulin. Three procedure-related deaths occurred (9%), 19 patients improved after treatment, and of those, 5 relapsed. Currently, several centers in the United States as well as in Europe are actively pursuing these types of therapies. A centralized registry has been formed by the European Group for Blood and Marrow Transplantation (EBMT) and the European League against Rheumatism (EULAR) [76].

In conclusion, high-intensity immunosuppressive therapy, with or without stem-cell transplantation, appears to have the potential of achieving treatment response in those few patients with very severe SLE that are refractory to conventional immunosuppressive treatments, and of achieving long-lasting treatment-free remissions in some but certainly not all patients. Whether such high-intensity regimens have advantages over conventional immunosuppressive treatment, for example, by shortening the duration of immunosuppression or by attaining more "remissions," remains to be seen. Remarkably, a more recent study by Gunnarsson *et al*. [77] did *not* show a clear dose response relationship for cyclophosphamide dosages and renal outcome. Moreover, the risks and side effects of high-intensity treatments are formidable obstacles. Most importantly, the main challenge with these extremely aggressive and high-risk therapeutic options for pa-tients with SLE remains the selection of appropriate patients for such treatment. The identification of more reliable prognostic indicators in SLE [78] will be of great importance in order to implement successfully the more aggressive therapies for SLE that will become available.

HORMONAL THERAPIES

The role that sex hormones play in the etiology and pathogenesis of SLE remains incompletely understood. Because of various observations suggesting important

roles for such hormones, including the female-to-male predominance in SLE and the changing disease patterns during periods of hormonal alteration, a potential therapeutic role for sex steroids has been discussed [79].

Dehydroepiandrosterone (DHEA)

The adrenal androgen DHEA (prasterone) appeared attractive as a candidate hormonal therapy for SLE based on a number of considerations. The use of androgens in general was appealing based on the relative abundance of the immunologically active 16α-hydroxylated estrogen metabolites [80], and the faster oxidative elimination of testosterone seen in this disease [81]. The fact that DHEA is known to have only mild intrinsic androgenic properties suggested that it might be much better tolerated by female patients than androgens such as testosterone. Moreover, serum levels of DHEA, as well as of the principal metabolite DHEA-sulfate, are lower than normal in patients with SLE, even in the absence of corticosteroid treatment or disease activity [82, 83]. Interestingly, studies done at Stanford also suggested a potentially important immunomodulatory effect of DHEA on human T cells [84]. Specifically, it was shown that *in vitro* IL-2 production by stimulated peripheral blood T cells (from healthy individuals) was enhanced by DHEA. Similar results in murine systems had been obtained by Daynes and others [85], who also reported additional effects on other cytokines, suggesting that DHEA might effect a shift in T-cell phenotype from the Th2 type (characterized by production of IL-4, IL-5, and the enhancement of humoral immune responses), to Th1 (characterized by production of IL-2 and γ-interferon (IFNγ) and the enhancement of cellular immune responses) [86]. Juxtaposed to the finding that patients with SLE exhibit lower-than-normal IL-2 production by peripheral blood T cells [87, 88] and that, in general, humoral immune responses might be most pivotal in the immunopathogenesis of SLE, these results suggested that the administration of exogenous DHEA to patients with SLE might restore a fundamental abnormality of immunological functioning. More recently, it has been demonstrated that the defective IL-2 production in patients with SLE is due to deficiency of endogenous DHEA, and that *in vitro* supplementation with exogenous DHEA is sufficient to restore normal levels of IL-2 secretion [89]. Lucas *et al*. [90] showed that DHEA was beneficial in the NZB/NZW mouse model of SLE, and we subsequently showed that this benefit could be attained at lower dosage levels and was associated with normalization of lymphocyte proliferative responses in such mice [91].

Based on these observations a number of studies of DHEA in patients with SLE were done at Stanford. In an uncontrolled trial, 10 premenopausal women with mild to moderate SLE received DHEA 200 mg/day for 6 months [92]. Average systemic lupus erythematosus disease activity index (SLEDAI) scores (a composite measure of lupus activity based on clinical symptoms, signs and laboratory values; Ref. 2) decreased significantly, while prednisone dosages also decreased. Subsequently, a randomized, double-blinded, placebo-controlled clinical trial was performed [93]. Twenty-eight female patients with mild to moderate SLE were randomized to receive either DHEA 200 mg/day or placebo for 3 months. During the trial period, corticosteroid dosages were adjusted as clinically indicated. At the end of the trial period, a decrease was seen in the SLEDAI scores of patients who had received DHEA, along with a reduction in prednisone dosage; whereas a slight increase was noted in patients on placebo, both in terms of lupus activity by SLEDAI and prednisone dosage. Correcting for the baseline imbalance, the patients on DHEA did achieve a statistically significant reduction in patients' global assessment compared to the control group. In addition, SLE flares occurred less frequently in the DHEA-treated group (three flares vs eight in the controls, $p < 0.06$). DHEA appeared safe in this study and was tolerated well.

Data on 50 patients treated with DHEA 50–200 mg/day for periods up to 1 year [94] suggested that in some patients who show initial responses to DHEA after 3 months, further improvement of lupus scores and reductions of prednisone dosages are seen when treatment is continued for 6–12 months. Side effects with DHEA in this dosage range are generally minor and include mild to moderate acne, changes in skin texture, and mild hirsutism.

In our patients, serum levels of DHEA and DHEA-sulfate while in the study showed wide variability and corresponded only weakly to clinical benefits, although a trend was seen toward optimal clinical responses at serum DHEA-sulfate levels of around 1000 μg% [95].

We also conducted a 6-month trial of DHEA vs placebo in patients with *severe* SLE [96]. In this group of patients, which had not been included in any other studies of DHEA to date, the trial drug was added to a conventional regimen of high-dose corticosteroids and immunosuppressives. The primary outcome was stabilization or improvement in the principal target organ involvement in each patient's disease. After 6 months, 7 of 9 patients on DHEA and 4 of 10 patients on placebo achieved the primary outcome ($p < 0.10$). An additional trend in SLEDAI scores favoring DHEA was observed but baseline imbalances impeded further interpretation of these findings.

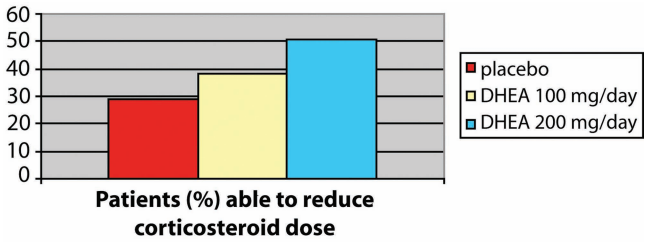


FIGURE 1 Results of the GL94-01 corticosteroid-sparing clinical trial of DHEA in SLE. Treatment with DHEA at 100–200 mg/day for 7–9 months allowed corticosteroid dosage reductions in a higher percentage of previously corticosteroid-dependent patients. Analyzed for those patients who at baseline hae disease activity (SLEDAI > 2). The difference between the DHEA 200 mg/day and placebo group is statistically significant ($p < 0.05$). Reformatted from published data [97].

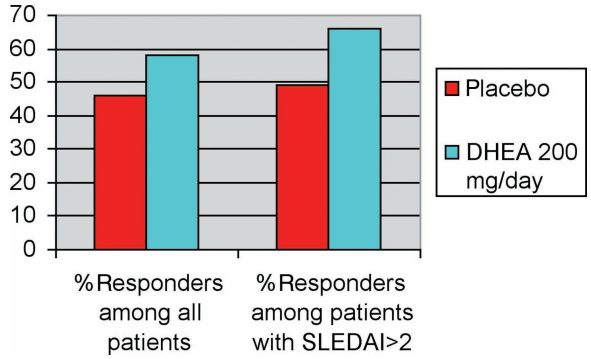


FIGURE 2 Treatment with DHEA 200 mg/day for 1 year in female patients with mild–moderate SLE. According to a predefined responder definition, a significantly higher percentage of patients on active treatment were responders ($p < 0.02$ for all patients, $p < 0.01$ for patients with SLEDAI > 2 at baseline). Reformatted from published data [98].

Based on these small studies, two large-scale, multicentered, randomized, placebo-controlled clinical trials of DHEA (prasterone, GL701) in SLE were conducted. The first of these two trials (the GL94-01 study) investigated the possible corticosteroid-sparing role of DHEA in patients with SLE who had been unsuccessful in attempts to taper prednisone dosages below 10 mg/day [97]. The results indicate that, in patients with at least some degree of disease activity (i.e., SLEDAI scores >2), among the patients on DHEA a significant and clinically meaningful increase was seen in the percentage who achieved the prospectively defined goal of a sustained reduction of corticosteroid dosage to ≤7.5 mg/day of prednisone. Moreover, a dose–response effect was seen, with patients on DHEA 200 mg/day showing higher rates of success than those on 100 mg/day (Fig. 1).

The second large-scale clinical trial of DHEA in SLE (the GL95-02 study) represents, in fact, the largest randomized placebo-controlled clinical trial to date in SLE [98]. Approximately 300 female patients were randomized to receive DHEA 200 mg/day for 1 year vs placebo. At the end of this year, the proportion of responders was determined for each group. In order to be qualified as a responder, patients had to have improved or stabilized with respect to all four of the following: SLAM score, SLEDAI score, patient's visual analog scale for disease activity, and a scale for fatigue. In addition, any lupus flare during the treatment year would also make the patient a nonresponder. Using this predefined criterion, 58% of patients in the DHEA group were responders, compared to 46% in the placebo-treated group (Fig. 2). For patients with disease activity at baseline (SLEDAI > 2) the difference was even more pronounced, 66% vs 49% ($p < 0.005$). Thus, DHEA treatment during 1 year imparted a statistically significant benefit with respect to global SLE activity.

An additional study of DHEA by Chang *et al.* [99] in 119 patients with SLE showed that during 6 months of treatment with DHEA 200 mg/day, the number of SLE flares was significantly reduced, from 34% in the placebo group to 18% in the treatment group ($p < 0.05$).

In addition to the overall benefits with respect to SLE activity, two separate analyses indicated that DHEA might have important benefits for bone metabolism in SLE as well. Thus, in the study of severe lupus [96], the placebo group showed significant reductions in bone mineral density at the lumbar spine after 6 months of treatment (with high-dose corticosteroids), whereas the group of patients receiving both high-dose corticosteroids and DHEA had no such decrease. In the GL95-02 study [98] a subset of patient underwent bone mineral densitometry: here a significant improvement was seen in the patients treated with DHEA compared to placebo.

In all these clinical studies, DHEA was generally well tolerated. Acne was a frequent but rarely problematic side effect. Hirsutism and other androgenic side effects occurred rarely. A decrease in HDL cholesterol was seen in some patients. There were no increases in infections, endocrinological disturbances, thromboembolic complications, or malignancies. Importantly, all these studies were limited to female patients with SLE.

In summary, several smaller studies, both controlled and uncontrolled, and three large clinical trials provide support for the hypothesis that DHEA may have therapeutic benefits for female patients with SLE. The significant gains in outcomes over intermediate periods of time coupled with good tolerability suggest that DHEA is likely to have an important role as a therapeutic

option in the long-term management of SLE, with improvements in overall disease activity, corticosteroid sparing effects, reductions in SLE flares, and beneficial effects on bone mineral metabolism.

The results of these trials have been under review by the FDA for approval of DHEA/prasterone as a prescription drug under the brand name Aslera. Meanwhile, in the United States, a number of products claiming to contain DHEA are being sold as nutritional supplements. In one study, these products were found to contain amounts of DHEA differing considerably from those indicated on the labels, and some such products did not contain DHEA at all [99a].

Other Hormonal Therapies

Besides DHEA, a variety of hormonal interventions have been employed in animal models as well as in patients with SLE, with different degrees of success.

Androgens

Danazol is a semisynthetic androgen with therapeutic efficacy in ITP, through a mechanism that is not well understood [100]. Not surprisingly, it also appears beneficial in patients with SLE-associated thrombocytopenia [101, 102], and it may be of benefit in lupus-related hemolytic anemia as well [103]. The possibility that danazol might be of benefit for nonhematological lupus manifestations was addressed in several papers. Case reports suggested benefits in patients with oral ulcers [104] and with discoid lesions [105]. A cross-over study in seven patients suggested modest clinical improvement with minimal side effects [106]. A 1-year controlled clinical trial in 40 patients showed fewer SLE flares in the patients randomized to danazol and lower corticosteroid requirements, but limiting side effects were common in this study, particularly gastrointestinal ones [107]. Thus, it appears that danazol may benefit patients with hematological lupus, and can perhaps ameliorate milder, generalized symptoms; but danazol is often not tolerated well by patients with SLE.

Treatment with the androgen 19-nortestosterone decanoate did not improve SLE in seven female patients and worsened disease in three male patients [108]. The disappointing results in the latter group may have been explained by down-regulation of endogenous androgen production. Moreover, 19-nortestosterone was not well tolerated by the female patients, resulting in severe masculinization.

One patient with Klinefelter's syndrome (XXY) and SLE showed sustained improvement on testosterone treatment [109]. In my own experience, many male patients with SLE have some degree of hypogonadism, most likely secondary to the chronic disease, and testosterone supplementation into the physiological range can bring about improvements in subjective well-being, muscle strength, and sexual function. Dyslipidemias, mood changes, and erythrocytosis can be limiting side effects.

GnRH Agonists and Antagonists

Continuous administration of gonadotropin releasing hormone (GnRH) suppresses ovarian estrogen production. A GnRH analog appeared to have short-term benefit in several patients [110], and a study of the GnRH agonist leuprolide showed improvement during 6 months of therapy with respect to SLAM scores, joint count, and lymphocytopenia [111]. Side effects in this study included hot flashes, decreased libido, and vaginal dryness. Moreover, a case report describing an SLE nephritis flare while receiving the GnRH agonist leuprorelin was also reported [111a]. GnRH *antagonists* were investigated in (SWR × NZB):F1 mice and had beneficial effects independently of regulation of gonadal steroid secretion [112].

Bromocriptine

Because of the proinflammatory and immunostimulatory properties of prolactin [113–116] it has been suggested that the prolactin-antagonist bromocriptine would be beneficial for patients with SLE. In an open-label study of seven patients, bromocriptine therapy led to significant decreases in SLAM and SLEDAI scores over a 6-month trial period. After discontinuation, however, prolactin levels appeared to rebound to levels above baseline, and lupus activity increased concomitantly in most of the patients [117]. One patient was found to have a pituitary microadenoma after the trial. Bromocriptine therapy was associated with few side effects in this study.

Subsequently, a double-blind, placebo-controlled, randomized clinical trial of bromocriptine for SLE was conducted [118]. Sixty-six patients with mild to moderate disease were randomized to receive bromocriptine 2.5 mg/day or placebo and followed for a mean of 12.5 months. After 1 year of follow-up, the bromocriptine-treated patients had significantly better SLEDAI scores than controls, and significantly fewer SLE flares had occurred in the treatment group. Headaches and nausea were treatment-limiting side effects in five patients.

In an additional randomized trial, 24 patients with mild to moderate SLE were randomized to receive bromocriptine or hydroxychloroquine for 1 year. A preliminary analysis revealed that over this period similar

treatment effects (improvements in SLAM scores) were seen in both groups [119].

These results point to a potential role for prolactin-antagonism in mild to moderate SLE. Bromocriptine therapy does not appear to carry major risks, but nausea, headaches, and mood changes can be treatment-limiting side effects for 10–20% of patients. Moreover, the significant rebound activation of SLE after discontinuation of bromocriptine noted in one study would limit the applicability of this treatment [120]. However, for patients with SLE who have prolactin-secreting microadenomas with hyperprolactinemia, the use of bromocriptine can be recommended.

Estrogen and Estrogen Antagonists

Carlsten *et al*. [121] showed that estrogen aggravates the immune complex disease in MRL/lpr mice—but also that it slows down the cell-mediated components of the autoimmune disease in the same mice, vasculitis and sialadenitis, pointing out the ambiguous role played by this hormone. A selective estrogen receptor modulator LY139478 was beneficial in MRL/lpr mice [122]. The estrogen antagonist Tamoxifen, was effective in the 16/6 Id [123] and the MRL/lpr [124] mouse models of lupus, but a small study in patients with SLE failed to show benefit [125].

MONOCLONAL ANTIBODIES AND OTHER BIOLOGICAL TREATMENTS

The availability of a number of biological agents including monoclonal antibodies (mAbs) as therapeutic agents for a variety of diseases, and most notably the spectacular therapeutic results seen in the treatment of rheumatoid arthritis with the anti-TNFα agents etanercept and infliximab, has fueled hopes that some such agents will assume a major role in the treatment of SLE as well. To date, many biological interventions have been employed in animal models of SLE, and increasingly, human trials are being conducted to explore these exciting possibilities.

Cell-Directed Biological Therapies

Earlier efforts utilized mAbs targeting subsets of lymphocytes. After the initially promising reports regarding successful treatment of murine lupus with anti-T-cell antibodies [126], anti-CD4 [127–129], and anti-B-cell antibodies [130, 131], human trials were eagerly awaited. In five patients with severe cutaneous lupus, anti-CD4 did appear to show some benefit [132]. However, the disappointing results with anti-CD4 in rheumatoid arthritis [133] put a serious damper on these efforts. More recently, nondepleting anti-CD4 mAbs have been developed and are being tested in various clinical settings. A non-depleting anti-CD4 mAb had modest benefits in MRL/lpr mice [134]. The T-cell specific immunoconjugate anti-CD5-ricin was given to six patients with SLE, of whom two improved clinically [135]. This treatment proved very toxic and is not being studied further in SLE.

The B-cell specific monoclonal anti-CD20 antibody rituximab (Rituxin, Mabthera), is used widely for the treatment of B-cell lymphomas [136, 137]. Logically, its use has now been reported in isolated cases of patients with other B-cell driven diseases [138–140]. In a small dose-escalation trial, 12 patients with SLE were given relatively low dosages of rituximab [141]. Significant depletion of circulating B cells was seen, and no adverse experiences occurred. Improvement in disease activity was seen in some patients.

At our institution, we treated two patients with highly refractory SLE, using a combination of pulse-dose corticosteroids, conventional-dose cyclophosphamide, and rituximab at the full lymphoma dose of $375\,mg/m^2$ weekly $\times$ 4. Both patients tolerated the treatment well. One of these patients had severe lupus nephritis and had received a prior 6-month course of cyclophosphamide without improvement; 4 months after the combined therapy, the nephritis was shown by biopsy to have nearly resolved. The other patient had persistent hypocomplementemia, active nephritis, and multiple other active lupus manifestations despite standard therapy with cyclophosphamide. After the combined treatment, her disease parameters improved dramatically, whereas repeat renal biopsy showed modest improvement. We continue to evaluate the efficacy and safety of combined cyclophosphamide plus rituximab as a salvage therapy for patients who fail conventional immunosuppressive treatment.

Cytokines and Cytokine Antagonists

The roles and interactions of the multitude of identified cytokines in the pathophysiology of SLE are being increasingly unraveled, but major gaps in our knowledge to date preclude accurate predictions of which cytokines or cytokine-antagonists would be most likely to benefit patients with lupus. Unfortunately, results from cytokine manipulation in animal models of lupus have not always provided clear answers to such questions, and as more and more cytokines/cytokine-antagonists are being developed for use in patients with other diseases, it is likely that the main advances in our understanding of these aspects of SLE will come from

carefully conducted observational studies or controlled clinical trials. For instance, antagonism of the broadly proinflammatory cytokines IL-1, IL-6, and IL-15 might be theoretically appealing in SLE. A recombinant form of the IL-1 receptor antagonist has more recently been approved for the treatment of rheumatoid arthritis [142], but there are no published data concerning its potential in SLE. Likewise, anti-IL6-receptor mAbs and anti-IL15 mAbs are being tested in rheumatoid arthritis, and may eventually be tried in SLE as well; anti-IL6-receptor was effective in MRL/lpr mice [143].

Levels of the pleiotropic cytokine IL-10 are known to be increased in SLE and to correlate with disease activity, playing an important role in the pathogenesis of the disease [144, 145]. Anti-IL10 was shown to be beneficial in NZB/NZW mice [146]. In a pilot study, six patients were treated with a murine mAb against IL-10 [147]. The intravenous treatment was given daily for 3 weeks and the patients followed for 6 months. During the entire follow-up period improvements were seen, and after 6 months, five of the six patients were considered to have inactive disease. These encouraging results will most likely have to be duplicated with humanized anti-IL10 before further progress can be made. In a case report, the platelet growth factor IL11 was effective for SLE-related thrombocytopenia [148].

Antagonism of TNFα has been established as a treatment for RA and other inflammatory disorders [149, 150]. However, in SLE the role of TNFα remains controversial. Some studies showed that in NZB/NZW mice, the administration of TNFα rather than its antagonist was beneficial [151, 152]. On the other hand, other studies have suggested a pathogenetic role for TNFα even in human SLE [153–155]. In the 16/6 Id mouse model of SLE, TNFα blockade with a mAb or with pentoxiphylline attenuated disease [156]. Interestingly, in some rheumatoid arthritis patients treated with anti-TNFα therapy, anti-DNA antibodies develop, and a few have developed transient SLE-like symptoms.

Other cytokines have been studied in murine models only. Gene transfer of IL-12 using a plasmid ameliorated disease in the MRL/lpr mouse [157]. Studies point to an important role for the α-interferon (IFNα) system in SLE [158]. Disease in NZB/NZW mice is suppressed by mAb to IFNα [159]. The role of IFNγ is less clear: soluble IFNγ receptor was beneficial in NZB/NZW mice [160] whereas anti-IFNγ mAbs did not appear beneficial in MRL/lpr mice [161].

Several case reports have documented the onset of SLE following thymectomy for myasthenia gravis [162–164]. It was assumed that defective thymic immune education was responsible. Interestingly, a case was described of one such patient who was successfully treated with thymosin [165]. An older report suggested that three out of four patients with SLE improved after receiving thymosin [166], although details were sketchy.

Second Signal Blockade

A new and fertile area of interest is the possibility to target therapeutically the cell-surface molecules responsible for cell–cell interactions. Thus, it has been recognized that *de novo* T-cell activation not only requires the recognition of an antigenic peptide in the context of an appropriate MHC molecule, but also the delivery of a "second signal": without the second signal, anergy rather than activation will result. There are, in fact, several different surface molecules that can mediate the "second signal," most importantly the interaction between CD40 and CD154 (CD40-ligand), and the one between B7 and CD28. Both sets of interactions can be blocked by mAbs directed at the individual molecules, and the B7–CD28 interaction can also be blocked by the fusion protein CTLA4-Ig. CTLA4-Ig was beneficial in BXSB mice [167] and particularly in NZB/NZW mice [168], where significant survival benefits were seen even when treatment was delayed to the point when severe nephritis was already established. In a remarkable study, the combination of CTLA4-Ig and cyclophosphamide was much more effective than either agent alone [169]. Likewise, in MRL/lpr mice, adenovirus mediated gene transfer of the CTLA4-Ig gene gave excellent results [170]. Clinical trials with CTLA4-Ig are currently underway for a variety of diseases other than SLE (RA, psoriasis). Similarly, an anti-CD154 mAb was effective in NZB/NZW mice [171, 172]. In human SLE, clinical trials of two different mAbs targeting CD154 (CD40 ligand) were reported. The first study [173] showed good tolerability but little to suggest efficacy in this small group of patients. The second study was prematurely terminated when a high frequency of thromboembolic events was noted in the treatment group [174]. A mechanism for this observation was suggested in that CD154 is expressed on platelets as well.

Conceptually, second-signal blockade may be of interest, yet it remains an antigen-nonspecific, broadly immunosuppressive strategy. Moreover, as the "second signal" is mostly involved in the activation of naive T lymphocytes, it remains to be seen whether established autoimmune responses can be down-regulated sufficiently, and if so, whether treatment must be continued indefinitely.

Other Biological Agents

An interesting study showed that polyclonal dextran-conjugated anti-IgD delays autoimmunity in MRL/lpr mice [175]. It was hypothesized that the anti-IgD treat-

ment prevented class switching of pathogenic autoreactive B cells, thus leading to production of the relatively nonpathogenic IgM isotype rather than more pathogenic IgG. This suggests that the elevated serum IgD levels found in NZB/NZW and other autoimmune-prone mice [176] may have pathogenic significance. In addition, it is conceivable that the interaction between IgD on B cells and the Fcδ receptor on T cells described by Coico *et al*. [177] mediates a "second signal" analogous to the ones discussed previously.

Anti-idiotypic antibody therapy has been investigated for its potential immunomodulatory role, but an anti-Id against anti-DNA only temporarily suppressed anti-DNA and disease activity in NZB/NZW mice [178]. Nine human patients were treated with the anti-idiotypic anti-DNA "vaccine" 3E10. In five, an anti-idiotypic response was seen. Inasmuch as the patients had quiescent disease at the onset, no conclusions regarding efficacy could be drawn [179].

Evidence has accumulated implicating dysregulation of apoptosis in the pathogenesis of SLE. It was therefore of interest that a mAb against the Fas ligand attenuated disease in the NZB/NZW mice [180] and that a synthetic apoptosis inhibitor ameliorated an SLE-like disease in IFNγ transgenic mice [181]. In other approaches, mAbs against complement C5 [182] were beneficial in NZB/NZW mice, and similar antibodies are being tested for use in rheumatoid arthritis. Antisense oligonucleotides were used to target the p50 subunit of NFκB, a regulatory protein of immune response genes and the primary target of corticosteroids. In the BXSB model this therapy reduced anti-DNA antibodies by 90% [183].

NOVEL IMMUNOSUPPRESSIVE AND IMMUNOMODULATORY PHARMACOLOGICAL AGENTS

Spurred by the spectacular growth of organ and bone-marrow transplantation, a number of novel immunosuppressive agents are currently in early or advanced development, or have become available for use in patients, while not specifically approved or recommended for use in SLE.

Mycophenolate Mofetil

The immunosuppressive agent mycophenolate mofetil (MMF) was effective in NZB/NZW mice [184] as well as in MRL/lpr mice [185]. In an uncontrolled study [186] 10 patients with SLE were treated with MMF 1500–2000 mg daily for 8–16 months. During the first 6 months, significant improvements were seen in SLAM scores, and prednisone dosages were significantly reduced. Side effects in this trial were minor. Subsequently, a controlled study was done in patients with diffuse proliferative lupus nephritis [187]. Forty-two patients were randomized to receive either cyclophosphamide for 6 months followed by azathioprine for 6 months, or MMF for 12 months. All patients received prednisone as well. After 1 year, the percentages of patients achieving a complete (renal) remission were similar in the two groups (81% on MMF and 76% on conventional therapy), and other efficacy parameters were also nearly identical. The side effects with cyclophosphamide/azathioprine were generally more severe, including two deaths. Thus, the results after 1 year suggested therapeutic equivalence and a more favorable side-effect profile. However, it is important to note the limitations of this study: the 1 year duration was too short to establish equivalence between treatments for this particular disease, and this group of patients appeared to have an overall better prognosis compared to other such studies, possibly suggesting milder disease. Moreover, in a study of 11 children with juvenile SLE and nephritis [188], significant global improvements were seen, but renal disease improved only in those patients who had membranous (WHO class V) nephritis and not in those with membranoproliferative disease (WHO class IV). Moreover, infections and other adverse events were a major concern.

Other Immunosuppressive Agents

The transplantation agent tacrolimus (FK506) is a fungal-derived complex molecule with immunosuppressive properties. Highly specific for T lymphocytes, it inhibits the production of several cytokines including IL-2 and IFNγ [189]. Tacrolimus is effective in the treatment of lupus-like disease in NZB/NZW and MRL/lpr mice [190–192], and was synergistic with cyclophosphamide in MRL/lpr mice [193]. In one report, 2 of 3 patients treated with tacrolimus for up to 9 months had good clinical responses [194]. Tacrolimus has toxicities similar to cyclosporin A.

Other immunosuppressives that are in development but not (yet) generally available include frentizole, which was successfully used in NZB/NZW mice [195]. In human patients, it resulted in modest improvements of SLE activity, but resulted in severe, and sometimes life-threatening, side effects [196–199]. Mizoribine was beneficial in MRL/lpr mice [200], and a case report showed a favorable response to treatment with mizoribine alone (i.e., without corticosteroids) in a patient with mild SLE [201]. Several immunosuppressive agents have been used with favorable results in murine lupus, but without human data so far. These include linomide

[202, 203], rapamycin [204], deoxyspergualin [205–208], cholera toxin [209], and OK-432 [210].

Immunomodulators

Thalidomide possesses immunomodulatory therapies and may down-regulate TNFα. It has shown benefits in various cutaneous autoimmune disorders, including E. nodosum and vasculitis, and is approved for use against oral aphthous ulcers in HIV infected patients [211]. Several large observational studies [212–216] have consistently shown that this agent can benefit many patients with severe and/or refractory cutaneous lupus. The agent does not appear to have major efficacy with respect to other lupus manifestations. Moreover, sedation, neuropathy, and devastating teratogenicity pose significant limitations on the use of this otherwise interesting agent.

Dinitrochlorobenzene (DNCB) administered topically to the skin appears to boost Th1 type immune responses [217]. An elderly patient with SLE was treated with topical DNCB with excellent clinical and immunological results [218]. Levamisole, an antihelminthic agent with immunostimulatory properties, was unsuccessful in a controlled trial [219]. The antiviral ribavirin appeared beneficial in NZB/NZW mice [220].

Arachidonic Acid Derivatives and Their Inhibitors

Prostaglandins and leukotrienes are important modulators of the inflammatory cascade, and selective inhibition or augmentation of such molecules is a logical target for intervention. Specifically in SLE, it was shown that intrarenal production of PGE2 and thromboxane was elevated in mice as well as in humans [221, 222]. In NZB/NZW mice, iloprost (a prostacyclin analog) and thromboxane were beneficial [223]. In patients with lupus nephritis, infusion of a selective thromboxane antagonist for 48 h was shown transiently to improve renal function [224].

Prostacyclin analogs are used increasingly in the treatment of pulmonary hypertension, and several reports suggest that patients with SLE who suffer from this complication can benefit from these as well [225, 226].

The 5-lipoxygenase (5-LO) inhibitor Zileuton was tried in an 8-week, double-blinded study of 40 patients with SLE [227]. Modest improvement in SLAM scores were seen in the treatment group with slight worsening in the controls ($p < 0.05$ for comparison of the two groups). 5-LO inhibition appears to be safe, but it is not clear whether the small effect size in this study would translate into a meaningful clinical benefit.

PROPHYLACTIC USE OF CORTICOSTEROIDS

Although corticosteroids are the most well-established agents in the treatment of SLE, a novel approach to using corticosteroids was suggested by Bootsma *et al*. [228]. ter Borg *et al*. [229, 230] demonstrated that rises in anti-DNA titers preceded the development of SLE flares, suggesting that by treating patients who exhibited rising anti-DNA titers even prior to any change in symptoms, lupus flares might be preventable. To put this hypothesis to the test, a total of 156 patients with stable SLE were monitored with serial anti-DNA determinations, and patients who showed a 25% rise in their titer were randomized to receive "early" therapy with prednisone 30–60 mg/day vs conventional therapy (watchful waiting). The trial showed that, indeed, a significant proportion of SLE flares could be prevented by such treatment: a total of 20 flares occurred in the patients under conventional treatment, and only 2 flares occurred with "early treatment." However, this success came at a price: the total amount of corticosteroids used in the patients receiving the "early" treatment was significantly higher (median prednisone dose 15.3 vs 10.0 mg/day) than that received by the conventionally treated patients (who did, of course, receive corticosteroids as treatment for their flares). These intriguing results suggest that monitoring of anti-DNA levels in patients with SLE, and treating the patients with rising titers even before any symptoms develop, could prevent many flares of SLE. However, it is an interesting question whether patients would actually prefer this, that is, would they be bothered more by having to take corticosteroids when feeling well as opposed to when feeling ill. Moreover, a study by Esdaile *et al*. [231] did not confirm the most important underlying assumption for this treatment, namely, that rising anti-DNA titers predict SLE flares. Clearly, more studies are needed before this treatment strategy can be adopted widely.

UV-A1 LIGHT THERAPY

The use of UV-A1 light therapy for SLE, as counterintuitive as it may seem, has been proposed based on specific immunomodulatory properties of electromagnetic waves in the 340–400 nm range [232]. Uncontrolled observations suggested possible benefits [233, 234]. In a double-blind, crossover study of 26 women with SLE, 3 weeks of treatment with UV-A1 appeared superior to placebo treatment with visible light [235]. Similar results were seen in a double-blinded, randomized, controlled crossover study in 11 patients [236]. Modest effect sizes,

technical details, and cost of the treatment have tempered enthusiasm for this therapy. It should be emphasized that UV light sources in commercial use (i.e., solariums) do not adequately filter out UVB to make them safe for patients with SLE.

"ALTERNATIVE THERAPIES"

During the past decade, interest has burgeoned in the use of "alternative" treatments, such as nutritional supplements, vitamins, antioxidants, herbs, acupuncture, homeopathy, and many others. A more recent study [237] found that nearly half of all lupus patients (whether it be in the United States, in Canada or in the United Kingdom) utilize alternative therapies.

Chinese Herbs and Other Herbal Therapies

IN VITRO AND ANIMAL STUDIES

Traditional Chinese medicine employs a great variety of herbal treatments. Efforts are underway to characterize some of the complex mixtures used in such treatments and analyze the active ingredients. Not surprisingly, varied biological effects have been found. For instance, "chi-han" was found to contain several immunomodulatory ingredients, some with opposite effects on IL1 or TNFα secretion *in vitro* [238]. Solamargine, extracted from the Chinese herb *Solanum incanum*, was shown to trigger expression of the TNFα receptor and to increase apoptosis [239]. Similarly, Saiboku-To, an herbal mixture used in Japan, was found to inhibit 5-lipoxygenase [240]. Some Chinese herbs were found to enhance IL2 production, and to decrease anti-DNA antibody titers and prolong the life of NZB/NZW mice [241]. More recently, an extract of the fungus *Ganoderma tsugae*, commonly used in traditional Chinese medicine, was shown to decrease proteinuria and anti-DNA antibody formation and prolong survival in NZB/NZW mice [242]. The Japanese herbal therapy ren-shen-yang-rong-tang was effective in MRL/lpr mice, but only when given in combination with corticosteroids [243]. In the same model, stagalin was shown to decrease immune complex depositions in the glomeruli [244], and the Japanese traditional herb hachimi-jio-gan was shown to decrease autoantibody formation and IL12 expression [245].

Experience in SLE

In a remarkable blend of traditional Chinese medicine and Western approaches, traditional Chinese herbal medicine was used in 41 patients with active lupus nephritis in conjunction with cyclophosphamide. These patients were compared with 35 patients treated with cyclophosphamide alone [246]. The patients receiving both conventional and traditional therapies were found to have significantly better outcomes after 6 months of treatment. However, this was not a randomized clinical trial, and details of the treatment regimens are unclear. The traditional Chinese medical diagnosis in these patients was a "deficiency of liver-kidney Yin."

Tripterygium Wilfordii Hook F

In Chinese herbal medicine, rheumatoid arthritis and other inflammatory conditions are frequently treated with extracts from the vinelike plant *Tripterygium wilfordii* Hook. f. (TW). Such an extract was found to inhibit animal models of arthritis [247] and, in a controlled study, to be efficacious in the treatment of rheumatoid arthritis [248]. Studies of this compound revealed that it had powerful immunomodulatory properties, inhibiting transcription of cytokine genes, including IL-1, -2, -4, -6, -8, and TNFα [249, 250]. Unfortunately, TW is associated with significant gastrointestinal side effects. A slow-release form of TW was shown to be equally efficacious but less toxic [251]. The active ingredients in TW are most likely the diterpenoid triepoxides, triptolide and tripdiolide [252]. TW has not been systematically studied in SLE. Benefit was shown in the MRL/lpr model of lupus [253, 254]. A single case report indicated improvement of severe lupus nephritis after treatment with TW [255].

Toxicities

Unlike a commonly held perception, Chinese herbs are not at all harmless. It stands to reason that any mixture with significant immunomodulatory effects would also engender potential risks, and it is becoming clear that this is indeed the case. The most significant risk appears to be a unique interstitial nephropathy, characterized by a relatively rapid progressive course, without much proteinuria, but with severe anemia [256, 257]. A severe but reversible dilated cardiomyopathy was linked to treatment with Chinese herbs in one patient [258].

Nutritional Therapies

Caloric restriction was shown to ameliorate murine lupus [259–261] but has not been studied in humans. Likewise, dietary modulation of fatty acid content has resulted in attenuation of murine lupus, for example, by

limiting essential fatty acids [262], or by supplementation with eicosapentaenoic acid [263, 264], or with other unsaturated fatty acids [265]. In a small pilot study, fish oil, which contains ω-3 fatty acids including eicosapentaenoic acid, was given to 12 patients with SLE [266], but no benefits with respect to SLE disease activity were seen.

Flaxseed contains α-linolenic acid, which has antiatherogenic properties, and lignans, which are antagonists of platelet activating factor. Flaxseed was beneficial in MRL/lpr mice. In an uncontrolled study, flaxseed was given to 9 patients with SLE and nephritis [267]. The creatinine clearance and proteinuria improved while on dosages of 30 or 45 g/day for 4 weeks. However, in a subsequent 1-year controlled trial in 23 patients with lupus nephritis, no clear benefits were seen [268]. The lignan precursor SDG was studied in the MRL/lpr mice and exerted a modest renoprotective effect [269]. The wheat germ-derived fermentation product Avemar attenuated the aberrant immune responses in the 16/6 Id model of lupus [270].

Antioxidants

The broad hypothesis that antioxidants can be of benefit in inflammatory diseases goes back to the original discovery of vitamin C by Pauling and Szent-Györgyi. More recently, a number of other antioxidants have become popularized in the health food and nutritional supplements arena. Only a few scientific studies relate to the use of antioxidants specifically in SLE. In one study, NZB/NZW mice were treated with the antioxidants *N*-acetylcysteine and cysteamine [271]. While *N*-acetylcysteine reduced anti-DNA antibodies and nephritis severity, cysteamine increased anti-DNA and kidney inflammation; however, both agents prolonged survival. Thus, these two antioxidants appear to have different immunomodulatory properties, while the effects on the overall inflammatory response appear to be favorable for both. The antioxidant Pycnogenol was reported to improve SLEDAI scores in a small controlled study [272]. The antioxidant Lobenzarit [273] was well tolerated and appeared beneficial in an uncontrolled 1-year trial in 15 patients with SLE [274].

Acupuncture

Acupuncture was reported to be of value in discoid lupus in one series of 15 patients [275]. However, a review of acupuncture for the treatment of various rheumatic diseases concluded that the 17 surveyed studies were all of such poor quality that "acupuncture cannot be recommended for treatment" as of yet [276].

Other Alternative Therapies

The list of therapies suggested for use in SLE is nearly endless. In addition to those discussed previously, various vitamins, plasma-derived proteins, various kinds of electromagnetic radiation, pulsed magnetic fields [277], heavy metals, photoferesis [278], and other more or less bizarre treatments have been proposed. It would seem prudent to exclude from consideration those treatments that lack a scientific rationale and are not supported by animal data and are successful only in uncontrolled circumstances.

CONCLUSION

During the first years of the third millennium, many significant developments in the area of new treatments for SLE are taking place. From bench work through animal studies and all the way into large clinical trials, promising novel approaches are being explored. It is unprecedented in the history of SLE that several novel therapeutic agents are in large-scale clinical trials at the same time. Among the new treatments, DHEA and high-intensity chemotherapeutic regimens illustrate approaches to opposite poles in the spectrum of lupus, the former of potential benefit for patients with mild or moderately active lupus, the latter for those with severe and refractory disease. These and many other new treatments, if successful, may herald in a new era in the treatment of SLE.

References

1. Liang, M. H., Socher, S. A., Larson, M. G., *et al.* (1989). Reliability and validity of six systems for the clinical assessment of disease activity in systemic lupus erythematosus. *Arthritis Rheum*. **32**, 1107–1118.
2. Bombardier, C., Gladman, D. D., Urowitz, M. B., Caron, D., and Chang, C. H. (1992). Derivation of the SLEDAI: A disease activity index for lupus patients. *Arthritis Rheum.* **35**, 630–640.
3. Schwartz, S. A. (1990). Intravenous immunoglobulin for the therapy of autoimmune disorders. *J. Clin. Immunol.* **10**, 81–89.
4. Fehr, J., Hofmann, V., and Kappeler, U. (1982). Transient reversal of thrombocytopenia in idiopathic thrombocytopenia purpura by high-dose intravenous gamma globulin. *N. Engl. J. Med.* **306**, 1254–1258.
5. Newburger, J. W., Takahashi, M., Burns, J. C., *et al.* (1986). The treatment of Kawasaki syndrome with intravenous gamma globulin. *N. Engl. J. Med.* **315**, 341–347.
6. Maier, W. P., Gordon, D. S., Howard, R. F., *et al.* (1990). Intravenous immunoglobulin therapy in systemic lupus erythematosus-associated thrombocytopenia. *Arthritis Rheum.* **33**, 1233.

7. Gaedike, G., Teller, W. M., Kohne, E., Dopfer, R., Niethammer, D. (1984). IgG therapy in systemic lupus erythematosus: Two case reports. *Blut* **48**, 387–390.
8. Francioni, C., Galeazzi, M., Fioravanti, A., Gelli, R., Megale, F., and Marcolongo, R. (1994). Long term I.V.Ig treatment in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **12**, 163–168.
9. Corvetta, A., Della Bitta, R., Gabrielli, A., Spaeth, P. J., and Danieli, G. (1984). Use of high-dose intravenous immunoglobulin in systemic lupus erythematosus: Report of three cases. *Clin. Exp. Rheumatol.* **7**, 295–299.
10. Tomer, Y., and Shoenfeld, Y. (1992). Successful treatment of psychosis secondary to SLE with high dose intravenous immunoglobulin. *Clin. Exp. Rheumatol.* **10**, 391–393.
11. Winder, A., Molad, Y., Ostfeld, I., Kenet, G., Pinkhas, J., and Sidi, Y. (1993). Treatment of systemic lupus erythematosus by prolonged administration of high dose intravenous immunoglobulin: Report of two cases. *J. Rheumatol.* **20**, 495–498.
12. Engel, G., and van Vollenhoven, R. (1997). Treatment of severe CNS lupus with IVIG: Case report. *J. Clin. Rheumatol.* **5**, 228–232.
13. Lin, C.-Y., Hsu, H.-C., and Chang, H. (1989). Improvement of histological and immunological change in steroid and immunosuppressive drug-resistant lupus nephritis by high-dose intravenous gamma globulin. *Nephron* **53**, 303–310.
14. Schroeder, J. O., Zeuner, R. A., Euler, H. H., and Loffler, H. (1996). High dose intravenous immunoglobulins in systemic lupus erythematosus: Clinical and serological results of a pilot study. *J. Rheumatol.* **1**, 71–75.
15. Aharon, A., Levy, Y., Bar-Dayan, Y., *et al.* (1997). Successful treatmen of early secondary myelofibrosis in SLE with IVIG. *Lupus* **6**, 408–411.
16. Lafferty, T. E., Smith, J. B., Schuster, S. J., and DeHoratius, R. J. (1997). Treatment of acquired factor VIII inhibitor using intravenous immunoglobulin in two patients with systemic lupus erythematosus. *Arthritis Rheum.* **40**, 775–778.
17. Sherer, Y., Levy, Y., and Shoenfeld, Y. (1999). Marked improvement of severe cardiac dysfunction after one course of intravenous immunoglobulin in a patient with systemic lupus erythematosus. *Clin. Rheumatol.* **18**, 238–240.
18. Lesprit, P., Mouloud, F., Bierling, P., *et al.* (1996). Prolonged remission of SLE-associated polyradiculoneuropathy after a single course of intravenous immunoglobulin. *Scand. J. Rheumatol.* **25**, 177–178.
19. Ballow, M., and Parke, A. (1989). The uses of intravenous immune globulin in collagen vascular disease. *J. Allergy Clin. Immunol.* **84**, 608–612.
20. De Pita, O., Bellucci, A. M., Ruffelli, M., Girardelli, C. R., and Puddu, P. (1997). Intravenous immunoglobulin therapy is not able to efficiently control cutaneous manifestations in patients with lupus erythematosus. *Lupus* **6**, 415–417.
21. Maltbaek, N., Harreby, M. S., and Thogersen, B. (1994). Intravenous immunoglobulin administration to a patient with systemic lupus erythematosus and pneumococcal septicemia. *Ugeskrift for Laeger* **156**, 4039–4041.
22. Arnout, J., Spitz, B., Wittevrongel, C., Vanrusselt, M., Van Assche, A., and Vermylen, J. (1994). High-dose intravenous immunoglobulin treatment of a pregnant patient with an antiphospholipid syndrome: Immunological changes associated with a successful outcome. *Thromb. Haemost.* **71**, 741–747.
23. Mouthon, L., Kaveri, S. V., Spalter, S. H., *et al.* (1996). Mechanisms of action of intravenous immune globulin in immune-mediated disease. *Clin. Exp. Immunol.* **1**, S3–S9.
24. Williams, R. C., Jr., Malone, C. C., Fry, G., and Silvestris, F. (1997). Affinity columns containing anti-DNA Id^+ human myeloma proteins adsorb human epibodies from intravenous gamma globulin. *Arthritis Rheum.* **40**, 683–693.
25. Heyneman, C. A., Gudger, C. A., and Beckwith, J. V. (1997). Intravenous immune globulin for inducing remissions in systemic lupus erythematosus. *Ann. Pharmacother.* **31**, 242–244.
26. Hashkes, P. J., and Lovell, D. J. (1996). Vasculitis in systemic lupus erythematosus following intravenous immunoglobulin therapy. *Clin. Exp. Rheumatol.* **14**, 673–675.
27. Pisetsky, D. S. (1993). Autoantibodies and their significance. *Curr. Opin. Rheumatol.* **5**, 549–556.
28. Battisto, J. R., and Miller, J. (1962). Immunological unresponsiveness produced in adult guinea pigs by parenteral introduction of minute quantities of hapten or protein antigen. *Proc. Soc. Exp. Biol. Med.* **111**, 111–120.
29. Siskind, G. W. (1984). Immunologic tolerance. In "Fundamental Immunology" (W. E. Paul, Ed.), pp. 537–565. Raven, New York.
30. van Vollenhoven, R. F., Nagler-Anderson, C., Soriano, A., *et al.* (1988). Tolerance induction by a poorly arthritogenic collagen II can prevent collagen-induced arthritis. *Cell Immunol.* **115**, 146–155.
31. Nossal, G. J. (1997). B lymphocyte physiology: The beginning and the end. *Ciba Found. Symp.* **204**, 220–230.
32. Jones, D. S., Barstad, P. A., Feild, M. J., *et al.* (1995). Immunospecific reduction of antioligonucleotide antibody-forming cells with a tetrakis–oligonucleotide conjugate (LJP 394), a therapeutic candidate for the treatment of lupus nephritis. *J. Med. Chem.* **38**, 2138–2144.
33. Plunkett, M. L., Iverson, G. M., Crisologo, J., *et al.* (1995). LPJ394: A novel clinical candidate for the treatment of lupus nephritis. *Lupus* **4**, 99.
34. Weisman, M. H., Bluestein, H. G., Berner, C. M., and de Haan, H. A. (1997). Reduction in circulating dsDNA antibody titer after administration of LJP394. *J. Rheumatol.* **24**, 314–318.
35. Furie, R. A., Cash, J. M., Cronin, M. E., *et al.* (2001). Treatment of systemic lupus erythematosus with LJP 394. *J. Rheumatol.* **28**, 257–265.
36. Doria, A., Piccoli, A., Vesco, P., *et al.* (1994). Therapy of lupus nephritis. A two-year prospective study. *Ann. Med. Intern.* **45**, 307–311.

37. Lewis, E. J., Hunsicker, L. G., Lan, S. P., Rohde, R. D., Lachin, J. M. (1992). A controlled trial of plasmapheresis therapy in severe lupus nephritis. The Lupus Nephritis Collaborative Study Group. *N. Engl. J. Med.* **326**, 1373–1379.
38. Euler, H. H., and the Lupus Plasmapheresis Study Group (1996). Presented for the LPSG Workshop at the ACR National Meeting, Orlando, Florida.
39. Wallace, D. J., Goldfinger, D., Pepkowitz, S. H., *et al.* (1998). Randomized controlled trial of pulse/synchronization cyclophosphamide/apheresis for proliferative lupus nephritis. *J. Clin. Apheresis* **13**, 163–166.
40. Jain, R., Chartash, E., Susin, M., and Furie, R. (1994). Systemic lupus erythematosus complicated by thrombotic microangiopathy. *Semin. Arthritis Rheum.* **24**, 173–182.
41. Nesher, G., Hanna, V. E., Moore, T. L., Hersh, M., and Osborn, T. G. (1994). Thrombotic microangiopathic hemolytic anemia in systemic lupus erythematosus. *Semin. Arthritis Rheum.* **24**, 165–172.
42. Hess, D. C., Sethi, K., and Awad, E. (1992). Thrombotic thrombocytopenic purpura in systemic lupus erythematosus and antiphospholipid antibodies: Effective treatment with plasma exchange and immunosuppression. *J. Rheumatol.* **19**, 1474–1478.
43. Hochfeld, M., Druzin, M. L., Maia, D., Wright, J., Lambert, R. E., and McGuire, J. (1994). Pregnancy complicated by primary antiphospholipid antibody syndrome. *Obstet. Gynecol.* **83**, 804–805.
44. Aringer, M., Smolen, J. S., and Graninger, W. B. (1998). Severe infections in plasmapheresis-treated systemic lupus erythematosus. *Arthritis Rheum.* **41**, 414–420.
45. el-Habib, R., Laville, M., and Traeger, J. (1984). Specific adsorption of circulating antibodies by extracorporeal plasma perfusions over antigen coated collagen flatmumbranes: Application to systemic lupus erythematosus. *J. Clin. Lab. Immunol.* **15**, 111–117.
46. Matsuki, Y., Suzuki, K., Kawakami, M., *et al.* (1996). High-avidity anti-DNA antibody removal from the serum of systemic lupus erythematosus patients by adsorption using dextran sulfate cellulose columns. *J. Clin. Apheresis* **11**, 30–35.
47. Hashimoto, H., Tsuda, H., Kanai, Y., *et al.* (1990). Selective removal of anti-DNA and anticardiolipin antibodies by adsorbent plasmapheresis using dextran sulfate columns in patients with systemic lupus erythematosus. *J. Rheumatol*. **18**, 545–551.
48. Kinoshita, M., Aotsuka, S., Funahashi, T., Tani, N., and Yokohari, R. (1989). Selective removal of anti-double-stranded DNA antibodies by immunoadsorption with dextran sulphate in a patient with systemic lupus erythematosus. *Ann. Rheum. Dis.* **48**, 856–860.
49. Schneider, M., Berning, T., Waldendorf, M., Glaser, J., and Gerlach, U. (1989). Immunoadsorbent plasma perfusion in patients with systemic lupus erythematosus. *J. Rheumatol.* **17**, 900–907.
50. Suzuki, K., Ishizuka, T., Harigai, M., *et al.* (1990). Continuous anti-ds DNA antibody apheresis in systemic lupus erythematosus. *Lancet* **336**, 753–754.
51. Balint, J. P., Cochran, S. K., and Jones, F. R. (1995). Modulation of idiotypic and antiidiotypic immunoglobulin G responses in an immune thrombocytopenic purpura patient as a consequence of extracorporeal protein A immunoadsorption. *Artificial Organs* **19**, 496–499.
52. Taniguchi, Y., Yorioka, N., Okushin, S., Oda, H., Usui, K., and Yamakido, M. (1995). Usefulness of immunoadsorption therapy for systemic lupus erythematosus associated with transverse myelitis. A case report. *Int. J. Artificial Organs* **18**, 799–801
53. Pfueller, B., Wolbart, K., Bruns, A., Burmester, G. R., and Hiepe, F. (2001). Successful treatment of patients with systemic lupus erythematosus by immunoadsorption with a C1q column: A pilot study. *Arthritis Rheum.* **44**, 1962–1963.
54. Berner, B., Scheel, A. K., Schettler, V., *et al.* (2001). Rapid improvement of SLE-specific cutaneous lesions by C1q immunoadsorption. *Ann. Rheum. Dis.* **60**, 898–899.
55. Fuks, Z., Strober, S., Bobrove, A. M., *et al.* (1976). Long-term effects of radiation of T and B lymphocytes in peripheral blood of patients with Hodgkin's disease. *J. Clin. Invest*. **58**, 803–810.
56. Kotzin, B. L., and Strober, S. (1979). Reversal of NZB/NZW disease with total lymphoid irradiation. *J. Exp. Med.* **150**, 371–378.
57. Slavin, S. (1979). Successful treatment of autoimmune disease in (NZB/NZW)F_1 female mice by using fractionated total lymphoid irradiation. *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5174–5182.
58. Solovera, J. J., Farinas, M. C., and Strober, S. (1988). Changes in B lymphocyte function in rheumatoid arthritis and lupus nephritis after total lymphoid irradiation. *Arthritis Rheum.* **31**, 1481–1488.
59. Strober, S., Field, E., Hoppe, R. T., *et al.* (1985). Treatment of intractable lupus nephritis with total lymphoid irradiation. *Ann. Intern. Med.* **102**, 450–458.
60. Strober, S., Farinas, M. C., Field, E. M., *et al.* (1987). Lupus nephritis after total lymphoid irradiation. *Ann. Intern. Med.* **107**, 689–699.
61. Ben-Chetrit, E., Gross, D. J., Braverman, A., *et al.* (1986). Total lymphoid irradiation in refractory systemic lupus erythematosus. *Ann. Intern. Med.* **105**, 58–65.
62. Westhovens, R., Verwilghen, J., and Dequeker, J. (1997). Total lymphoid irradiation in rheumatoid arthritis: A ten-year followup. *Arthritis Rheum.* **40**, 426–429.
63. Genovese, M. C., Uhrin, Z., Bloch, D. A., *et al.* (2002). Long-term followup in patients treated with total lymphoid irradiation for lupus nephritis. *Arthritis Rheum.* in press.
64. Euler, H. H., Schroeder, J. O., Harten, P., Zeuner, R. A., and Gutschmidt, H. J. (1994). Treatment-free remission in severe systemic lupus erythematosus following synchronization of plasmapheresis with subsequent pulse cyclophosphamide. *Arthritis Rheum.* **37**, 1784–1794.
65. Schroeder, J. O., Schwab, U., and Zeuner, R. (1997). Plasmapheresis and subsequent pulse cyclophosphamide in severe lupus erythematosus. *Arthritis Rheum.* **40**, S325.

66. Petri, M., Jones, R., and Brodsky, R. (2001). High dose immunoablative cyclophosphamide (HDIC) in SLE: Complete responders and durability of response in the open label trial. *Arthritis Rheum.* **44**, S387.
67. Marmont, A. M. (1997). Stem cell transplantation for severe autoimmune disorders, with special reference to rheumatic diseases. *J. Rheumatol.* **48**, S13–S18.
68. Brooks, P. M. (1997). Hematopoietic stem cell transplantation for autoimmune diseases. *J. Rheumatol.* **48**, S19–S22.
69. Sullivan, K. M., and Furst, D. E. (1997). The evolving role of blood and marrow transplantation for the treatment of autoimmune diseases. *J. Rheumatol.* **48**, S1–S4.
70. Marmont, A. M. (1993). Immune ablation with stem-cell rescue: A possible cure for systemic lupus erythematosus? *Lupus* **2**, 151–156.
71. Euler, H. H., Marmont, A. M., Bacigalupo, A., *et al.* (1996). Early recurrence or persistence of autoimmune diseases after unmanipulated autologous stem cell transplantation. *Blood* **88**, 3621–3625.
72. Rosen, O., Thiel, A., Massenkeil, G., *et al.* (2000). Autologous stem-cell transplantation in refractory autoimmune diseases after *in vivo* immunoablation and *ex vivo* depletion of mononuclear cells. *Arthritis Res.* **2**, 327–336.
73. Wulffraat, N. M., Sanders, E. A., Kamphuis, S. S., *et al.* (2001). Prolonged remission without treatment after autologous stem cell transplantation for refractory childhood systemic lupus erythematosus. *Arthritis Rheum*. **44**, 728–731.
74. Traynor, A. E., Schroeder, J., Rosa, R. M., *et al.* (2000). Treatment of severe systemic lupus erythematosus with high-dose chemotherapy and haemopoietic stem-cell transplantation: A phase I study. *Lancet* **356**, 701–707.
75. Tyndall, A. (2001). Immunoablation and haemopoietic stem cell transplantation for severe autoimmune disease with special reference to systemic lupus erythematosus. *Lupus* **10**, 214–215.
76. Bingham, S. J., Snowden, J. A., and Emery, P. (2000). Autologous blood stem cell transplantation as therapy for autoimmune diseases. *Ann. Med.* **32**, 615–621.
77. Gunnarsson, I., *et al.* (2002). *J. Rheumatol.* in press.
78. Abu-Shakra, M., Murrary, B. U., Gladman, D. G., and Gough, J. (1995). Mortality studies in sysemic lupus erythematosus. Results from a single center. II. Predictor variables for mortality. *J. Rheumatol.* **22**, 1265–1270.
79. Van Vollenhoven, R. F., and McGuire, J. L. (1994). Estrogen, progesterone, and testosterone: Can they be used to treat autoimmune diseases? *Cleveland Clin. J. Med.* **61**, 276–284.
80. Lahita, R. G., Bradlow, H. L., Kunkel, H. G., and Fishman, J. (1979). Alterations of estrogen metabolism in systemic lupus erythematosus. *Arthritis Rheum.* **22**, 1195–1198.
81. Lahita, R. G., Kunkel, H. G., and Bradlow, H. L. (1983). Increased oxidation of testosterone in systemic lupus erythematosus. *Arthritis Rheum.* **26**, 1517–1521.
82. Jungers, P., Hahoul, K., Pelissier, C., Dougados, M., Tron, F., and Bach, J.-F. (1982). Low plasma androgens in women with active or quiescent systemic lupus erythematosus. *Arthritis Rheum*. **25**, 454–462.
83. Lahita, R. F., Bradlow, H. L., Ginzler, E., Pang, S., and New, M. (1987). Low plasma androgens in women with systemic lupus erythematosus. *Arthritis Rheum.* **30**, 241–249.
84. Suzuki, T., Suzuki, N., Daynes, R. A., and Engleman, E. G. (1991). Dehydroepiandrosterone enhances IL2 production and cytotoxic effector function of human T cells. *Clin. Immunol. Immunopathol.* **61**, 202–211.
85. Daynes, R. A., Dudley, D. J., and Araneo, B. A. (1990). Regulation of murine lymphokine production *in vivo*. II. Dehydroepiandrosterone is a natural enhancer of interleukin 2 synthesis by helper T cells. *Eur. J. Immunocl.* **20**, 793–799.
86. Daynes, R. A., and Araneo, B. A. (1992). Natural regulators of T-cell lymphokine production *in vivo*. *J. Immunother.* **12**, 174–179.
87. Linker-Israeli, M., Bakke, A. C., Kitridou, R. C., *et al.* (1983). Defective production of interleukin 1 and interleukin 2 in patients with systemic lupus erythematosus (SLE). *J. Immunol.* **130**, 2651–2655.
88. Alcocer-Varela, J., and Alarcon-Segovia, D. (1982). Decreased production of and response to interleukin-2 by cultured lymphocytes from patients with systemic lupus erythematosus. *J. Clin. Invest.* **69**, 1388–1392.
89. Suzuki, T., Suzuki, N., Engleman, E. G., Mizushima, Y., and Sakane, T. (1995). Low serum levels of dehydroepiandrosterone may cause deficient IL-2 production by lymphocytes from patients with systemic lupus erythematosus (SLE). *Clin. Exp. Immunol.* **99**, 251–255.
90. Lucas, J. A., Ahmed, A. A., Casey, M. L., and MacDonald, P. C. (1985). Prevention of autoantibody formation and prolonged survival in New Zealand Black/New Zealand White F1 mice fed dehydroisoandrosterone. *J. Clin. Invest.* **75**, 2091–2093.
91. van Vollenhoven, R. F., and McDevitt, H. O. (1992). Studies of the treatment of nephritis in NZB/NZW mice with dehydroepiandrosterone. *Arthritis Rheum.* **35**, S207.
92. van Vollenhoven, R. F., Engleman, E. G., and McGuire, J. L. (1994). An open study of dehydroepiandrosterone in systemic lupus erythematosus. *Arthritis Rheum.* **37**, 1305–1310.
93. van Vollenhoven, R. F., Engleman, E. G., and McGuire, J. L. (1995). Dehydroepiandrosterone (DHEA) in systemic lupus erythematosus: Results of a double-blinded, placebo-controlled, randomized clinical trial. *Arthritis Rheum.* **38**, 1826–1831.
94. van Vollenhoven, R. F., Morabito, L. M., Engleman, E. G., and McGuire, J. L. (1997). Treatment of systemic lupus erythematosus with dehydroepiandrosterone: 50 patients treated for up to 12 months. *J. Rheumatol.* **25**, 285–290.
95. Barry, N. N., McGuire, J. L., and van Vollenhoven, R. F. (1998). Dehydroepiandrosterone in systemic lupus erythematosus: Relationship between dosage, serum levels and clinical resonse. *J. Rheumatol.* **25**, 2352–2356.

96. van Vollenhoven, R. F., Park, J. L., Genovese, M. C., West, J. P., and McGuire, J. L. (1999). A double-blind, placebo-controlled, clinical trial of dehydroepiandrosterone in severe systemic lupus erythematosus. *Lupus* **8**, 181–187.
97. Petri, M., Lahita, R., McGuire, J., *et al.* (1997). Results of the GL701 (DHEA) multicenter steroid-sparing SLE study. *Arthritis Rheum.* **40**, S327.
98. Mease, P. J., Merrill, J. T., Lahita, R. G., *et al.* (2000). GL701 (prasterone, dehydroepiandrosterone) improves systemic lupus erythematosus. *Arthritis Rheum.* **43**, S271.
99. Chang, D. M., Lan, J. L., Lin, H. Y., and Luo, S. F. (2000). GL701 (prasterone, dehydroepiandrosterone) significantly reduces flares in female patients with mild to moderate systemic lupus erythematosus (SLE). *Arthritis Rheum.* **43**, S241.
99a. Parasrampuria, J., Schwartz, K., and Petesch, R. (1998). Quality control of dehydroepiandrosterone dietary supplement products. *JAMA* **280**, 1565.
100. Ahn, Y. S., Rocha, R., Mylvaganam, R., *et al.* (1989). Long-term danazol therapy in autoimmune thrombocytopenia: Unmaintained remission and age-dependent response in women. *Ann. Intern. Med.* **111**, 723–729.
101. West, S. G., and Johnson, S. C. (1988). Danazol for the treatment of refractory autoimmune thrombocytopenia in systemic lupus erythematosus. *Ann. Intern. Med.* **108**, 703–706.
102. Marino, C., and Cook, P. (1985). Danazol for lupus thrombocytopenia. *Arch. Intern. Med.* **145**, 2251–2252.
103. Chan, A. C., and Sack, K. (1991). Danazol therapy for autoimmune hemolytic anemia associated with systemic lupus erythematosus. *J. Rheumatol.* **18**, 280–282.
104. Jungers, P., Liote, F., Pelissier, C., Viriot, J., *et al.* (1986). The hormonomodulation in systemic lupus erythematosus: Preliminary results with Danazol (D) and cyproterone-acetate (CA). *Ann. Med. Interne* **137**, 313–319.
105. Torrelo, A., Espana, A., Medina, S., *et al.* (1990). Danazol and discoid lupus erythematosus. *Dermatologica* **182**, 239–245.
106. Agnello, V., Pariser, K., Gell, J., *et al.* (1983). Preliminary observations on danazol therapy of systemic lupus erythematosus: Effects on DNA antibodies, thrombocytopenia and complement. *J. Rheumatol.* **10**, 682–687.
107. Dougados, M., Job-Deslandre, Amor, B., *et al.* (1987). Danazol therapy in systemic lupus erythematosus. A one-year prospective controlled trial on 40 female patients. *Clin. Trials J.* **24**, 191–200.
108. Lahita, R. G., Cheng, C. Y., Monder, C., and Bardin, C. W. (1992). Experience with 19-nortestosterone in the therapy of systemic lupus erythematosus: Worsened disease after treatment with 19-nortestosterone in men and lack of improvement in women. *J. Rheumatol.* **19**, 547–555.
109. Olsen, N. J., and Kovacs, W. J. (1995). Case report: Testosterone treatment of systemic lupus erythematosus in a patient with Klinefelter's syndrome. *Am. J. Med. Sci.* **310**, 158–160.
110. Catania, A., Mangone, I., Motta, P., and Zanussi, C. (1989). Admininstation of gonadotrophin-releasing hormone analog as adjunctive therapy in women with systemic lupus erythematosus. *Arthritis Rheum.* **32**, 1186–1188.
111. Brickman, C. M. (1996). Safety, tolerance and efficacy of leuprolide acetate depot supension (lupron) in the treatment of lupus patients: A pilot study. *Arthritis Rheum.* **39**, S1360.
111a. Metcalfe, W., Boulton-Jones, J. M. (1997). Exacerbation of lupus nephritis in association with leuprorelin injection for uterine leiomyoma. *Nephrol. Dial. Transplant.* **12**, 1699–1700.
112. Jacobson, J. D., Nisula, B. C., and Steinberg, A. D. (1994). Modulation of the expression of murine lupus by gonadotropin-releasing hormone analogs. *Endocrinology* **134**, 2516–2523.
113. Cross, R. J., Campbell, J. L., and Roszman, T. L. (1989). Potentiation of antibody responsiveness after the transplantation of a syngeneic pituitary gland. *J. Neuroimmunol.* **25**, 29–35.
114. McMurray, R., Keisler, D., Kanuckel, K., *et al.* (1991). Prolactin influences autoimmune disease activity in the female B/W mouse. *J. Immunol.* **147**, 3780–3787.
115. Clevenger, C. V., Russell, D. H., Appasamy, P. M., *et al.* (1990). Regulation of interleukin-2 driven T-lymphocyte proliferation by prolactin. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6460–6464.
116. McMurray, R. W., Hoffman, R. W., and Walker, S. E. (1991). *In vivo* prolactin manipulation alters *in vitro* IL-2, IL-4 and IFN-γ mRNA levels in female B/W mice. *Clin. Res.* **39**, 734A.
117. McMurray, R. W., Weidensaul, D., Allen, S. H., and Walker, S. E. (1995). Efficacy of bromocriptine in an open label therapeutic trial for systemic lupus erythematosus. *J. Rheumatol.* **22**, 2084–2091.
118. Alvarez-Nemeguei, J., Cobarrubias-Cobbs, A., Escalante-Triay, F., *et al.* (1998). Bromocriptine in systemic lupus erythematosus: A double-blind, randomized, placebo-controlled study. *Lupus* **7**, 414–419.
119. Walker, S. E., Reddy, G. H., Miller, D., *et al.* (1999). Treatment of systemic lupus erythematosus (SLE) with the prolactin (PRL) lowering drug, bromocriptine (BC): Comparison with hydroxychloroquine (HC) in a randomized, blinded one-year study. *Arthritis Rheum.* **42**, S282.
120. Walker, S. E. (2001). Treatment of systemic lupus erythematosus with bromocriptine. *Lupus* **10**, 197–202.
121. Carlsten, H., Nilsson, N., Jonsson, R., Backman, K., Holmdahl, R., and Tarkowski, A. (1992). Estrogen accelerates immune complex glomerulonephritis but ameliorates T cell-mediated vasculitis and sialadenitis in autoimmune MRL lpr/lpr mice. *Cell. Immunol.* **144**, 190–202.
122. Apelgren, L. D., Bailey, D. L., and Fouts, R. L. (1996). The effect of a selective estrogen receptor modulator on the progression of spontaneous autoimmune disease in MRL lpr/lpr mice. *Cell. Immunol.* **173**, 55–63.

123. Sthoeger, Z. M., Bentwich, Z., Zinger, H., and Mozes, E. (1994). The beneficial effect of the estrogen antagonist, tamoxifen, on experimental systemic lupus erythematosus. *J. Rheumatol.* **21**, 2231–2238.
124. Wu, W. M., Suen, J. L., Lin, B. F., and Chiang, B. L. (2000). Tamoxifen alleviates disease severity and decreases double negative T cells in autoimmune MRL-lpr/lpr mice. *Immunology* **100**, 110–118.
125. Strugess, A. D., Evans, D. T., Mackay, I. R., *et al.* (1984). Effects of the oestrogen antagonist tamoxifen on disease indices in systemic lupus erythematosus. *J. Clin. Lab. Immunol.* **13**, 11–14.
126. Wofsy, D., Ledbetter, J. A., Hendler, P. L., *et al.* (1985). Treatment of murine lupus with monoclonal anti-T cell antibody. *J. Immunol.* **134**, 852–857.
127. Wofsy, D., and Seaman, W. E. (1985). Successful treatment of autoimmunity in NZB/NZW F_1 mice with monoclonal antibody to L3T4. *J. Exp. Med.* **161**, 378–387.
128. Wofsy, D., and Seaman, W. E. (1987). Reversal of advanced murine lupus in NZB/NZW F_1 mice by treatment with monoclonal antibody to L3T4. *J. Immunol.* **138**, 3247–3252.
129. Tomer, Y., Blank, M., and Shoenfeld, Y. (1994). Suppression of experimental antiphospholipid syndrome and systemic lupus erythematosus in mice by anti-CD4 monoclonal antibodies. *Arthritis Rheum.* **37**, 1236–1244.
130. Carny, A., Starolinski, M., Hugin, A. W., *et al.* (1987). Treatment with high doses of anti-IgM prevents but with lower doses accelerates autoimmune disease in (NZW × BXSB) F_1 hybrid mice. *J. Immunol.* **138**, 4222–4228.
131. Yakura, H., Ashida, T., Kawabata, I., *et al.* (1989). Alleviation of autoimmunity in BXSB mice by monoclonal alloantibody to Ly-5 (CD45). *Eur. J. Immunol.* **19**, 1505–1511.
132. Prinz, J. C., Meurer, M., Reiter, C., Rieber, E. P., Plewig, G., and Riethmuller, G. (1996). Treatment of severe cutaneous lupus erythematosus with a chimeric CD4 monoclonal antibody, cM-T412. *J. Am. Acad. Dermatol.* **34**, 244–252.
133. van der Lubbe, P. A., Dijkmans, B. A., Markussee, H. M., *et al.* (1995). A randomized, double-blind, placebo-controlled study of CD4 monoclonal antibody therapy in early rheumatoid arthritis. *Arthritis Rheum.* **38**, 1097–1106.
134. Harper, J. M., and Cook, A. (2001). Beneficial effects of non-depleting anti-CD4 in MRL/Mp-lpr/lpr mice with active systemic lupus erythematosus and microscopic angiitis. *Autoimmunity* **33**, 245–251.
135. Stafford, F. J., Fleisher, T. A., Lee, G., *et al.* (1994). A pilot study of anti-CD5 ricin A chain immunoconjugate in systemic lupus erythematosus. *J. Rheumatol.* **21**, 2068–2070.
136. Davis, T. A., White, C. A., Grillo-Lopez, A. J., *et al.* (1999). Single-agent monoclonal antibody efficacy in bulky non-Hodgkin's lymphoma: Results of a phase II trial of Rituximab. *J. Clin. Oncol.* **17**, 1851–1857.
137. Coiffier, B., Haioun, C, Ketterer, N., *et al.* (1998). Rituximab (anti-CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: A multicenter phase II study. *Blood* **92**, 1927–1932.
138. Weide, R., Heymanns, J., and Koppler, H. (2000). The polyneuropathy associated with Waldenstrom's macroglobulinaemia can be treated effectively with chemotherapy and the anti-CD20 monoclonal antibody rituximab. *Br. J. Hematol.* **109**, 838–841.
139. Zaja, F., Russo, D., Fuga, G., *et al.* (1999). Rituximab for the treatment of type II mixed cryoglobulinemia. *Haematologica* **84**, 1157–1158.
140. Levine, T. D., and Pestronk, A. (1999). IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using rituximab. *Neurology* **52**, 1701–1704.
141. Anolik, J. H., Campbell, D., Ritchlin, C., *et al.* (2001). B lymphocyte depletion as novel treatment for systemic lupus erythematosus (SLE): Phase I/II trial of rituximab (Rituxan) in SLE. *Arthritis Rheum.* **44**, S387.
142. Jiang, Y., Genant, H. K., Watt, I., Cobby, M., Bresnihan, B., Aitchison, R., and McCabe, D. (2000). A multicenter, double-blind, dose-ranging, randomized, placebo-controlled study of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis: Radiologic progression and correlation of Genant and Larsen scores. *Arthritis Rheum.* **43**, 1001–1009.
143. Kiberd, B. A. (1993). Interleukin-6 receptor blockage ameliorates murine lupus nephritis. *J. Am. Soc. Nephrol.* **4**, 58–61.
144. Park, Y., Lee, S. K., Kim, D. S., *et al.* (1998). Elevated interleukin-10 levels correlated with disease activity in systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **16**, 283–288.
145. Rönnelid, J., and Nilsson, B. (1998). Immune complexes from SLE sera induce IL-10 production. A possible vicious circle maintaining B cell hyperactivity in SLE. *Arthritis Rheum.* **41**, S67.
146. Ishida, H., Muchamuel, T., Sakaguchi, S., *et al.* (1994). Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. *J. Exp. Med.* **179**, 305–310.
147. Llorente, L., Richaud-Patin, Y., Garcia-Padilla, *et al.* (2000). Clinical and biologic effects of anti-interleukin-10 monoclonal antibody administration in systemic lupus erythematosus. *Arthritis Rheum.* **43**, 1790–1800.
148. Feinglass, S., and Deodhar (2001). Treatment of lupus-induced thrombocytopenia with recombinant human interleukin-11. *Arthritis Rheum.* **44**, 170–175.
149. Moreland, L. W., Baumgartner, S. W., Schiff, M. H., *et al.* (1997). Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (pg75)-Fc fusion protein. *N. Engl. J. Med.* **337**, 141–147.
150. Maini, R. N., Breedveld, F. C., Kalden, J. R., *et al.* (1998). Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum.* **41**, 1552–1563.
151. Jacob, C. D., and McDevitt, H. O. (1988). Tumor necrosis factor-α in murine autoimmune lupus nephritis. *Nature* **331**, 356–357.

152. Gordon, C., Ranges, G. E., Greenspan, J. S., *et al.* (1989). Chronic therapy with recombinant tumor necrosis factor-α in autoimmune NZB/NZW F_1 mice. *Clin. Immunol. Immunopathol.* **52**, 421–425.
153. Robak, E., Sysa-Jedrzejewska, A., Dziankowska, B., *et al.* (1998). Association of interferon gamma, tumor necrosis factor alpha and interleukin 6 serum levels with systemic lupus erythematosus activity. *Arch. Immunol. Ther. Exp.* (*Warsz.*) **46**, 375–380.
154. Alvarado-de la Barrera, C., Alcocer-Varela, J., Richaud-Patin, Y., Alarcon-Segovia, D., and Llorente, L. (1998). Differential oncogene and TNF-alpha mRNA expression in bone marrow cells from systemic lupus erythematosus patients. *Scand. J. Immunol.* **48**, 551–556.
155. Herrera-Esparza, R., Barbosa-Cisneros, O., Villalobos-Hurtado, R., and Avalos-Diaz, E. (1998). Renal expression of IL-6 and TNFalpha genes in lupus nephritis. *Lupus* **7**, 154–158.
156. Segal, R., Dayan, M., Zinger, H., and Mozes, E. (2001). Suppression of experimental systemic lupus erythematosus (SLE) in mice via TNF inhibition by an anti-TNFalpha monoclonal antibody and by pentoxiphylline. *Lupus* **10**, 23–31.
157. Hagiwara, E., Okubo, T., Aoki, I., *et al.* (2000). IL-12-encoding plasmid has a beneficial effect on spontaneous atuoimmune disease in MRL/MP-lpr/lpr mice. *Cytokine* **12**, 1035–1041.
158. Ronnblom, L., and Alm, G. V. (2001). An etiopathogenic role for the type I IFN system in SLE. *Trends Immunol.* **22**, 427–431.
159. Jacob, C. O., Meide, P. H., and McDevitt, H. O. (1987). In vivo treatment of (NZB × NZW) F_1 lupus-like nephritis with monoclonal antibody to α interferon. *J. Exp. Med.* **166**, 798–804.
160. Ozmen, L., Roman, D., Fountoulakis, M., Schmid, G., Ryffel, B., and Garotta, G. (1995). Experimental therapy of systemic lupus erythematosus: The treatment of NZB/W mice with mouse soluble interferon-gamma receptor inhibits the onset of glomerulonephritis. *Eur. J. Immunol.* **25**, 6–12.
161. Nicoletti, F., Meroni, P., Di Marco, R., *et al.* (1992). In vivo treatment with a monoclonal antibody to interferon-gamma neither affects the survival nor the incidence of lupus-nephritis in the MRL/lpr-lpr mouse. *Immunopharmacology* **24**, 11–16.
162. Alarcon-Segovia, D. (1963). Systemic lupus erythematosus following thymectomy for myasthenia gravis. *Lancet* **2**, 662.
163. Peterson, P., and Lund, J. (1969). Systemic lupus erythematosus following thymectomy for myasthenia gravis. *Dan. Med. Bull.* **16**, 179.
164. Grinlinton, F. M., Lynch, N. M., and Hart, H. H. (1991). A pair of monozygotic twins who are concordant for myasthenia gravis but became discordant for systemic lupus erythematosus post-thymectomy. *Arthritis Rheum.* **34**, 916.
165. Balo-Banga, J. M., Pinter, E., and Nemeth, G. (1993). Incidence of SLE in a patient thymectomized for myasthenia gravis. *Orvosi Hetilap* **134**, 1369–1372.
166. Goldstein, A. L., Zatz, M. M., Low, T. L., *et al.* (1981). Potential role of thymosin in the treatment of autoimmune diseases. *Ann. N. Y. Acad. Sci.* **377**, 486–495.
167. Chu, E. B., Hobbs, M. V., Wilson, C. B., Romball, C. G., Linsley, P. S., and Weigle, W. O. (1996). Intervention of $CD4^+$ cell subset shifts and autoimmunity in the BXSB mouse by murine CTLA4Ig. *J. Immunol.* **156**, 1262–1268.
168. Finck, B. K., Linsley, P. S., and Wofsy, D. (1994). Treatment of murine lupus with CTLA4Ig. *Science* **265**, 1225–1227.
169. Daikh, D. I., and Wofsy, D. (2001). Cutting edge: Reversal of murine lupus nephritis with CTLA4Ig and cyclophosphamide. *J. Immunol.* **166**, 2913–2916.
170. Takiguchi, M., Murakami, M., Nakagawa, I., Saito, I., Hashimoto, A., and Uede, T. (2000). CTLA4IgG gene delivery prevents autoantibody production and lupus nephritis in MRL/lpr mice. *Life Sci.* **66**, 991–1001.
171. Early, G. S., Zhao, W., and Burns, C. M. (1996). Anti-CD40 ligand antibody treatment prevents the development of lupus-like nephritis in a subset of New Zealand black × New Zealand white mice. Response correlates with the absence of an anti-antibody response. *J. Immunol.* **157**, 3159–3164.
172. Kalled, S. L., Cutler, A. H., Datta, S, K., and Thoma, D. W. (1998). Anti-CD40 ligand antibody treatment of SNF1 mice with established nephritis: Preservation of kidney function. *J. Immunol.* **160**, 2158–2165.
173. Davis, J. C., Totoritis, M. C., Rosenberg, J., Sklenar, T. A., and Wofsy, D. (2001). Phase I clinical trial of a monoclonal antibody against CD40-ligand (IDEC-131) in patients with systemic lupus erythematosus. *J. Rheumatol.* **28**, 95–101.
174. Kalunian, K. I., Davis, J., Merrill, J. T., *et al.* (2000). Treatment of SLE by inhibition of T cell costimulation. *Arthritis Rheum.* **43**, S271.
175. Shirai, A., Aoki, I., Otani, M., Mond, J. J., and Klinman, D. M. (1994). Treatment with dextran-conjugated anti-IgD delays the development of autoimmunity in MRL-lpr/lpr mice. *J. Immunol.* **153**, 1889–1894.
176. van Vollenhoven, R. F., Swenson, C. D., Soriano, A. *et al.* (1989). Serum IgD levels in mice: Effect of strain, age and autoimmune disease. *J. Autoimmun.* **2**, 259–267.
177. Coico, R. F., Xue, B., Wallace, D., Pernis, B., Siskind, G. W., and Thorbecke, G. J. (1985). T cells with receptors for IgD. *Nature* **316**, 744–746.
178. Hahn, B. H., and Ebling, F. M. (1984). Suppression of murine lupus nephritis by administration of an anti-idiotypic antibody to anti-DNA. *J. Immunol.* **132**, 187–197.
179. Spertini, F., Leimgruber, A., Morel, B., *et al.* (1999). Idiotypic vaccination with a murine anti-dsDNA antibody: Phase I study in patients with nonactive systemic lupus erythematosus with nephritis. *J. Rheumatol.* **26**, 2602–2608.
180. Nakajima, A., Hirai, H., Kayagaki, N., *et al.* (2000). Treatment of lupus in NZB/W F1 mice with monoclonal antibody against Fas ligand. *J. Autoimmun.* **14**, 151–157.

181. Seery, J. P., Cattell, V., and Watt, F. M. (2001). Cuting edge: Amelioration of kidney disease in transgenic mouse model of lupus nephritis by administration of the caspase inhibitor carbobenzoxy-valyl-aspartyl-(beta-o-methyl)-fluoromethylketone. *J. Immunol.* **167**, 2452–2455.
182. Wang, Y., Hu, Q., Madri, J. A., Rollins, S. A., Chodera, A., and Matis, L. A. (1996). Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8563–8568.
183. Khaled, A. R., Soares, L. S., Butfiloski, E. J., Stekman, I., Sobel, E. S., and Schiffenbauer, J. (1997). Inhibition of the p50 (NKkappaB1) subunit of NF-kappaB by phosphorothioate-modified antisenseoligodeoxynucleotides reduces NF-kappaB expression and immunoglobulin synthesis in murine B cells. *Clin. Immunol. Immunopathol.* **83**, 254–263.
184. Corna, D., Morigi, M., Facchinetti, D., Bertani, T., Zoja, C., and Remuzzi, G. (1997). Mycophenolate mofetil limits renal damage and prolongs life in murine lupus autoimmune disease. *Kidney Int.* **51**, 1583–1589.
185. Jonsson, C. A., Erlandsson, M., Svensson, L., Molne, J., and Carlsten, H. (1999). Mycophenolate mofetil ameliorates perivascular T lymphocyte inflammation and reduces the double-negative T cell population in SLE-prone MRLlpr/lpr mice. *Cell. Immunol.* **197**, 136–144.
186. Gaubitz, M., Schorat, A., Schotte, H., Kern, P., and Domschke, W. (1999). Mycophenolate mofetil for the treatment of systemic lupus erythematosus: An open pilot trial. *Lupus* **8**, 731–736.
187. Chan, T. M., Li, F. K., Tang, C. S., *et al.* (2000). Efficacy of mycophenolate mofetil in patients with difuse proliferative lupus nephritis. Hong Kong–Guangzhou Nephrology Study Group. *N. Engl. J. Med.* **343**, 1156–1162.
188. Buratti, S., Szer, I., Spencer, C. H., Bartosh, S., and Reiff, A. (2001). Mycophenolate mofetil treatment of severe renal disease in pediatric onset systemic lupus erythematosus. *J. Rheumatol.* **28**, 2103–2108.
189. Kino, T., Hatanaka, H., Miyata, S., *et al.* (1987). FK-506, a novel immunosuppressant isolated form a *Streptomyces*. **II**. Immunosuppressive effect of FK-506 in vitro. *J. Antibiot.* **40**, 1256.
190. Takabayashi, K., Koike, T., Kurasawa, K., *et al.* (1989). Effect of FK-506, a novel immunosuppressive drug on murine systemic lupus erythematosus. *Clin. Immunopathol.* **51**, 110–114.
191. Furukawa, F., Imamura, S., and Takigawa, M. (1995). FK506: Therapeutic effects on lupus dermatoses in autoimmune-prone MRL/Mp-lpr/lpr mice. *Arch. Dermatol. Res.* **287**, 558–563.
192. Entani, C., Izumino, K., Iida, H., *et al.* (1993). Effect of a novel immunosuppressant, FK506, on spontaneous lupus nephritis in MRL/MpJ-lpr/lpr mice. *Nephron* **64**, 471–475.
193. Woo, J., Wright, T. M., Lemster, B., Borochovitz, D., Nalesnik, M. A., and Thomson, A. W. (1995). Combined effects of FK506 (tacrolimus) and cyclophosphamide on atypical $B220^+$ T cells, cytokine gene expression and disease activity in MRL/MpJ-1pr/lpr mice. *Clin. Exp. Immunol.* **100**, 118–125.
194. Duddridge, M., and Powell, R. J. (1997). Treatment of severe and difficult cases of systemic lupus erythematosus with tacrolimus. A report of three cases. *Ann. Rheum. Dis.* **56**, 690–692.
195. Walker, E. W., Solsky, M., and Schnitzer, B. (1982). Prolonged lifespans in female NZB/NZW mice treated with the experimental immunoregulatory drug frentizole. *Arthritis Rheum.* **25**, 1291–1295.
196. Valentine, T. V., Kay, D. R., Walker, S. E., *et al.* (1978). Frentizole therapy of active systemic lupus erythematous. *Arthritis Rheum.* **21**, 599–606.
197. Sabharwal, U. K., Vaughan, J. H., Kaplan, R. A., *et al.* (1980). Frentizole therapy in systemic lupus erytematosus. *Arthritis Rheum.* **23**, 1376–1380.
198. Kay, D. R., Valentine, V., Walker, S. E., *et al.* (1980). Frentizole therapy of active systemic lupus erythematosus. *Arthritis Rheum.* **23**, 1381–1387.
199. Bang, N. U. (1980). Frentizole in systemic lupus erythematosus: Current status. *Arthritis Rheum.* **23**, 1388–1390.
200. Soga, T., Ishigatsubo, Y., Kitamura, H., and Okubo, T. (1996). Effect of mizoribine on pulmonary lesions in MRL/lpr/lpr mice. *Autoimmunity* **25**, 9–18.
201. Iwasaki, T., Hamano, T., Aizawa, K., Kobayashi, K., and Kakishita, E. (1994). A case of systemic lupus erythematosus (SLE) successfully treated with mizoribine (Bredinin). *Ryumachi.* **34**, 885–889.
202. Zandman-Goddard, G., George, J., and Levy, Y. (1996). Modulation of experimental systemic lupus erythematosus with linomide. *Lupus* **5**, 328–333.
203. Hortelano, S., Diaz-Guerra, M. J., Gonzalez-Garcia, A., Leonardo, E., Gamallo, C., Bosca, L., and Martinez, A. C. (1997). Linomide administration to mice attenuates the induction of nitric oxide synthase elicited by lipopolysaccharide-activated macrophages and prevents nephritis in MRL/Mp-lpr/lpr mice. *J. Immunol.* **158**, 1402–1408.
204. Warner, L. M., Adams, L. M., and Sehgal, S. N. (1994). Rapamycin prolongs survival and arrests pathophysiologic changes in murine systemic lupus erythematosus. *Arthritis Rheum.* **37**, 289–297.
205. Okubo, M., Inone, K., Umetansi, N., *et al.* (1988). Lupus nephropathy in New Zealand F_1 hybrid mice treated by 15-deoxyspergualin. *Kidney Int.* **34**, 467–474.
206. Ito, S., Ueno, M., Arakawa, M., *et al.* (1991). Therapeutic effect of 15-deoxyspergualin on progression of lupus nephritis in MRL mice. *Clin. Exp. Immunol.* **81**, 446–454.
207. Okubo, M., Inoue, K., Amemiya, K., Kobayashi, N., Masaki, Y., and Kamata, K. (1993). High-dose 15-deoxyspergualin monotherapy surpasses methylprednisolone in its therapeutic effect on advanced lupus nephritis in New Zealand black/white F1 hybrid mice, and low-dose combination may be synergistic. *Transplantation* **8**, 720–724.

208. Nemoto, K., Mae, T., Saiga, K., Matsuura, E., and Koike, T. (1995). Autoimmune-prone (NZW × BXSB) F1 (W/BF1) mice escape severe thrombocytopenia after treatment with deoxyspergualin, an immunosuppressant. *Br. J. Haematol.* **91**, 691–696.
209. Fan, J. L., Himeno, K., Tsuru, S., *et al.* (1987). Treatment of immune MRL/Mp-lpr/lpr mice with cholera toxin. *Clin. Exp. Immunol.* **70**, 94–99.
210. Mihara, M., and Ohsugi, Y. (1989). The biological response modifier OK-432 inhibits the development of autoimmune kidney disease in NZB/W F_1 hybrid mice. *Int. Arch. Allergy Appl. Immunol.* **90**, 37–42.
211. Jacobson, J. M., Greenspan, J. S., Spritzler, J., *et al.* (1997). Thalidomide for the treatment of oral aphthous ulcers in patients with human immunodeficiency virus infection. National Institute of Allergy and Infectious Diseases Aids Clinical Trials Group. *N. Engl. J. Med.* **336**, 1487–1493.
212. Knop, J., Bonsmann, G., Gapple, R., *et al.* (1983). Thalidomide in the treatment of sixty cases of chronic discoid lupus erythematosus. *Br. J. Dermatol.* **108**, 461–466.
213. Stevens, R. J., Andujar, C., Edwards, C. J., *et al.* (1997). Thalidomide in the treatment of the cutaneous manifestations of lupus erythematosus: Experience in sixteen consecutive patients. *Br. J. Rheumatol.* **36**, 353–359.
214. Duong, D. J., Spigel, G. T., Moxley, R. T., and Gaspari, A. A. (1999). American experience with low-dose thalidomide therapy for severe cutaneous lupus erythematosus. *Arch. Dermatol.* **135**, 1079–1087.
215. Sato, E. I., Assis, L. S., Lourenzi, V. P., and Andrade, L. E. (1998). Long-term thalidomide use in refractory cutaneous lesions of systemic lupus erythematosus. *Rev. Assoc. Med. Bras.* **44**, 289–293.
216. Ordi-Ros, J., Cortres, F., Curucull, E., *et al.* (2000). Thalidomide in the treatment of cutaneous lupus refractory to conventional therapy. *J. Rheumatol.* **27**, 1429–1433.
217. Stricker, R. B., Elswood, B. F., Goldberg, *et al.* (1994). Clinical and immunologic evaluation of HIV-infected patients treated with dinitrochlorobenzene (DNCB). *J. Am. Acad. Dermatol.* **31**, 462–466.
218. Stricker, R. B., Goldberg, B., and Epstein, W. L. (1995). Immunological changes in patient with systemic lupus erythematosus treated with topical dinitrochlorobenzene. *Lancet.* **345**, 1505–1506.
219. Hadidi, T., Decker, J. L., El-Nagdy, L., *et al.* (1981). Ineffectivenesss of levamisole in systemic lupus erythematosus: A controlled trial. *Arthritis Rheum.* **24**, 60–67.
220. Klassen, L. W., Budman, D. R., Williams, G. W., *et al.* (1977). Ribavirin: Efficacy in the treatment of murine autoimmune disease. *Science* **195**, 787–788.
221. Kelley, V. E., Snene, S., and Musinski, S. (1986). Increased renal thromboxane production in murine lupus nephritis. *J. Clin. Invest.* **77**, 252–256.
222. Kimberly, R. P., Gill, J. R., Bowden, R. E., *et al.* (1978). Elevated urinary prostaglandins and the effects on renal function in lupus erythematosus. *Ann. Intern. Med.* **89**, 336–341.
223. Clark, W. F., Parbtani, A., McDonald, J. W. D., *et al.* (1987). The effects of a thromboxane synthetase inhibitor a prostacyclin analog and PGE_1 on the nephritis of the NZB/W F_1 mouse. *Clin. Nephrol.* **28**, 288–291.
224. Pierucci, A., Simonetti, B. M., Perri, G., *et al.* (1989). Improvement of renal function with selective thromboxane antagonism in lupus nephritis. *N. Engl. J. Med.* **320**, 421–424.
225. Robbins, I. M., Gaine, S. P., Schilz, R., *et al.* (2000). Epoprostenol for treatment of pulmonary hypertension in patients with systemic lupus erythematosus. *Chest* **117**, 14–18.
226. Horn, E. M., Barst, R. J., and Poon, M. (2000). Epoprostenol for treatment of pulmonary hypertension in patients with systemic lupus erythematosus. *Chest* **118**, 1229–1230.
227. Hackshaw, K. V., Shi, Y., Brandwein, S. R., Jones, K., and Westcott, J. Y. (1995). A pilot study of zileuton, a novel selective 5-lipoxygenase inhibitor, in patients with systemic lupus erythematosus. *J. Rheumatol.* **22**, 462–468.
228. Bootsma, H., Spronk, P., Derkensen, R., *et al.* (1995). Prevention of relapses in systemic lupus erythematosus. *Lancet* **345**, 1595–1599.
229. ter Borg, E. J., Horst, G., Hummell, E. J., *et al.* (1990). Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus, a long term, prospective study. *Arthritis Rheum.* **33**, 634–643.
230. ter Borg, E. J., Horst, G., Hummell, E. J., *et al.* (1990). Changes in plasma levels of interleukin-2 receptor in relation to disease exacerbations and levels of anti-dsDNA and complement in systemic lupus erythematosus. *Clin. Exp. Immunol.* **82**, 21–26.
231. Esdaile, J. M., Abrahamowicz, M., Joseph, L., *et al.* (1996). Laboratory tests as predictors of disease exacerbations in systemic lupus erythematosus: Why some tests fail. *Arthritis Rheum.* **39**, 370–378.
232. Andreu, G., Boccaccio, C., Leguen, J. P., *et al.* (1992). Ultraviolet light-induced immunomodulation: A possible new tool in organ transplantation. *Ann. Med. Interne* **143**, S52–S56.
233. McGrath, H. (1994). Ultraviolet-A1 irradiation decreases clinical disease activity and autoantibodies in patients with systemic lupus erythematosus. *Clin. Exp. Rheumatol.* **12**, 129–135.
234. Sonnichsen, N., Meffert, H., Kunzelmann, V., and Audring, H. (1993). UV-A-1 therapy of subacute cutaneous lupus erythematosus. *Hautarzt.* **44**, 723–725.
235. McGrath, H., Martinez-Osuna. P., Lee, FA. (1996). Ultraviolet-A1 (340–400 nm) irradiation therapy in systemic lupus erythematosus. *Lupus* **5**, 269–274.
236. Polerman, M. C., Huizinga, T. W., Le Cessie, S., and Pavel, S. (2001). UVA-1 cold light treatment of SLE: A double blind, placebo controlled crossover trial. *Ann. Rheum. Dis.* **60**, 112–115.
237. Moore, A. D., Petri, M. A., Manzi, S., *et al.* (2000). The use of alternative medical therapies in patients with

systemic lupus erythematosus. Trination study group. *Arthritis Rheum.* **43**, 1410–1418.
238. Chang, J. Y., Yang, T. Y., Chang, C. P., and Chang, J. G. (1996). The effect of "chi-han (hot nature)" Chinese herbs on the secretion of IL-1 beta and TNF-alpha by mononuclear cells. *J. Med. Sci.* **12**, 18–24.
239. Hsu, S. H., Tsai, T. R., Lin, C. N., *et al.* (1996). Solamargine purified from *Solanum incanum* Chinese herb triggers gene expression of human TNFR 1 which may lead to cell apoptosis. *Biochem. Biophys. Res. Commun.* **299**, 1–5.
240. Kobayashi, I., Hamasaki, Y., Sato, R., *et al.* (1995). Saiboku-To, a herbal extract mixture, selectively inhibits 5-lipoxygenase activity and leukotriene synthesis in rat basophilic leukemia-1 cells. *J. Ethnopharmacol.* **48**, 33–41.
241. Chen, J. R., Yen, J. H., Lin, C. C., *et al.* (1993). The effects of Chinese herbs on improving survival and inhibiting anti-ds DNA antibody production in lupus mice. *Am. J. Chin. Med.* **21**, 257–262.
242. Lai, N. S., Lin, R. H., Lai, R. S., Kun, U. C., and Leu, S. C. (2001). Prevention of autoantibody formation and prolonged survival in New Zealand Black/New Zealand White F1 mice with an ancient Chinese herb, *Ganoderma tsugae. Lupus* **10**, 461–465.
243. Zhou, N. N., Nakai, S., Kawakita, T., Oka, M., Nagasawa, H., Himeno, K., and Nomoto, K. (1994). Combined treatment of autoimmune MRL/MP-lpr/lpr mice with a herbal medicine, Ren-shen-yang-rong-tang (Japanese name: Ninjin-youei-to) plus suboptimal dosage of prednisolone. *Immunopharmacology* **16**, 845–854.
244. Chen, X., Yu, L., and Lu, Y. (1995). Image analysis for intercell adhesion molecule-1 expression in MRI/lpr mice: Effects of Chinese herb medicine. *Chin. Med. J.* **75**, 204–206.
245. Furuya, Y., Kawakia, T., and Nomoto, K. (2001). Immunomodulating effect of a traditional Japanese medicine, hachimi-jio-gan (ba-wei-di-huang-wan), on Th1 predominance in autoimmune MRL/MP-lpr/lpr mice. *Int. Immunopharmacol.* **1**, 551–559.
246. Ruan, J., and Ye, R. G. (1994). Lupus nephritis treated with impact therapy of cyclophosphamide and traditional Chinese medicine. *Chung-Ku Chung Hsi I Chieh Ho Tsa Chih.* **14**, 276–278.
247. Yu, K. T., Nuss, G., and Boyce, R. (1994). Inhibition of IL-1 release from human monocytes and suppression of streptococcal cell wall and adjuvant-induced arthritis in rats by an extract of *Tripterygium wilfordii* Hook. *Gen. Pharmacol.* **25**, 1115–1122.
248. Xu, D. S., Shen, Z. Y., and Lu, W. (1996). Clinical and experimental study of RA mixture in treatment of rheumatoid arthritis. *Chung-Kuo Chung Hsi I Chieh Ho Tsa Chih.* **16**, 14–17.
249. Dong, Y. (1993). The suppressive effect of *Tripterygium wilfordii* Hook. f. on the IL-2 autocrine loop of human T cells. *Acta Acad. Med. Sin.* **15**, 193–196.
250. Chang, D. M., Chang, W. Y., Kuo, S. Y., *et al.* (1997). The effects of traditional antirheumatic herbal medicines on immune response cells. *J. Rheumatol.* **24**, 436–441.
251. Li, R. L., Liu, P. L., and Wu, X. C. (1996). Clinical and experimental study on sustained release tablet of *Tripterygium wilfodii* in treating rheumatoid arthritis. *Chung-Kuo Chung Hsi I Chieh Ho Tsa Chih* **6**, 10–13.
252. Gu, W. Z., Chen, R., Brandwein, S., *et al.* (1995). Isolation, purification, and characterization of immunosuppressive compounds from triptrerygium: triptolide and tripdiolide. *Int. J. Immunopharmacol.* **17**, 351–356.
253. Gu, W. Z., Banerjee, S., Rauch, J., and Brandwein, S. R. (1992). Suppression of renal disease and arthritis, and prolongation of survival in MRL-lpr mice treated with an extract of *Tripterygium wilfordii Hook f. Arthritis Rheum.* **35**, 1381–1386.
254. Zhang, X. Y., Tsuchiya, N., Dohi, M., *et al.* (1992). Prolonged survival of MRL-lpr/lpr mice treated with *Tripterygium Wilfordii* Hook. f. *Clin. Immunol. Immunopathol.* **62**, 66–71.
255. Kao, N. L., Richmond, G. W., and Moy, J. N. (1993). Resolution of severe lupus nephritis associated with *Tripterygium wilfordii* Hook. f. ingestion. *Arthritis Rheum.* **12**, 1751–1752.
256. Depierreux, M., Van Damme, B., Vanden Houte, K., *et al.* (1994). Pathologic aspects of a newly described nephropathy related to the prolonged use of Chinese herbs. *Am. J. Kidney Dis.* **24**, 172–180.
257. Reginster, F., Jadoul, M., van Ypersele, and de Strihou, C. (1997). Chinese herbs nephropathy presentation, natural history and fate after transplantation. *Transplantation* **12**, 81–86.
258. Ferguson, J. E., Chalmers, R. J., and Rowlands, D. J. (1997). Reversible dilated cardiomyopathy following treatment of atopic eczema with Chinese herbal medicine. *Br. J. Dermatol.* **136**, 592–593.
259. Dubois, E. L., and Strain, L. (1973). Effect of diet on survival and nephropathy of NZB/NZW hybrid mice. *Biochem. Med.* **7**, 336–343.
260. Friend, P. S., Fernandes, G., Good, R. A., *et al.* (1978). Dietary restrictions: Early and late effects on the nephropathy of the NZB × NZW mouse. *Lab. Invest.* **38**, 629–637.
261. Izui, S., Fernandes, G., Hara, I., *et al.* (1981). Low calorie diet selectively reduces expression of retroviral envelope glycoprotein gp 70 in sera of NZB × NZW F_1 hybrid mice. *J. Exp. Med.* **154**, 1116–1121.
262. Hurd, E. R., Johnston, J. M., Okita, J. R., *et al.* (1981). Prevention of glomerulonephritis and prolonged survival in New Zealand Black/New Zealand White F_1 hybrid mice fed an essential fatty acid-defficient diet. *J. Clin. Invest.* **67**, 476–484.
263. Prickett, J. D., Robinson, D. R., and Steinberg, D. (1981). Dietary enrichment with polyunsaturated fatty acid eicosapentaenoic acid prevents proteinuria and prolongs survival in NZB × NZW F_1 mice. *J. Clin. Invest.* **68**, 556–564.
264. Prickett, J. D., Robinson, D. R., and Steinberg, D. (1983). Effects of dietary enrichment with eicosapentaeoic acid upon autoimmune nephritis in female NZB × NZW F_1 mice. *Arthritis Rheum.* **26**, 133–138.

265. Watson, J., Godfrey, D., Stinson, W. H., *et al.* (1988). The therapeutic effects of dietary fatty acid supplementation in the autoimmune disease of the MRL-mp-lrp/lpr mouse. *Int. J. Immunopharmacol.* **10**, 467–474.
266. Clark, W. F., Parbtani, A., Huff, M. W., *et al.* (1989). Omega-3 fatty acid dietary supplementation in systemic lupus erythematosus. *Kidney Int.* **36**, 653–659.
267. Clark, W. F., Parbtani, A., Huff, M. W., *et al.* (1995). Flaxseed: A potential treatment for lupus nephritis. *Kidney Int.* **48**, 475–480.
268. Clark, W. F., Kortas, C., Heidenheim, A. P., Garland, J., Spanner, E., and Parbtani, A. (2001). Flaxseed in lupus nephritis: A two-year nonplacebo-controlled crossover study. *J. Am. Coll. Nutr.* **20**, 143–148.
269. Clark, W. F., Muir, A. D., Westcott, N. D., and Parbtani, A. (2000). A novel treatment for lupus nephritis: Lignan precursor derived from flax. *Lupus* **9**, 429–436.
270. Ehrenfeld, M., Blank, M., Shoenfeld, Y., and Hidvegi, M. (2001). AVEMAR (a new benzoquinone-containing natural product) administration interferes with the Th2 response in experimental SLE and promotes amelioration of the disease. *Lupus* **10**, 622–627.
271. Suwannaroj, S., Lagoo, A., Keisler, D., and McMurray, R. W. (2001). Antioxidants suppress mortality in the female NZB × NZW F_1 mouse model of systemic lupus erythematosus (SLE). *Lupus* **10**, 258–265.
272. Stefanescu, M., Matache, C., Onu, A., *et al.* (2001). Pycnogenol(R) efficacy in the treatment of systemic lupus erythematosus patients. *Phytother. Res.* **15**, 698–704.
273. Gonzalez, R., and Remirez, D. (1997). Current views on the pharmacological properties of the immunomodulator, lobenzarit disodium. *J. Invest Allergol. Clin. Immunol.* **7**, 77–82.
274. Hirohata, S., Ohnishi, K., and Sagawa, A. (1994). Treatment of systemic lupus erythematosus with lobenzarit: An open clinical trial. *Clin. Exp. Rheumatol.* **12**, 261–265.
275. Chen, Y. S., and Hu, X. E. (1983). Auriculo-acupuncture in 15 cases of discoid lupus erythematosus. *J. Tradit. Chin. Med.* **5**, 261–262.
276. Lautenschlager, J. (1997). Acupuncture in treatment of inflammatory rheumatic diseases. *Z. Rheumatol.* **56**, 8–20.
277. Khamaganova, I. V., Berlin, Iu. V., Volkov, V. E., *et al.* (1995). The use of a pulsed magnetic field in the treatment of lupus erythematosus. *Terapevticheskii Arkhiv.* **67**, 84–87.
278. Knobler, R. M., Graninger, W., Graninger, W., Lindmaier, A., Trautinger, F., and Smolen, J. S. (1992). Extracorporeal photochemotherapy for the treatment of systemic lupus erythematosus. A pilot study. *Arthritis Rheum.* **35**, 319–324.

INDEX

D

E

F

G

H

I

L

M

N

O

P

Q

R

U

V

W

X

Z